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ORIGINAL ARTICLE

Prevalence of Shiga toxin-producing *Escherichia coli* in pasture-based dairy herds

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Significance and Impact of the Study: Shiga toxin-producing *Escherichia coli* (STEC) are important food-borne pathogens that can cause severe illness in humans. Cattle are asymptomatic reservoirs for STEC, and transmission to humans can be by consumption of food products or water contaminated with cattle faeces. Our study investigated the prevalence of O157:H7 and six *E. coli* serogroups of STEC (O26, O103, O45, O145, O121, O111) over time in the dairy reservoir and increases the knowledge and understanding of these pathogens on pasture-based farms. Such information is required to develop risk-assessment models aiming at limiting transmission of these STEC to human.

Keywords
calf, dairy, detection, ERIC-PCR, faeces, farm effluent, food safety, Shiga-toxin-producing *E. coli*.

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Abstract

Shiga toxin-producing *Escherichia coli* strains (STEC) are food-borne pathogens. While *E. coli* O157:H7 is commonly associated with cattle, less is known about the prevalence of non-O157 STEC serogroups in bovines. This study evaluated the prevalence and virulence status of O157:H7 and six *E. coli* O-serogroups (O26, O103, O45, O145, O121, O111) in New Zealand dairy farms using molecular as well as culture-based methods. Fresh farm dairy effluent (FDE) (n = 36) and composite calf faeces (n = 12) were collected over three samplings from 12 dairy farms. All seven target serogroups were detected through molecular techniques. Of the 202 isolates which were serologically confirmed following traditional culturing and immunomagnetic separation (IMS), O103, O26, O45 and O121 were the most common serogroups, being found in 81, 47, 42 and 32% of the FDE and in 17, 33, 25 and 9% of the calf faeces respectively. The majority (157/202) of the isolates were negative for *stx* and *eae* virulence genes. The prevalence of the seven target STEC was low, and only nine O26 isolates (4%) were recovered from four of the farms. The study has highlighted the need for improving the isolation of Top 7 STEC from the *stx*-negative populations present in fresh dairy effluent and calf faeces.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are an important group of food-borne-associated human disease world-wide. Exposure to STEC may result in a wide range of symptoms from asymptomatic infection to life-threatening sequelae such as haemorrhagic colitis (HC) or haemolytic uremic syndrome (HUS). While *Escherichia coli* O157:H7 is the most well-known STEC serotype, STEC belonging to six other serogroups, namely O26, O45, O103, O111, O121 and O145 have increasingly been associated with sporadic cases and large outbreaks (Johnson *et al.* 2006; Gould *et al.* 2013). In the last decades, the number of O157 STEC isolates epidemiologically linked to disease has decreased, whereas that of non-O157 STEC has steadily increased (Gould *et al.* 2013). STEC are characterized by the possession of the Shiga toxins, primary factor during infection. These toxins are encoded by *stx1* and *stx2* genes and halt or alter protein synthesis within the intestinal and vascular cells, inducing apoptosis. Another factor for infection includes the pathogenicity island known as the locus of enterocyte effacement (LEE), which contains the *eae* (*E. coli* attachment effacement) gene encoding the intimin protein and governing the formation of attaching-and-effacing

(A/E) lesions on enteric epithelial cells. Intimin-positive STEC, including non-O157 strains, are more likely to cause severe disease than intimin-negative strains (Paton and Paton 1998; Girardeau *et al.* 2005).

Cattle herds are asymptomatic reservoirs for both O157:H7 and non-O157 STEC, and transmission to humans can be by consumption of food products or water contaminated with cattle faeces (Mathusa *et al.* 2010). Food of bovine origin that has been positively associated with STEC infection include poorly handled or inadequately cooked ground beef, and as a consequence, the United States Department of Agriculture – Food Safety Inspection Service (USDA-FSIS) declared O157:H7 and STEC O26, O103, O45, O145, O121 and O111 (collegially called Top 7 STEC) to be food adulterants, with zero tolerance in raw ground beef and non-intact beef products (Anonymous 2011). Raw or inadequately pasteurized milk (Mathusa *et al.* 2010; Pihkala *et al.* 2012), consumption of fresh produce contaminated by animal manure (Hilborn *et al.* 1999), direct contact with infected animals or animal manure (Jaros *et al.* 2013), and exposure to contaminated recreational water (Vally *et al.* 2012; Jaros *et al.* 2013) have also subsequently emerged as risk factors.

In New Zealand, dairy production is typically pasture-based with a limited use of housed wintering systems. The dairy sector contributes significantly to the beef production, with *c.* 40% of the livestock processed at export plants and abattoirs comprising cull cows, heifers and young calves (Morris and Kenyon 2014; Beef and Lamb New Zealand 2016). Beef and dairy farms are further connected through the supply of young (4–10 day old) and weaned (3–4 month-old) calves to beef farmers and the use of 'beef' land for 'dairy' activities such as winter grazing of dairy cows or rearing dairy heifer replacements. Currently, however, there is limited information available on the extent of the Top 7 STEC reservoir in New Zealand dairy herds to inform risk-assessment models for human consumption of beef products.

The aim of this study was to obtain an overview of the prevalence of the *E. coli* serogroups O26, O103, O45, O145, O121, O111 and O157:H7 as well as the virulence status (presence of *stx*1/2 and *eae* genes) of these organisms in fresh dairy farm effluent and calf faeces in the Waikato region of New Zealand. A secondary aim was to provide information on the genetic relationship among a selection of isolates obtained from different farms and at different times of the year using Enterobacteriaceae Repetitive Intergenic Consensus (ERIC)-PCR.

Results and discussion

This study investigated the prevalence of the Top 7 STEC and serogroups in dairy cows and calves on 12 pasture-based dairy farms over time. O26, O103, O45 and O121 were the serogroups most frequently detected by molecular methods, as their molecular markers were each found in >94% of the FDE and in >75% of calf faeces (Table 1). The molecular prevalence of O26, O103, O45 and O121 in the enriched FDE were significantly greater than those of the remaining three serogroups ($P < 0{\cdot}005$). These four serogroups were also the most prevalent by the culture-based method, with isolates recovered from 32 to 81% of the FDEs and from 9 to 25% of the calf faeces. O145, O157 and O111 were the least prevalent serogroups, with molecular markers found in <40% of the calf faeces and FDEs. O145 was detected in 3% of FDE and 9% of calf faeces by culture-based methods, whereas no isolates from the O111 or O157 serogroups were obtained. The molecular prevalences in our study are in general greater than those reported in New Zealand (Irshad *et al.* 2016; Jaros *et al.* 2016). It is possible that the greater prevalences observed in our study resulted from analysing faecal material that was naturally shed by several animals, which may have attenuated the animal effect. It is also possible that the enrichment conditions chosen for the study were favourable to the growth of the target STEC serogroups, as proven for food analyses (Gill *et al.* 2012), and care should be taken when comparing absolute prevalences among the different studies. Nonetheless, relatively stable molecular prevalence of the target serogroups in the FDEs over the study and recovery of individual serogroups with no apparent relationship with sampling visits confirmed the ubiquitous presence of O26, O121, O45 and O103 serogroups in the investigated herds over time. There was no significant difference in the abundance of serogroups O103, O45 and O121 over time, estimated from the Ct-values obtained in the molecular method. The abundance of serogroup O26 on the contrary varied significantly over time, with mean Ct-values of 22·1, 27·2 and 25·9 in April, July and January respectively ($LSD_{5\%} = 4{\cdot}1$). This result suggests that the abundance of the O26 serogroup in the herds might change over time, at least in the Waikato region of New Zealand. Drivers for such a change warrant further investigation. The tested virulence genes (*stx*1/2; *eae*) were widespread in the tested herds, being found in all of the FDE samples and in 11 of the 12 calf faecal samples (Table 1). As observed for the O-serogroup, abundance of the virulence genes can vary over time. The *eae* gene sequence was more abundant in the FDEs collected in April (mean Ct-values: 22·6) and January (mean Ct-values: 25·2) compared to July (mean Ct-values: 27·7) ($LSD_{0{\cdot}05} = 1{\cdot}9$), whereas the abundance of *stx1/2* genes in the FDEs did not vary significantly over time ($P = 0{\cdot}789$) (Fig. 1). Possible reasons for this temporal variation warrant further investigations.

Nine isolates were confirmed as STEC. These nine STEC all belonged to serogroup O26 and were recovered from FDEs from four different farms (Farms 4, 5, 9 and 12) (Table 2). Interestingly, STEC O26 isolates were all recovered at the summer sampling, which matches previous reports of the temporal pattern of STEC prevalence in farms following various management practices (Cobbold *et al.* 2004; Pearce *et al.* 2004; Baltasar *et al.* 2014). Whole Genome Sequencing analysis of four of these STEC revealed that they carry *stx1a* genes. There were two different variants of the *stx1a* gene (one carried by isolates from FDE Farm 4, FDE Farm 9 and FDE Farm 12; the other carried by one isolate from FDE Farm 5). Each variant was found to be 100% identical to *stx1a* sequences of O26:H11 found in clinical cases in the Waikato region (data not shown). This finding suggests a possible link between STEC O26 in dairy herds and illness in humans.

The majority (157/202) of the recovered isolates were negative for *stx1/2*, which was in contrast to the ubiquitous nature of *stx*-gene sequences observed in the FDEs and calf faeces. There are many examples of molecular detection of *stx* genes where the corresponding STEC has been unable to be cultured from bovine faecal samples (Cobbold and Desmarchelier 2000; Pearce *et al.* 2004; Fremaux *et al.* 2006). The discrepancy between molecular and culture-based detection of Top 7 STEC could be attributed to several causes, including the presence of other STEC serogroups associated with dairy animals (Cookson *et al.* 2006; Fremaux *et al.* 2006) or other Stx-producing bacteria (Paton and Paton 1998). Another hypothesis to explain low isolation rate of Top 7 STEC strains excreted by dairy cattle is that in a mixed enrichment culture, the *stx*-positive population may be present in low abundance compared to *stx*-negative bacterial cells of the same serogroup and may be diluted in the faecal sample prior to enrichment and/or during selection process.

Epidemiological analysis of the O26 isolates ($n = 67$) revealed a high diversity in the ERIC-PCR genetic fingerprints, with a total of 10 different ERIC types identified from 15 FDEs and three calf faeces (Fig. 2). One ERIC type (type 3) was common among the STEC O26 isolates from three geographically distant farms, suggesting a STEC O26 population well-adapted to the bovine reservoir. This STEC population was not dominant all year round. Of the three ERIC types (ERIC types 1, 2 and 5) identified in the *eae*-positive *stx*-negative O26 population, one type (ERIC type 1) was widely dispersed among farms (found in 9 of the 12 farms) and was found repeatedly over successive samplings. Constant presence of an *eae*-positive population in the bovine reservoir might be significant from a human health perspective. The *eae* gene is located in the LEE (locus of enterocyte effacement) pathogenicity island alongside other genes involved in the pathogenic process and associated with the development of the attaching and effacing (A/E) lesions (Trabulsi *et al.* 2002). In our study, cluster analysis identified that the nine STEC O26 isolates that were recovered from four different farms and all carried the *eae* gene appeared to be more closely related (>90% similarity) to each other than to the *stx*-negative isolates, and genetically closer to the *eae*-positive isolates than to *eae*-negative

Table 1 Prevalence of the Top 7 O-serogroups in farm dairy effluents (FDE) and composite calf faeces on 12 pasture-based farms

Molecular detection* (%)						Culture-based method† (%)				
	Farm dairy effluents				Calf faeces	Farm dairy effluents				Calf faeces
	April ($n = 12$)	July ($n = 12$)	Jan ($n = 12$)	Total FDE ($n = 36$)	July ($n = 12$)	April ($n = 12$)	July ($n = 12$)	Jan ($n = 12$)	Total FDE ($n = 36$)	July ($n = 12$)
O157	5	3	0	8 (22%)	4 (33%)	0	0	0	0	0
O26	12	12	11	35 (97%)	9 (75%)	5	4	8	17 (47%)	3 (25%)
O103	12	12	12	36 (100)	12 (100%)	11	6	12	29 (81%)	2 (17%)
O45	12	12	12	36 (100)	10 (83%)	4	3	8	15 (42%)	3 (25%)
O121	11	12	11	34 (94%)	11 (92%)	1	4	6	11 (32%)	1 (9%)
O145	7	1	6	14 (39%)	3 (25%)	0	0	1	1 (3%)	1 (9%)
O111	0	2	0	2 (5%)	1 (8%)	0	0	0	0	0

*Enriched samples were tested by the RapidFinder™ Screening Assay for the presence/absence of the O157-molecular marker and by the MLG assay for the presence/absence of the molecular markers of O26, O103, O45, O121, O145 and O111.

†Enriched samples, subjected to serogroup-specific IMS beads, were plated onto non modified RBA and RBA modified to include 10 mg l^{-1} Novobiocin and 0·8 mg l^{-1} Potassium Tellurite and then a minimum of five well-separated colonies per sample were tested by the agglutination assay and MLG assays. In total 65% (31/48), 48% (21/44), 39% (18/46), 27% (12/45) and 12% (11/44) of the samples positive by molecular detection for O103, O26, O45, O121 and O145, respectively, were also found positive by culture-based method.

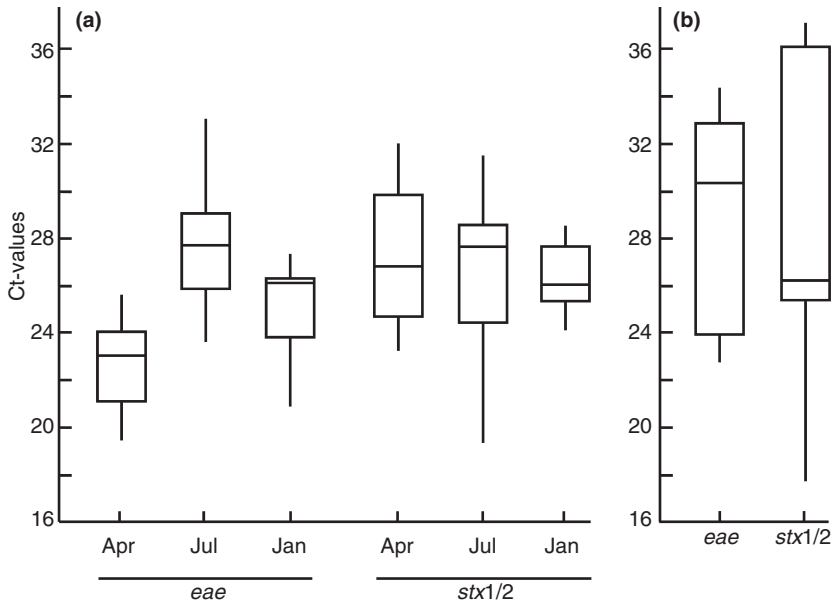

Figure 1 Box plots showing the Ct values (abundance) of *eae* and *stx1/2* gene sequences in (a) farm dairy effluents (FDE) collected from 12 dairy farms over three occasions and (b) composite calf faeces collected from 12 dairy farms in July. Median is shown as the horizontal line inside of each box; top and bottom edges of the box correspond to the 75th and 25th percentiles respectively (i.e. each box shows the middle of 50% of the data). Whiskers show the maximum and minimum values. No 'outlier' samples, whose values are more than 1·5 times the difference between 3rd and 1st quartiles were observed.

Table 2 Number of recovered *Escherichia coli* isolates testing for the targeted virulence genes (*stx*1/2, *eae*). In bracket the number of farms from which isolates were recovered. No O111 or O157 isolates were recovered. No *stx*1/2 + *eae* − isolate was recovered

		Farm dairy effluents			Calf faeces
Serogroup	Virulence profile	April	July	Jan	July
O26	*stx*1/2 + *eae* +	–	–	9 (4 farms)	–
	*stx*1/2 − *eae* +	15 (5 farms)	1 (1 farm)	8 (4 farms)	3 (1 farm)
	*stx*1/2 − *eae* −	–	9 (3 farms)	9 (6 farms)	13 (3 farms)
O103	*stx*1/2 + *eae* +	–	–	–	–
	*stx*1/2 − *eae* +	–	–	3 (1 farm)	–
	*stx*1/2 − *eae* −	17 (11 farms)	12 (6 farms)	28 (11 farms)	2 (2 farms)
O45	*stx*1/2 + *eae* +	–	–	–	–
	*stx*1/2 − *eae* +	–	–	–	–
	*stx*1/2 − *eae* −	9 (4 farms)	11 (3 farms)	17 (8 farms)	7 (3 farms)
O121	*stx*1/2 + *eae* +	–	–	–	–
	*stx*1/2 − *eae* +	–	–	–	–
	*stx*1/2 − *eae* −	1 (1 farm)	11 (4 farms)	10 (6 farms)	1 (1 farm)
O145	*stx*1/2 + *eae* +	–	–	–	–
	*stx*1/2 − *eae* +	–	–	3 (1 farm)	3 (1 farm)
	*stx*1/2 − *eae* −	–	–	–	–

isolates. Although the genetic evolution of STEC is still unclear, recent evidence obtained from whole genome sequencing has established that human O26 strains associated with severe symptoms of human infection can be closely related to bovine O26 strains that are lacking the *stx* genes (Norman *et al.* 2015). Accurate assessment of the importance of the bovine reservoir as a risk for human health would require studies involving a larger number of farms and isolates of both bovine and human origin to obtain sufficient data to explore the dynamics of the Top 7 STEC and *eae*-positive *E. coli* populations in detail and to understand temporal changes in prevalence.

In conclusion, STEC O26 was identified as the most prevalent Top 7 STEC on the dairy farms included in the study, and was with three other serogroups (O103, O45 and O121) prevalent in all of the enriched FDEs and calf faeces. No STEC belonging to any of these three serogroups was isolated despite the constant presence of the target serogroups and *stx* and *eae* genes in the bovine reservoir. Overall, the study has highlighted the need for improving the detection of STEC Top 7 in fresh environmental samples such as dairy effluents and calf faeces. Further research on isolation of Top 7 STEC from the *stx*-negative populations present in such samples is recommended.

Materials and methods

Study sites

Twelve commercial dairy farms located in the Waikato region of New Zealand were enrolled in the study on the basis of the willingness of the herd owner to participate. Farms were allocated a sequential number from 1 to 12. The median number of cows in the milking herds was 325 cows (range: 215–1200 cows), with the median stocking rate of 3·2 cow ha^{-1} (range: 2·7–4·7 cow ha^{-1}). The main calving season was spread between May and October with a peak in July–August. On all farms, calves were housed together in a covered barn on bedding materials of wood shavings or chip fines. All calves were fed colostrum milk.

Sample collection

Samples were collected over three periods: April 2014 (autumn), between July and September 2014 (late winter/early spring) and January 2015 (summer). Each farm was visited once at each sampling period, for a total of three sampling visits per farm. At each visit, a 1 l grab sample of farm dairy effluent (FDE) was collected from the exit point of the milking parlour after the final wash-down following morning milking. Fresh dairy farm effluent was considered a naturally composite sample as it has been reported to contain about 11% of faeces and urine excreted daily by the milking herd (Vanderholm 1984). Faeces from young calves (<7 day old) were collected when available (i.e. visit during the July–September period). For each farm, a 50 g composite of up to five freshly voided calf faeces were collected using an individual plastic spatula to transfer the sample into a sterile screw capped container. Faeces was collected from the bedding material of the calf holding pens except at Farm 4 where it was collected from the wooden slats of a holding pen. All collected samples were transported to the laboratory in an insulated container and processed within 6 h of collection.

Enrichment of FDEs and composite calf faeces by culture in mTSB

One millilitre of each collected FDE sample was homogenized with 9 ml of modified tryptone soya broth (mTSB; Fort Richard Laboratories, Auckland, New Zealand). Each composite calf faeces sample was thoroughly mixed by stirring with a spatula until consistency and colour were homogeneous following a visual assessment; a 0·1 g-subsample was transferred to a clean sterile container using a sterile spatula and homogenized with 10 ml of mTSB. All FDE- and calf faeces-inoculated broths were incubated at 42°C for 15–22 h. After incubation, a 1 ml aliquot of each enrichment broth was removed for total DNA extraction using a High-Pure DNA preparation kit (Roche Diagnostics NZ Ltd, Auckland, New Zealand) as described in Brightwell and Clemens (2012).

Molecular detection of *stx, eae* and O-antigen gene cluster

For each sample, extracted DNA (30 μl) was screened for the presence of Shiga toxin (*stx1* and *stx2*) genes, intimin (*eae*) gene and O157:H7 by a TaqMan quantitative PCR using the RapidFinder™ STEC Screening Assay (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA). Results were analysed using the RapidFinder™ express software ver. 1.2.1. (Applied Biosystems). The genes encoding for serogroup-specific O-antigens of O26 (*wzx*O26), O111 (*wbdI*), O103 (*wzx*O103), O145 (*wzx*O145), O45 (*wzx*O45) and O121 (*wzx*O121) were detected in the samples testing positive for *stx1/2* and/or *eae* using the Custom TaqMan® STEC O26 & O111 MLG, STEC O103 & O145 MLG and STEC O45 & O121 MLG assays (Applied Biosystems). All the PCR assays, which were provided by the manufacturer as a lyophilized bead format, were used according to the manufacturer's instructions. All qPCR assays were carried out in an ABI 7500 Fast qPCR instrument (Applied BioSystems) and results analysed using SDS software ver. 2.0.6. The number of PCR cycles needed for the fluorescence intensity to raise above a specific threshold (Ct-value) was used as an indication of the amount of target DNA sequence in the sample.

Recovery and confirmation procedures of pure isolates of the target O-serogroups

The enriched faecal samples that were positive in the molecular screening steps for O26, O45, O121, O103, O111, O145 and/or O157 were subjected to immunomagnetic separation (IMS) procedure using the appropriate O-serogroup-specific IMS beads (Abraxis LLC., Warminster, PA) according to

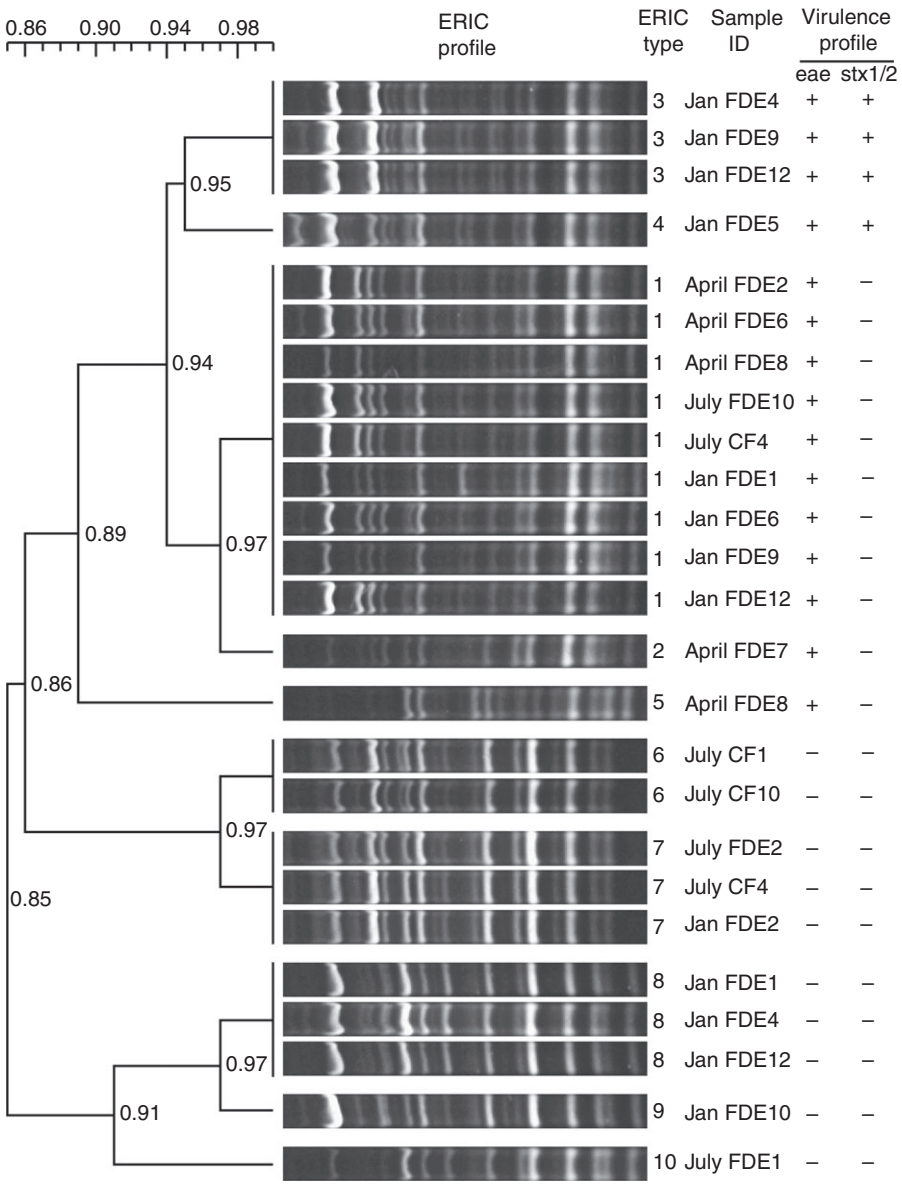

Figure 2 Dendogram of ERIC profile showing genetic relationship of *Escherichia coli* O26 isolates purified from farm dairy effluents (FDE) and calf faeces (CF) on 12 pasture-based farms over time (April, July, January). The presence of *stx1/2* and *eae* genes in each isolate as determined by the Taqman® STEC STX & EAE MLG assay (Life Technologies) are indicated by – (negative) or + (positive). The dendrogram was constructed using UPGMA with a 1% band tolerance. Each number (1–10) represents an ERIC type.

the manufacturer's protocol. Briefly, one ml of the enriched faecal sample was added to 20 μl of IMS beads and incubated for 15 min at room temperature with moderate shaking (*c.* 800 rev min^{-1}). After washing, the beads were released in Abraxis elution buffer as per the manufacturer's protocol. A 100 μl volume of the undiluted bead suspension and a 1 : 100 diluted bead suspension was spread-plated onto Rainbow® Agar O157 (RBA) (Biolog, Hayward) and/or RBA with 0·8 mg l^{-1} Potassium Tellurite (Fort Richard Laboratories) and 10 mg l^{-1} Novobiocin (SERVA, Boehringer, Heidelburg, Germany) (NT-RBA). Inoculated plates were incubated for 16–24 h at 42°C. A minimum of five colonies, which included at least one colony of each colony morphology type observed on the plate, were tested by O-antisera agglutination using the appropriate (O103, O145, O157, O26, O121, O45 or O111) *E. coli* O-antiserum (SSI® *E. coli* OK O-antisera, Statens Serum Institut, Copenhagen, Denmark) according to the manufacturer's instructions. Colonies that confirmed as O-antisera agglutination-positive were individually inoculated onto RBA and

incubated for 16–24 h at 42°C before genomic and biochemical confirmation.

Genomic DNA of purified colonies was prepared by suspending a well-separated colony harvested from RBA in 500 μl of sterile molecular grade water, boiling for 10 $\pm$ 1 min then centrifugation at 20 000 ***g*** for 5 min. The recovered colonies were confirmed as one of the target O-serogroup by Real-time PCR using the Custom TaqMan MLG assays (Life Technologies) according to the manufacturer's instructions. All colonies confirmed as one of the target O-serogroup were biochemically identified as *E. coli* by Microbact 12A + 12B (Oxoid, Thermo Scientific).

Virulence profile

Determination of the virulence profile (presence/absence of *stx* and *eae* genes) of the recovered colonies was by Real-time PCR using the Taqman® STEC STX & EAE, MLG assay (Life Technologies).

Genotyping of recovered colonies by ERIC-PCR

Molecular typing of each confirmed isolate was done by ERIC-PCR using ERIC 2 primer (Versalovic *et al.* 1991) and following the amplification procedure described the protocol of Weijtens *et al.* (1999). ERIC-PCR banding patterns were analysed using Quantity-One software ver. 4.5.2 (Bio-Rad Laboratories, Hercules, CA). UPGMA cluster analysis with a 1% band tolerance was used to determine relationships between isolates. Isolates that grouped at a $\geq$ 90% similarity level were considered the same type. Different types were assigned a number (i.e. ERIC type 1 to ERIC type 10).

Statistical analysis

To assess whether the molecular prevalence of each serogroup differed, a logistic mixed model was fitted followed by post-hoc pairwise comparisons (adjusted using Tukey's method) at the 5% significance level. To assess whether the Ct-values of the DNA sequences targeting *stx1*/*2*, *eae* or the serogroups genes in the enriched FDEs differed between the three sampling times, ANOVA blocked by farm was used followed by protected Fisher's least significant difference (LSD) post hoc tests at the 5% significance level. A linear mixed effects model was used to assess whether the average Ct-value differed between samples from which isolates were successfully recovered and those from which isolates were not recovered. Data from all serogroups was jointly analysed, with the fixed model including treatment factors for the serogroup and recovery status (i.e. recovered or not). As there was no evidence of a serogroup by recovery status interaction (P = 0·612), this term was omitted from the fixed model. Random effects of farm, sample time and the interactions between farm and sample time, farm and serogroup and sample time and serogroup were included to account for the structure of the data.

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Conflict of Interest

No conflict of interest declared.

References

Anonymous (2011) USDA Takes New Steps To Fight *E. coli*, Protect The Food Supply – Designation Extends Zero Tolerance Policy for *E. coli* O157:H7 to Six Additional *E. coli* Serogroups. Release No. 0400.11 USDA-FSIS Office of Communication, Washington, DC. Available at https://content.govdelivery.com/accounts/USDAOC/bulletins/126020

Baltasar, P., Milton, S., Swecker, W. Jr, Elvinger, F. and Ponder, M. (2014) Shiga toxin–producing *Escherichia coli* distribution and characterization in a pasture-based cow-calf production system. *J Food Protect* **77**, 722–731.

Beef and Lamb Zealand Economic Service (2016) Mid-season update 2015–2016. Available at: http://www.beeflambnz.com/Documents/Information/Compendium%20of%20New%20Zealand%20farm%20facts.pdf (accessed February 2016).

Brightwell, G. and Clemens, R. (2012) Development and validation of a real-time assay specific for *Clostridium estertheticum*-like psychrotolerant bacteria. *Meat Sci* **92**, 697–703.

Cobbold, R. and Desmarchelier, P. (2000) A longitudinal study of Shiga-toxigenic *Escherichia coli* (STEC) prevalence in three Australian dairy herds. *Vet Microbiol* **71**, 125–137.

Cobbold, R.N., Rice, D.H., Szymanski, M., Call, D.R. and Hancock, D.D. (2004) Comparison of Shiga-toxigenic *Escherichia coli* prevalences among dairy, feedlot, and cow-calf herds in Washington state. *Appl Environ Microbiol* **70**, 4375–4378.

Cookson, A.L., Taylor, S.C., Bennett, J., Thomson-Carter, F. and Attwood, G.T. (2006) Serotypes and analysis of distribution of Shiga toxin producing *Escherichia coli* from cattle and sheep in the lower North Island, New Zealand. *NZ J Vet Res* **54**, 78–84.

Fremaux, B., Raynaud, S., Beutin, L. and Vernozy Rozand, C. (2006) Dissemination and persistence of Shiga toxin-producing *Escherichia coli* (STEC) strains on French dairy farms. *Vet Microbiol* **117**, 180–191.

Gill, A., Martinez-Perez, A., McIlwham, S. and Blais, B. (2012) Development of a method for the detection of verotoxin-producing *Escherichia coli* in food. *J Food Prot* **75**, 827–837.

Girardeau, J.P., Dalmasso, A., Bertin, Y., Ducrot, C., Bord, S., Livrelli, V., Vernozy-Rozand, C. and Martin, C. (2005) Association of virulence genotype with phylogenetic background in comparison to different seropathotypes of Shiga toxin-producing *Escherichia coli* isolates. *J Clin Microbiol* **43**, 6098–6107.

Gould, L.H., Mody, R.K., Ong, K.L., Clogher, P., Cronquist, A.B., Garman, K.N., Lathrop, S., Medus, C. *et al.* (2013) Increased recognition of non-O157 Shiga toxin–producing *Escherichia coli* infections in the United States during 2000–2010: epidemiologic features and comparison with *E. coli* O157 infections. *Foodborne Path Dis* **10**, 453–460.

Hilborn, E.D., Mermin, J.H., Mshar, P.A., Hadler, J.L., Voetsch, A., Wojtkunski, C., Swartz, M., Mshar, R. *et al.* (1999) A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. *Arch Int Med* **159**, 1758–1764.

Irshad, H., Cookson, A.L., Ross, C.M., Jaros, P., Prattley, D.J., Donnison, A., McBride, G., Marshall, J. *et al.* (2016) Diversity and relatedness of Shiga toxin-producing *Escherichia coli* and *Campylobacter jejuni* between farms in a dairy catchment. *Epidem Infect* **144**, 1406–1417.

Jaros, P., Cookson, A.L., Campbell, D.M., Besser, T.E., Shringi, S., Mackereth, G.F., Lim, E., Lopez, L. *et al.* (2013) A prospective case-control and molecular epidemiological study of human cases of Shiga toxin–producing *Escherichia coli* in New Zealand. *BMC Infect Dis* **13**, 450.

Jaros, P., Cookson, A.L., Reynolds, A., Prattley, D.J., Campbell, D.M., Hathaway, S. and French, N.P. (2016) Nationwide prevalence and risk factors for faecal carriage of *Escherichia coli* O157 and O26 in very young calves and adult cattle at slaughter in New Zealand. *Epidem Infect* **6**, 1–12.

Johnson, K.E., Thorpe, C.M. and Sears, C.L. (2006) The emerging clinical importance of non-O157 Shiga toxin-producing *Escherichia coli*. *Clin Infect Dis* **43**, 1587–1595.

Mathusa, E.C., Chen, Y., Enache, E. and Hontz, L. (2010) Non-O157 Shiga toxin–producing *Escherichia coli* in foods. *J Food Prot* **73**, 1721–1736.

Morris, S.T. and Kenyon, P.R. (2014) Intensive sheep and beef production from pasture – a New Zealand perspective of concerns, opportunities and challenges. *Meat Sci* **98**, 330–335.

Norman, K.N., Clawson, M.L., Strockbine, N., Mandrell, R.E., Johnson, R., Ziebell, K., Zhao, S., Fratamico, P.M. *et al.* (2015) Comparison of whole genome sequences from human and non-human *Escherichia coli* O26 strains. *Front Cell Infect Microbiol* **5**, 21.

Paton, J.C. and Paton, A.W. (1998) Pathogenesis and diagnsotic of Shiga toxin-producing *Escherichia coli* infections. *Clin Microbiol Rev* **11**, 450–479.

Pearce, M.C., Jenkins, C., Vali, L., Smith, A.W., Knight, H.I., Cheasty, T., Smith, H.R., Gunn, G.J. *et al.* (2004) Temporal shedding patterns and virulence factors of *Escherichia coli* serogroups O26, O103, O111, O145, and O157 in a cohort of beef calves and their dams. *Appl Environ Microbiol* **70**, 1708–1716.

Pihkala, N., Bauer, N., Eblen, D., Evans, P., Johnson, R., Webb, J. and Williams, C. (2012) Risk profile for pathogenic non-O157 Shiga toxin-producing *Escherichia coli* (non-O157 STEC) U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Office of Policy and Program Development. 1–64. Available at: http://www.fsis.usda.gov/shared/PDF/Non_O157_STEC_Risk_Profile_May2012.pdf?redirecthttp=true (accessed 18 March 2014).

Trabulsi, L.R., Keller, R. and Tardelli Gomes, T.A. (2002) Typical and atypical enteropathogenic *Escherichia coli*. *Emerg Infect Dis* **8**, 508–513.

Vally, H., Hall, G., Dyda, A., Raupach, J., Knope, K., Combs, B. and Desmarchelier, P. (2012) Epidemiology of Shiga toxin producing *Escherichia coli* in Australia, 2000–2010. *BMC Public Health* **12**, 63.

Vanderholm, D.H. (1984) Agricultural Waste Manual NZAEI Project report No. 32, New Zealand Agricultural Engineering Institute, Lincoln College, Canterbury.

Versalovic, J., Koeuth, T. and Lupski, R. (1991) Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* **19**, 6823–6831.

Weijtens, M.J.B.M., Reinder, R.D., Urlings, H.A.P. and Van der Plas, J. (1999) *Campylobacter* infections in fattening pigs; excretion pattern and genetic diversity. *J Appl Microbiol* **86**, 63–70.