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Diversity and relatedness of Shiga toxin-producing *Escherichia coli* and *Campylobacter jejuni* between farms in a dairy catchment

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SUMMARY

The aim of this study was to examine the population structure, transmission and spatial relationship between genotypes of Shiga toxin-producing *Escherichia coli* (STEC) and *Campylobacter jejuni*, on 20 dairy farms in a defined catchment. Pooled faecal samples ($n = 72$) obtained from 288 calves were analysed by real-time polymerase chain reaction (rtPCR) for *E. coli* serotypes O26, O103, O111, O145 and O157. The number of samples positive for *E. coli* O26 (30/72) was high compared to *E. coli* O103 (7/72), O145 (3/72), O157 (2/72) and O111 (0/72). Eighteen *E. coli* O26 and 53 *C. jejuni* isolates were recovered from samples by bacterial culture. *E. coli* O26 and *C. jejuni* isolates were genotyped using pulsed-field gel electrophoresis and multilocus sequence typing, respectively. All *E. coli* O26 isolates could be divided into four clusters and the results indicated that *E. coli* O26 isolates recovered from calves on the same farm were more similar than isolates recovered from different farms in the catchment. There were 11 different sequence types of *C. jejuni* isolated from the cattle and 22 from water. An analysis of the population structure of *C. jejuni* isolated from cattle provided evidence of clustering of genotypes within farms, and among groups of farms separated by road boundaries.

Key words: *Campylobacter*, public health, Shiga-like toxin-producing *E. coli*.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) and *Campylobacter* spp. are important bacterial causes of gastroenteritis in humans worldwide and cattle are considered an important reservoir for both pathogens [1, 2]. STEC have emerged globally as an important food-borne human zoonotic pathogen associated with outbreaks and sporadic cases of diarrhoea, haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) [1]. There are more than 100 STEC serogroups that have been associated with human disease [3], but seven (O26, O45, O103, O111, O121, O145, O157) are considered to be the most important due to their association with large outbreaks and many of the HUS and HC cases [4]. STEC-associated disease outbreaks have been

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reported worldwide [1] with the first STEC-associated human case from New Zealand reported in 1980 [5]. The rate of reported STEC infections in New Zealand has increased from 1·3 cases/100 000 population in 1998 to 5·1 cases/100 000 population in 2013 [6, p. 53] and is presently higher than that from the UK (2·4 cases/100 000 population) [7] and from the USA (2·2 cases/100 000 population) [8]. Ruminants are considered to be the main reservoir of STEC [1] and in New Zealand the greatest risk factors for human infection are proximity to cattle, contact with animal manure and contact with recreational water [9]. *Campylobacter jejuni* and *C. coli* are the most important gastroenteritis-causing *Campylobacter* spp. worldwide [10]. Campylobacteriosis, enteric infection associated with *C. jejuni* is the most frequently reported notifiable infectious disease in New Zealand. In 2013, 6837 (152·9 cases/100 000 population) human cases of campylobacteriosis were notified [6, pp. 25–26]. Chicken, red meat, milk, water (for drinking and recreational activities) and contact with farm animals are considered to be the most common sources of transmission of *Campylobacter* to humans [10] and, since the reduction in cases associated with controls in the poultry industry in New Zealand in 2007/2008, ruminant sources (both bovine and ovine) have become relatively more important [11].

Water channels and rural streams can become contaminated with *Campylobacter* and *E. coli* by direct deposition of cattle faeces and inputs from surface and subsurface flows following rainfall or irrigation. Elevated *Campylobacter* concentrations in stream water during flood events appear to arise from local land run-off whereas elevated *E. coli* concentration can be dominated by sources further upstream [12]. In New Zealand, high levels of faecal bacteria have been reported from rural streams including the catchment where this study was conducted [13] in which dairy farming is the major land use. Better understanding of population structure and transmission of zoonotic pathogens would assist with devising appropriate control strategies aimed at reducing environmental contamination, which in turn would reduce transmission to animals and humans. This study was conducted to determine the degree of relatedness of STEC and *C. jejuni* within farm and also between farms as a function of their separation by a road network and the extent to which they are connected by a network of streams. Understanding the population structure of STEC and *C. jejuni* within and between communities of farms will allow the development of effective mitigation strategies to reduce both within- and between-farm spread.

MATERIALS AND METHODS

The dairy catchment (~15 km^2) where the study was conducted was selected because 70% of catchment land is used for dairy farming and catchment boundaries are well defined. There are 23 farms in this catchment including 18 dairy farms, four dry-stock farms and one horse farm. Characteristics of the catchment and initial observations of the behaviour of generic *E. coli* and *Campylobacter* spp. in the waterway draining the dairy farming area have been described previously [14].

Farms that shared a boundary (excluding farms separated by roads, but including those separated by a stream) were grouped together (Fig. 1*a*). The region was also divided into farms adjacent to the main stream, and those in the periphery of the catchment (Fig. 1*b*).

Animal sampling was carried out over a 3-month period from October to December 2009. Faecal samples were collected from 288 animals, which were either born in July/August 2009 (Y0; average 3 or 4 months) or July/August 2008 (Y1; average 15 or 16 months), from 20 of the farms and each farm was sampled only once. The three farms not sampled were a dry-stock farm that had no calves during the study period and a horse farm. The remaining dairy farm had no Y0 calves but the Y1 animals which were grazed on a neighbouring farm were sampled. Different farms were sampled at each occasion and samples were collected from a freshly voided faecal pat shed by an individual animal.

Sets of four faecal pat samples (grouped on the basis of farm and age group) were thoroughly mixed to form a single composite sample for microbiological analysis. This was done in order to reduce the number of samples required for microbiological analysis, while focusing on the recovery of isolates for genotyping and subsequent phylogenetic comparisons between populations. In total, 72 pooled faecal samples from the 20 farms in the catchment area were transported under cold conditions to the laboratory. These samples were analysed for the presence of STEC (O26, O103, O111, O145, O157) and *C. jejuni*. In addition 33 water samples were collected from a single site at end of the main stream of the catchment (Fig. 1*a*) as part of a separate study that examined changes in total *Campylobacter* and *C. jejuni* numbers associated with two separate flood events (11–15 September and 29 September–1 October 2008).

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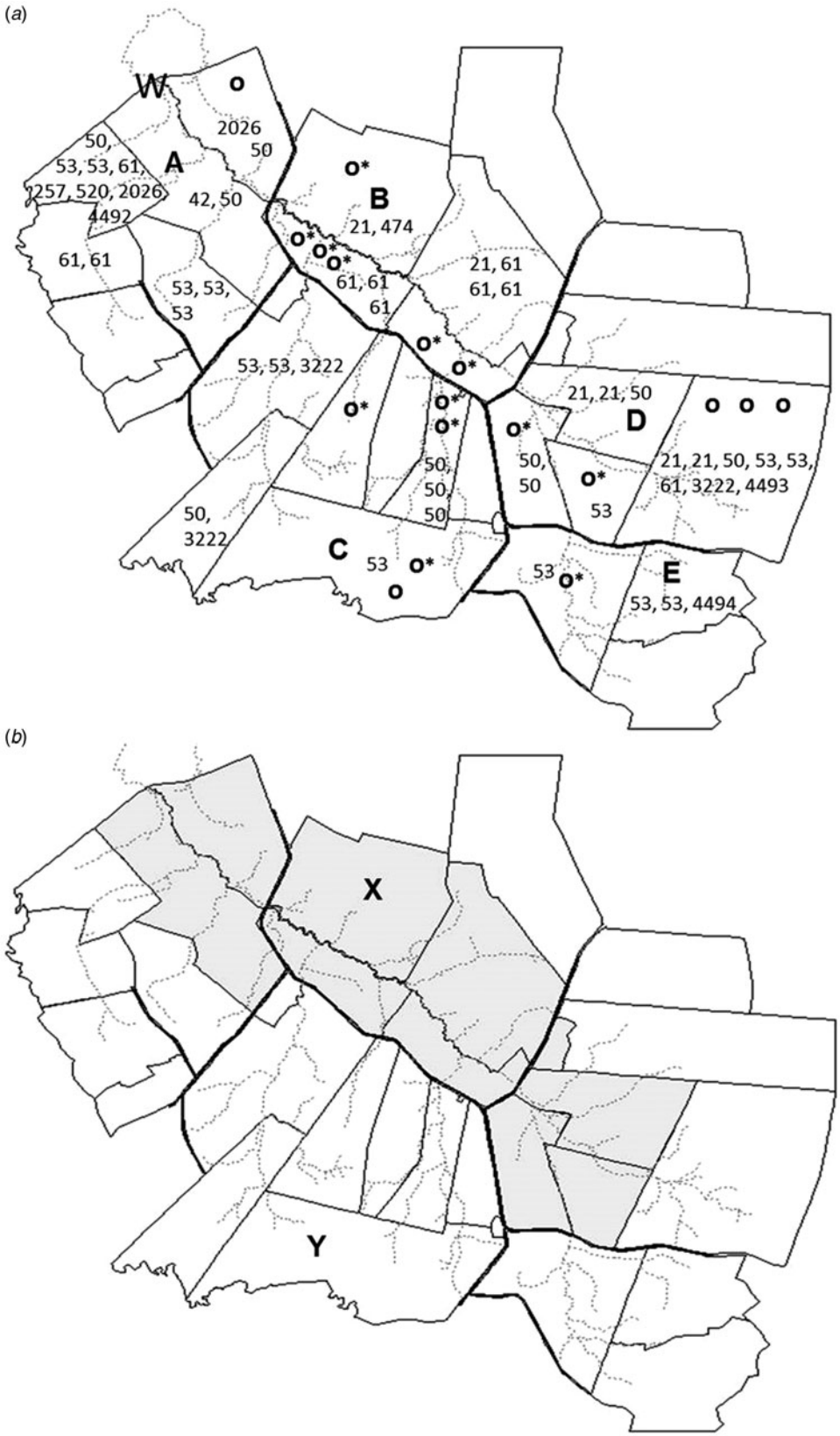

Fig. 1. River catchment mapping. (*a*) Five distinct groups marked A–E were evident. The road network is shown in heavy black lines and the stream network in broken grey lines. Presence of *E. coli* O26 (O), STEC O26 (O*) and *C. jejuni* sequence types shown at the farm level. W, Water sampling site. (*b*) An alternative grouping of farms according to whether they were adjacent to the main stream (X, in grey) or away from the main stream (Y, in white).

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Isolation and characterization of STEC

For STEC analysis, 2 g of each composite sample was enriched in 18 ml buffered peptone water (BPW) for 24 h at 37 °C. The Isolate Faecal DNA kits (Bioline, New Zealand) were used for isolation of DNA from enriched faecal samples according to the manufacturer's instructions. DNA samples were then tested by real-time polymerase chain reaction (rtPCR) for the presence of *wzx* (O26) [15], *wzx* (O103) [16], *wbdI* (O111) [15], *wzx1* (O145) [17] and *rfbE* (O157) [15] using previously published methods.

Briefly SYTO-9 was used as the fluorescent nucleic acid stain in rtPCR reactions with the thermal melt from 75 °C to 95 °C at a rate of 0·05 °C/s used to confirm the amplification of each serogroup-specific amplicon.

Enriched faecal samples that were *E. coli* O26, O103, O111, O145 or O157 rtPCR-positive underwent immunomagnetic separation (IMS) for isolation of the respective serogroup using previously published methods [18]. The bead suspension (100 µl) was inoculated onto sorbitol MacConkey agar supplemented with cefixime (50 µg/ml) and potassium tellurite (2·5 mg/ml) (CT-SMAC; Fort Richard, New Zealand) for isolation of O157, rhamnose MacConkey agar supplemented with cefixime and potassium tellurite (CT-RMAC; Fort Richard) for isolation of *E. coli* O26 and sorbitol MacConkey agar (SMAC; Fort Richard) for isolation of O103, O111 and O145. The plates were incubated at 37 °C for 24 h and observed for the presence of STEC O157 colonies (grey colour/colourless colonies on CT-SMAC), STEC O26 colonies (grey colour/colourless colonies on CT-RMAC) and O103, O111 and O145 colonies (pink/purple colonies on SMAC). Suspect colonies were subcultured on MacConkey agar. O157 suspect colonies were identified using *E. coli* O157 latex agglutination kits (Oxoid, New Zealand) and colonies suspected positive for *E. coli* O26, O103, O111 and O145 were identified by using rtPCR. The allelic profile of positive isolates was then confirmed by multiplex PCR using the method previously described by Paton & Paton [19] to detect the presence of Shiga toxin 1(*stx1*), Shiga toxin 2 (*stx2*), *E. coli* attaching and effacing (*eae*) and enterohaemolysin (*ehxA*) genes.

Molecular typing of each isolate was done by pulsed-field gel electrophoresis (PFGE) following the standard procedure described by PulseNet USA [20]. Bionumerics software (v. 7·2, www. applied-maths. com) was used to analyse and compare PFGE profiles of the *E. coli* O26 isolates, and to create a dendrogram applying the unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis using the Dice similarity coefficient, with >80% similarity cut-off, and 1% band-matching tolerance.

Isolation and characterization of *Campylobacter*

Campylobacter isolation was undertaken from 1 g aliquots of each composite sample using the New Zealand Reference Method [21]. Briefly, 1 g of each composite faecal sample was enriched in 9 ml Exeter broth for 48 h at 42 °C. A loopful of enriched broth was plated onto modified charcoal cefoperazone deoxycholate agar (mCCDA) and plates were incubated at 42 °C for 48 h under microaerobic conditions. *C. jejuni* was confirmed using DNA obtained from individual colonies by PCR as described previously [22].

In total 33 water samples were collected from a single site in the main stream of the catchment. *C. jejuni* were isolated as part of a study enumerating presumptive *Campylobacter* from the water samples using the most probable number (MPN) method in the format of 100 ml, 10 ml and 1 ml of each water sample in replicates of three (count data not shown here). Water samples of 100 ml and 10 ml were filtered through 0·45 µm filters. Each filter was placed in a tube containing 30 ml and 9 ml Exeter broth, respectively, while each 1 ml water sample was mixed with 9 ml Exeter broth without filtration. These tubes were incubated at 37 °C for 24 h and transferred to 42 °C for a further 24 h. A loopful of inoculum from each tube was inoculated onto mCCDA plates and incubated in a gas jar under microaerobic conditions for 24–48 h. The suspected *C. jejuni* colonies were identified using PCR as described previously,

Multilocus sequence typing (MLST) of *C. jejuni* isolates was performed using seven house-keeping genes: *aspA* (aspartase A), *glnA* (glutamine synthase), *gltA* (citrate synthase), *glyA* (serine hydroxyl methyltransferase), *pgm* (phosphor glucomutase), *tkt* (transketolase) and *uncA* (ATP synthase alpha subunit) based on the method according to Dingle, without modifications [23]. Sequence data were collated and alleles assigned using the *Campylobacter* PubMLST database (http://pubmlst.org/Campylobacter/).

Statistical analysis

The relationship between genotypes isolated from cattle and water was examined by constructing

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Table 1. *Composite faecal samples (n = 72) obtained from cattle in a dairy catchment were analysed for* E. coli *O157 and non-O157 serogroups using real-time PCR (rtPCR). The samples positive by rtPCR were subjected to isolation. The virulence profile (stx1, stx2, eae, ehxA) of isolates obtained was determined using multiplex PCR*

	Pooled faecal samples rtPCR positive				Virulence profiles (*stx1, stx2, eae, ehxA*)	
Serogroup	Total	Y0*	Y1†	Isolates obtained	*stx1,eae,ehxA*	*eae,ehxA*
O157	2	2	0	0	0	0
O26	30	28	2	18	13	5
O103	7	4	3	2	0	2
O111	0	0	0	0	0	0
O145	3	3	0	1	0	1

* Calves born in 2009.
† Calves born in 2008.

minimum spanning trees (MSTs) and by calculating the proportional similarity index (PSI) [24].

This index provides a value of between 0 and 1, where 1 indicates complete identity between populations from the two sources and 0 indicates no similarity [24]. Bootstrap confidence intervals (CIs) for PSI were calculated using the method described by Garrett *et al.* [25]. The MST was created using Bionumerics v. 7·2 (www.applied-maths.com).

Population structure and differentiation was examined by conducting a permutational multivariate analysis of variance (PERMANOVA) [26]. The dissimilarity measure used was the number of allele differences across all seven loci. The population of *C. jejuni* isolated on each farm was examined to estimate the components of allelic variation and test the hypotheses that networks of farms that share a boundary (i.e. are not separated by the road network) have isolates that are clustered/more similar compared to those that do not share a boundary (Fig. 1*a*, groups A–E); and that farms that are located either side of the main catchment stream have isolates that are clustered/more similar compared to farms that are located away from the main catchment stream (Fig. 1*b*, groups X and Y).

RESULTS

Population structure and characterization of STEC

In total, 72 pooled faecal samples collected from Y0 ($n = 46$) and Y1 ($n = 23$) calves were analysed by rtPCR to detect the presence of *E. coli* O26, O103, O111, O145 and O157. The age of the animals from the three remaining samples was not recorded. Using rtPCR, *E. coli* O26 was the most commonly detected *E. coli* serogroup (30/72 samples, 41·6%) compared to O103 (9·7%), O145 (4·1%) and O157 (2·7%), or O111, where no samples were positive (Table 1). The 30 *E. coli* O26 rtPCR-positive faecal samples were obtained from 13/20 (65%) farms, from which 18 isolates were recovered from 11 farms (Fig. 1*a*). Of these 18 isolates, 13 were positive for *stx1, eae*, and *ehxA* using multiplex PCR, while the remaining five were positive for *eae*, and *ehxA*. Similarly, samples positive for O103 and O145 originated from six and three farms, respectively. Both O157 rtPCR-positive samples were from the same farm, but no O157 isolates were recovered from these enrichments despite the use of serogroup-specific IMS beads and selective media (CT-SMAC). Both of the O103 isolates and a single O145 isolate recovered on SAMC agar were *eae, ehxA* positive. Most of the composite faecal samples rtPCR positive for *E. coli* O26, O103, O145 and O157 were from Y0 calves (Table 1). Given the low numbers of PCR-positive samples and isolates of other serotypes, all subsequent analyses focused on the distribution of *E. coli* O26.

The 18 *E. coli* O26 isolates recovered from the 17 composite faecal samples could be broadly grouped into four clusters (>80% similarity) based on their PFGE profile (Fig. 2). All 12 isolates from cluster 4 were *stx1*-positive and were derived from four regions (B–E). The remaining six isolates, five of which were *stx* negative, were more heterogeneous based on PFGE profile analysis and were separated into three clusters represented by one isolate (cluster 3), two isolates (cluster 1) and three isolates (cluster 2), respectively. Unlike regions B, C and D (see Fig. 1*a*) which produced six, four and five *E. coli* O26 isolates,

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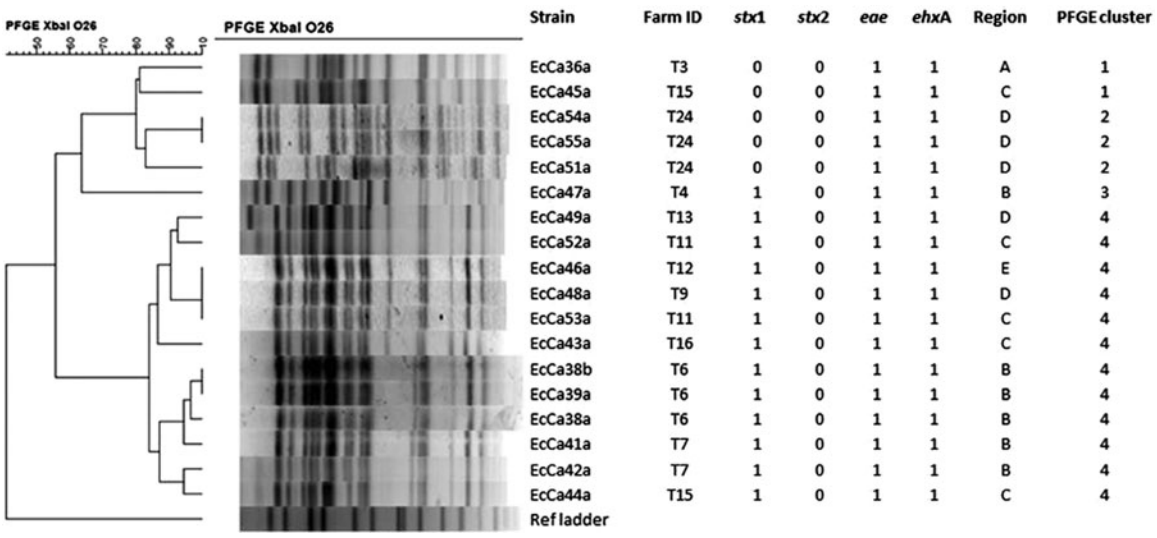

Fig. 2. Clustering (unweighted pair group method arithmetic mean, UPGMA dendrogram) of the PFGE profiles of 18 *E. coli* O26 isolates from 17 composite faecal samples taken from 11 farms in a catchment. Each farm is symbol coded and the catchment region is indicated. Note two isolates from sample EcCa38 are included in the analysis. The last lane is the *Salmonella* serotype Braenderup reference standard (H9812).

respectively, only a single isolate was identified from regions A and E. The isolate from region A was *stx* negative and present in cluster 1 while the isolate from region E was *stx* positive and present in cluster 4. Isolates recovered from cattle on the same farm were generally more similar than isolates from different farms although two farms had isolates from different clusters. There was no obvious association between the regions identified in Figure 1(*a* or *b*) and genotype of STEC O26, and given the small number of recovered isolates no further analyses were conducted.

Population structure and characterization of *C. jejuni*

Fifty (69·4%) of the 72 pooled faecal samples yielded *C. jejuni* isolates (Fig. 1*a*). Faecal samples from Y0 calves ($n = 46$) provided 35 (76·1%) *C. jejuni* isolates whereas samples from Y1 calves ($n = 23$) provided 15 (65·2%) isolates. Similarly, the water samples yielded 75 *C. jejuni* isolates. The distribution of sequence types (STs) from cattle and catchment stream are shown in Table 2. The diversity of genotypes of *C. jejuni* was higher in the catchment stream compared to the cattle population. Figure 3 shows the rarefaction curves with 95% CIs for the cattle and water samples. For a given sample size (e.g. 50 samples) the number of STs observed was higher in the water samples (22 STs) compared to the cattle samples (11 STs). There was a total of 11 different STs isolated from the cattle, six (54·5%) (STs 42, 50, 53, 61, 474, 2026) of which were also recovered from the catchment stream and these have all been associated with human clinical cases in New Zealand [2]. Conversely, there was much greater diversity in the water isolates; a total of 22 different *C. jejuni* STs were isolated from water and of these water *C. jejuni* STs only 31·8% (7/22) were also recovered from cattle in the catchment. Most of the water isolates (46/75, 61·3%) have previously been associated with wildlife sources, such as ducks, starlings (*Sturnus vulgaris*) and pukeko (*Porphyrio porphyria*, a member of the Rallidae family) in New Zealand [27] and these are rarely associated with human disease. The *C. jejuni* population structure displayed as a MST illustrates genotypes, such as ST42 and ST50 that were common to both water and cattle (Fig. 4).

The area of overlap of the genotype distributions from cattle and water (PSI) was low-moderate, PSI = 0·21 (95% bootstrapped CI = 0·13–0·29).

The PERMANOVA analysis (Tables 3 and 4) indicated that, while most of the allelic variation (72–73%) was residual between-animal variation that could not be accounted for by farm or groups of farms, there was significant clustering of genotypes at the farm level and a component of this may be attributed to the adjacency of farms contained within the road network (22%), but not their adjacency to the main catchment stream (<4%) (Tables 3 and 4). This is consistent with transmission being more likely to occur between

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Table 2. *The distribution of* C. jejuni *sequence types (STs) isolated from cattle and water from the catchment stream.*

ST	Cattle	Water
21	6	0
42	1	7
43	0	1
45*	0	6
50	11	6
53	14	2
61	10	1
257	1	1
474	1	3
520	2	0
677*	0	1
1324*	0	1
2026	2	3
2233	0	1
2378*	0	2
2381*	0	16
2538*	0	1
3675*	0	2
3845*	0	4
4336*	0	13
4342	0	1
4491	0	1
4492	1	0
4494	1	0
4495	0	1
7217	0	1

* Indicates STs associated with wildlife in New Zealand.

farms that share a road boundary, than through the stream network.

DISCUSSION

This study provides information about the genetic diversity of STEC and *C. jejuni* between farms in a defined catchment environment. It was a small-scale study, limited to a single dairy catchment (~15 km^2) and aimed to recover a representative set of isolates for molecular genotyping from as many farms as possible, rather than to conduct a large-scale analysis of the prevalence of carriage of *E. coli* and *C. jejuni* in different livestock groups on each farm. Therefore, the strategy of compositing faecal material from four animals for each sample was adopted in an attempt to maximize the recovery of isolates while reducing the number of samples required for culture. The recovery rate for *E. coli* O26 isolates was higher (60%) compared to O103 (28·5%) and O145 (33·3%) and may reflect the availability of selective media

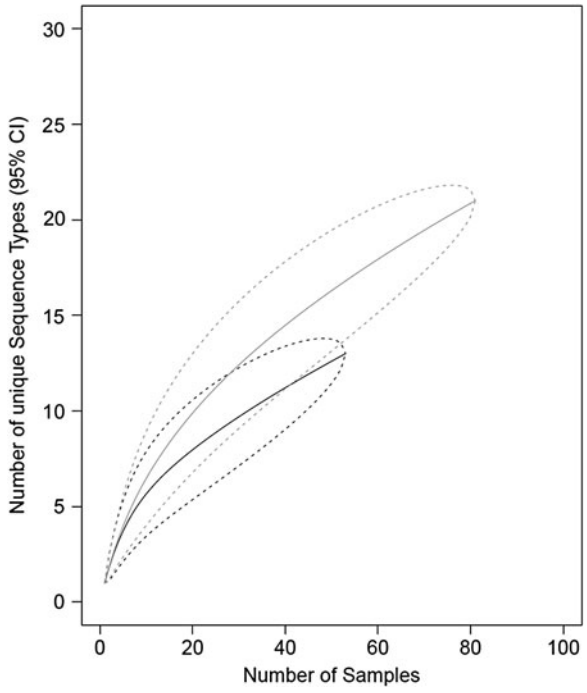

Fig. 3. Genotype (as defined by contrasting *C. jejuni* sequence types; STs) richness in cattle and water samples. Rarefaction curves indicating the estimated number of unique STs in cattle (black) and water (grey) for varying sample sizes, with 95% confidence intervals (CI).

(CT-RMAC) for *E. coli* O26, whereas no well-established selective media is available for isolation of O103 and O145.

The prevalence of *E. coli* O26 (41·6%) was higher compared to other serotypes included in this study. This finding has been noted previously in other studies [28, 29] although they were not conducted in a single catchment. For example, in a Scottish national survey which focused exclusively on non-O157 STEC, 6086 faecal samples were collected from calves aged >1 year and from 338 farms to determine the prevalence of *E. coli* O26, O103, O111 and O145 using IMS. The weighted mean prevalence of *E. coli* O26 (4·6%) was higher than O103 (2·7%), O145 (0·7%) and O111, which was absent. An Italian study which analysed 182 faecal samples collected from cattle from slaughter plants also reported the higher prevalence of *E. coli* O26 (3%) compared to O157 (0·5%) using IMS; however, no O103, O111 or O145 isolates were obtained [28]. The basis for the higher prevalence of *E. coli* O26 compared to other serogroups is uncertain but *E. coli* O26 may be better adapted to colonize cattle compared to other clinically important STEC serogroups, such as O103, O111, O145 and O157 [30].

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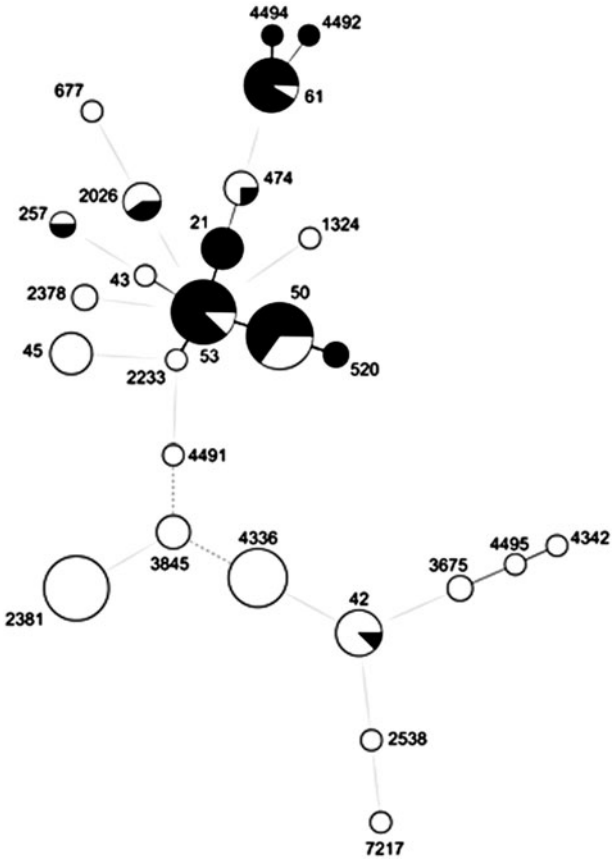

Fig. 4. Minimum spanning tree showing the multilocus sequence types (STs) of *C. jejuni* isolated from cattle (black) and water (white) in the catchment stream. Each circle represents a ST; the size of the circle is proportional to the number of isolates and the area of each circle the relative frequency of each source. Isolates are grouped according to their genetic relatedness; short solid lines between STs indicate they vary at just one of the seven MLST loci (i.e. they are single locus variants).

The current New Zealand on-farm prevalence of STEC O26 has not been determined. However an initial cross-sectional study to determine the prevalence of *E. coli* O26 from recto-anal mucosal swabs (RAMS) taken from 883 cattle and 695 calves (aged <7 days) at slaughter revealed an overall prevalence of 2·0% and 3·5% of *stx*-positive and *stx*-negative *E. coli* O26, respectively [31]. All twelve *stx*-positive isolates from cluster 4 were grouped with other *stx*-positive *E. coli* O26, clusters 1 and 2 grouped closely with *stx*-negative *E. coli* O26, and the single cluster 3 isolate was an outlier in an additional *stx*-positive group of isolates, distinct from cluster 4.

Infection with STEC O26 is often associated with mild diarrhoeal disease or asymptomatic cases and may not be reported to health practitioners, but may cause complications associated with HUS [32]. Even where cases may be reported, there is a potential underestimation of the rate of infection due to STEC O26 as many laboratories in New Zealand (and internationally) may only test young children with bloody diarrhoea for STEC and that non-bloody diarrhoeal faecal samples may not be assessed for the presence of STEC O26 or other non-O157 STEC [33]. The presence of *stx1, eae*, and *ehxA* virulence genes in *E. coli* O26 isolates from calves has great public health significance as this is the most common virulence profile observed in *E. coli* O26 isolates from human cases [34]. Several other studies have also reported the presence of *stx1* and *eae* virulence genes in *E. coli* O26 isolates from cattle [29, 35]. Although, carriage of the *stx2* gene in *E. coli* O26 isolates has also been reported in German cattle [36], no *stx2*-positive STEC O26 isolates were identified in this study. Five O26, two O103 and one O145 isolates were *eae, ehx*A-positive which is indicative of the atypical Enteropathogenic *E. coli* (aEPEC) pathotype. aEPEC has been associated with prolonged diarrhoeal disease and several studies have demonstrated the close genetic relationship and common virulence determinants between aEPEC and STEC strains suggesting a dynamic relationship between aEPEC and STEC through the loss and acquisition of *stx*-encoding bacteriophage [37, 38].

PFGE was used in this study to explore the genetic diversity of *E. coli* O26 isolates and their spatial separation or relatedness from separate farms. Water may represent an important transmission source for a number of zoonotic pathogens including STEC which have previously been isolated from various water sources in New Zealand [39]. Although our study did not have the power to explore the population structure of STEC between farms in detail, our data are consistent with a previous molecular epidemiological investigation of 163 STEC O157 isolates that indicated isolates from the same farms had similar molecular profiles compared to isolates from different farms indicating limited transmission of STEC O157 between farms [40].

In this study the prevalence of *C. jejuni* in cattle faeces was high (70·8%). Other studies have also reported the high prevalence (50–100%) of *C. jejuni* in cattle faeces [41, 42]. Cattle are considered an important reservoir of *C. jejuni* and potential source of infection to humans [41]. A New Zealand study reported cattle (11–18% of human cases) as the second most

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Table 3. *Permutational multivariate analysis of molecular variance as defined using farm structure grouped according to road network (Fig. 1a)*

Source of variation	Sum of squares	Variance components	% of variation	*P* (random value ⩾ observed value)
Among groups (A–E)	29 392	541·97	21·8	0·038
Among population within groups	33 986	149·21	6·0	0·309
Within population	52 112	1797	72·2	
Total	115 490	2488·2		

Table 4. *Permutational multivariate analysis of molecular variance as defined using farm structure grouped according to proximity to main stream (Fig. 1b)*

Source of variation	Sum of squares	Variance components	% of variation	*P* (random value ⩾ observed value)
Among groups (X and Y)	3699·8	80·8	3·3	0·13
Among population within groups	59 678	581·3	23·6	0·035
Within population	52 112	1797	73·1	
Total	115 490	2459·1		

important source of transmission of *C. jejuni* to humans after poultry (58–76% of human cases) [2]. Six of the 11 STs (42, 50, 53, 61, 474, 2026) isolated from cattle in this study are recognized as important human pathogens in New Zealand [2]. As noted in previous studies [43, 44] more samples were positive from Y0 calves compared to Y1 calves. Similar to the contrasting STEC prevalence rates associated with Y0 calves and Y1 adult cattle, an increased susceptibility of young calves to colonization with a variety of *Campylobacter* STs observed in this study and others may be associated with an age-dependent reduction in infection by enteric pathogens [45] due to development of mature intestinal microbiota [46].

Water is considered an important source of transmission of *Campylobacter*, and with water-borne campylobacteriosis outbreaks have been reported [47]. *Campylobacter* have been isolated from rivers and waterways [48] and transmission of *Campylobacter* from water to livestock has been indicated through the isolation of indistinguishable *Campylobacter* biotypes from water and cattle [42]. In this study, the genetic diversity of *C. jejuni* isolates from the catchment stream was higher compared to cattle isolates; observations also noted in other New Zealand studies [48, 49]. However, the water samples included in this study were collected during two separate storm events a year earlier than the cattle faecal samples which may contribute to the variation between the *C. jejuni* STs obtained from water and cattle samples. In the study by Devane *et al*. [49], 32 heat-stable (HS) serotypes were identified among the 616 isolates examined. Of the 32 HS serotypes 28 were present in isolates from water samples whereas only 12 were present in the isolates from dairy cattle faeces. The contrasting diversity of isolates obtained from cattle and catchment streams, and the association between non-cattle-associated STs and wildlife, suggests that a high proportion of the water isolates may originate from other sources inhabiting the area such as ducks, starlings and pukeko [27]. A study conducted in Cheshire, UK to investigate the molecular epidemiology of *C. jejuni* in dairy cattle, wildlife and the environment also reported that most of the water-related *C. jejuni* were similar to those present in wild birds [50]. We observed that the most prevalent ST in water was ST2381 and this ST is also the most prevalent isolate in many other river systems in New Zealand [48]. To date this ST has only been isolated from pukeko [27] and takahe (*Porphyrio hochstetteri*) [51], both members of the Rallidae family, and has not been recognised as a human pathogen. However, it is also important to note that some of the STs recovered from water may have originated in cattle, but not been recovered from the faeces sampled. For example, ST45 has been recovered from multiple sources,

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including cattle, and is strongly associated with human disease in New Zealand, particularly in rural areas and in children [52, 53]. Kwan *et al.* [50] also reported water as a potential source of infection to humans in the UK as six of the eight clonal complexes isolated from environmental water were similar to those isolated from human patients. However, a study conducted in the Taieri river catchment in New Zealand indicated that there was no overlap between genotypes from environmental water and human cases when PFGE profiles and Penner serotyping were used together [54].

A low-moderate area of overlap of the genotype distributions from cattle and water (PSI = 0·21) was observed. This is greater than the estimated overlap between water isolates and those recovered from human cases (PSI = 0·18) reported in earlier studies, but lower than the PSI for most other source pairwise comparisons (e.g. the PSI for cattle compared to sheep isolates was estimated to be 0·50 and cattle compared to human isolates was estimated to be 0·34 [2, 25].

PERMANOVA provides evidence of clustering of *C. jejuni* populations at the farm level and between farms grouped by geographical boundaries. Grove-White *et al.* [55] also reported a similar proportion of genetic variation associated with ruminant *C. jejuni* populations at the farm level (16–20%, $P < 0{\cdot}001$). This finding could have important implications for transmission and control of *C. jejuni*. Direct contact between cattle in neighbouring farms, sharing of equipment and personnel, and surface water run-off between farms could be important mechanisms for local transmission, underlining the importance of maintaining good between-farm biosecurity.

In summary, STEC and *C. jejuni* were prevalent in cattle in the catchment, and individual farms were associated with a subset of genotypes circulating among the young stock located on that farm. The pattern of genotypes of *E. coli* O26 and *C. jejuni* was suggestive of a high level of within farm transmission, and limited between-farm transmission. Although this study was conducted over a short time-frame on a small number of farms in a single catchment, it has revealed some significant associations that provide insight into the nature and scale of dissemination of potential human pathogens within and between farms. These observations will assist in the development of potential on-farm mitigation strategies, such as restricting animal movement between farms and maintaining individual animal cohorts, to reduce the prevalence of zoonotic pathogens.

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DECLARATION OF INTEREST

None.

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