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Ecology of *Escherichia coli* O157:H7 in Commercial Dairies in Southern Alberta

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ABSTRACT

Shedding of *Escherichia coli* O157:H7 was monitored monthly over a 1-yr period by collecting pooled fecal pats (FECAL) and manila ropes orally accessed for 4 h (ROPE) from multiple pens of cattle in 5 commercial dairies in southern Alberta, Canada. Using immunomagnetic separation, *E. coli* O157:H7 was isolated from cows on 4 of the dairies and from 13.5% of FECAL and 1.1% of ROPE samples. Pulsed-field gel electrophoresis of *Xba*I- and *Spe*I-digested bacterial DNA of the 65 isolates produced 23 unique restriction endonuclease digestion patterns, although 92% of the isolates belonged to 3 restriction endonuclease digestion pattern clusters sharing a minimum 90% homology. Collection of positive isolates was 15 times more likely from June through September. Across dairies, peak somatic cell count occurred in July, August, September, and November. The likelihood of positive isolates was 2.6 times higher in calves and heifers compared with mature cows. This study indicates that ROPE would be of little value for the detection of *E. coli* O157:H7 in dairy herds unless oral contact with ROPE could be increased in mature animals. Additionally, mitigation strategies for *E. coli* O157:H7 should be targeted to the months of July, August, and September and toward immature animals for maximum impact. All farms displayed unique combinations of seasonality of shedding and diversity of *E. coli* O157:H7 subtypes. The fact that seasonal prevalence of *E. coli* O157:H7 largely coincided with peak somatic cell count within climatically controlled dairy barns suggests that similar environmental factors may be enhancing fecal shedding *E. coli* O157:H7 and the incidence of mastitis.
(**Key words:** *Escherichia coli* O157:H7, dairy cattle, dairy cattle environment, foodborne pathogen)

Abbreviation key: FECAL = pooled fecal pats, **REPC** = restriction endonuclease digestion cluster, **ROPE** = orally accessible manila rope.

INTRODUCTION

On-farm food safety programs are gradually being instituted (Powell et al., 2002; Lardy et al., 2003), although the feasibility and mechanics of monitoring and mitigating all pathogens of concern presents a formidable challenge. Cattle are recognized reservoirs of the pathogen *Escherichia coli* O157:H7 (Bach et al., 2002), with human disease outbreaks linked to contamination of meat (Bell et al., 1994), unpasteurized milk (Murinda et al., 2002), and the environment (Varma et al., 2003). Due to the potential severity of human infection (Bach et al., 2002) and the scale of disease outbreaks (Synge et al., 2003; Bender et al., 2004), *E. coli* O157:H7 is a pathogen worthy of monitoring in an on-farm food safety program.

Escherichia coli O157:H7 has previously been monitored in individual dairy cattle by use of rectally collected fecal samples (Garber et al., 1995; Shere et al., 1998; Fitzgerald et al., 2003) or fecal swabs (Rahn et al., 1997; Mechie et al., 1997; Rice et al., 1999). Although individual monitoring of lactating animals may be feasible during routine milking, the transient and intermittent nature of shedding *E. coli* O157:H7 (Bach et al., 2002) would require collection of samples from all cattle in the dairy to reliably determine colonization status (Stanford et al., 2005). Dry cows, heifers, and weaned calves are commonly managed in groups or pens (Losinger and Heinrichs, 1996). Monitoring animals individually would require restraint, potentially causing injury, as well as considerable effort and expense. For practical, cost-effective implementation of on-farm food safety programs, methods of monitoring pathogens are required for dairy cattle housed in groups.

Collection of fecal pat samples has been used for monitoring groups of dairy cattle (Hancock et al., 1998; Cobbold et al., 2004), although pathogen levels measured may be reduced compared with rectally collected fecal samples (Lahti et al., 2003). Allowing oral access

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to ropes, which can then be cultured for the presence of *E. coli* O157:H7 is another method for monitoring groups of cattle, although all previous studies have been conducted in beef feedlots (Irwin et al., 2002; Smith et al., 2003; Stanford et al., 2005). Irwin et al. (2002) determined that in a pen averaging 123 cattle, 43.3% of animals orally accessed ropes in 2 h, but repeated use of ropes by individual animals does occur (Stanford et al., 2005). The main objective of the present study was to identify factors influencing the transmission and maintenance of *E. coli* O157:H7 in dairy farms by monitoring all groups/pens of cattle on 5 dairy farms for a 1-yr period. The secondary objective was to compare the utility of fecal pats and ropes for monitoring colonization of dairy cattle at all ages and stages of production. Pulsed-field gel electrophoresis was used to assess the degree of genetic diversity among the *E. coli* O157:H7 isolates.

MATERIALS AND METHODS

Collection of samples

Over a 1-yr period, samples were collected monthly from all cattle pens at 5 commercial dairies (A, B, C, D, and E) in southern Alberta, Canada. Dairies were selected based on diverse hygiene and manure management practices, and were located within 30 km of each other to minimize the effects of climate and geography on shedding of *E. coli* O157:H7. Management practices including importation of cattle, density of animals within pens, movement of cattle among pens, calf rearing, and pen drainage were considered. For each pen, number of cattle and stage of production (lactating cow; dry cow; heifer; milk-fed calf, age 1 to 12 wk; older calf, age 3 to 9 mo; calving cow) were recorded. For the lactating herd at each farm, monthly bulk tank milk samples (100 mL) were stored in a Whirl-Pak (Nasco Canada, Newmarket, ON, Canada) bag and shipped at 4°C to the Central Alberta Milk Testing Laboratory (Edmonton AB, Canada) for determination of SCC using a Fossomatic 360 (N.E. Foss Electric, Hillerød, Denmark).

One manila rope (120 cm, **ROPE**) was tied along the fence line of each pen of dairy cattle in a location accessible for oral contact. The ROPE was removed after 4 h, cut in half, and placed into a 1-L Nalgene bottle containing 500 mL of buffered peptone water (Difco, Ottawa, ON, Canada). For each pen, random fresh fecal pat subsamples of 3 to 5 g were collected for every 20 animals in the pen and pooled in a Whirl-Pak bag (**FECAL**). Nalgene bottles and FECAL samples were packed in insulated chests with ice packs and transported to the laboratory within 8 h after collection.

Isolation and Enumeration of *E. coli* O157:H7

Upon return to the laboratory, samples from ROPE were first shaken 3 times for 5 min each at 10-min intervals in buffered peptone water and then incubated at 37°C for 18 to 24 h. A single aliquot of buffered peptone water (10 mL) from the incubated ROPE was then added to 90 mL of modified tryptic soy broth, shaken, and incubated at 37°C for 18 to 24 h. For FECAL, a 10-g subsample was enriched in 90 mL of modified tryptic soy broth for 6 h at 37°C. After enrichment, samples from ROPE and FECAL were subjected to immunomagnetic separation using Dynabeads anti-*E. coli* O157 (Dynal, Lake Success, NY). A 50-μL aliquot of the antibody-coated bead suspension was plated onto sorbitol MacConkey agar with 0.5 mg/L of cefixime and the plate incubated at 37°C for 18 to 24 h. Three sorbitol-negative colonies from the plate were tested for the presence of the O157 antigen using the *E. coli* O157 latex kit (Oxoid, Nepean, ON, Canada). The presence of *vt*, *eaeA*, and *flicC* (H7) genes in sorbitol-negative, latex-positive isolates determined in multiplex PCR assays (Gannon et al., 1997) was used as a final confirmation of *E. coli* O157:H7.

Pulsed-Field Gel Electrophoresis-DNA Fingerprinting

All isolates confirmed as *E. coli* O157:H7 (n = 66) were first subtyped by pulsed-field gel electrophoresis using *Xba*I restriction according to the standard 1-d protocol (CDC, 1998) as previously described by Bach et al. (2004). Only 1 isolate of *E. coli* O157:H7 from each positive sample was typed. Banding patterns were viewed with UV illumination, and photographed using the Speedlight Platinum Gel Documentation System (BioRad, Mississauga, ON, Canada). Banding patterns in the digital images were classed as unique or grouped into restriction endonuclease digestion pattern clusters (**REPC**; 90% or greater homology) using Dice similarity coefficients, unweighted pair group methods arithmetic average algorithm, 1% position tolerance, and 0.5% optimization (BioNumerics 3.5, Applied Maths BVBA, Sin-Martens-Latem, Belgium). Within REPC, isolates separated by farm of origin or by at least 3-mo intervals were further subtyped (n = 12) using *Spe*I restriction and the appropriate protocol (CDC, 1998).

Statistical Analyses

Somatic cell count data were transformed ($\log_{10}$) before using the GLM procedure of SAS (SAS Institute, 1999) to compare farms and months of data collection. Orthogonal contrasts and odds ratios within the GENMOD procedure of SAS weighted by the number of ani-

Table 1. Number of pens in 5 commercial dairies in southern Alberta that tested positive for *Escherichia coli* O157:H7 as assessed by pooled fecal samples (FECAL) or manila ropes (ROPE) over a 1-yr period.

Dairy ID	Sample type[1]	No. of pens sampled	2002			2003									Total
			Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	
A	FECAL	8	2	0	0	2	0	0	0	0	1	4	6	4	19/98[2]
	ROPE	8	0	0	0	0	0	0	0	0	0	1	0	1	2/98[2]
B	FECAL	6	0	0	0	0	0	0	0	0	0	5	4	3	12/72
	ROPE	6	0	0	0	0	0	0	0	0	0	1	0	0	1/72
C	FECAL	8	2	1	0	1	2	0	0	2	4	4	3	2	21/72
	ROPE	8	0	0	0	0	1	0	0	1	0	0	0	0	2/72
D	FECAL	9	0	0	0	0	0	0	0	0	2	4	3	0	9/105[3]
	ROPE	9	0	0	0	0	0	0	0	0	0	0	0	0	0/105[3]
E	FECAL	7	0	0	0	0	0	0	0	0	0	0	0	0	0/96
	ROPE	7	0	0	0	0	0	0	0	0	0	0	0	0	0/96
Total	FECAL (% positive)		10.5	2.6	0	8.1	5.4	0	0	5.3	18.4	44.7	42.1	23.7	13.5
	ROPE (% positive)		0	0	0	0	2.6	0	0	2.6	0	4.8	0	2.6	1.1

[1]Pooled fecal samples (1 per pen, FECAL) were collected that contained 3 to 5 g of feces per 20 animals in the pen. A single manila rope (ROPE) was hung in each pen for oral access by cattle for one 4-h period per month.

[2]In November 2002 and April 2003, 9 pens were sampled at dairy A.

[3]In January, February, and March 2003, only 8 pens were sampled at dairy D.

mals per pen were used to compare utility of the monitoring methods and to determine the impacts of management and animal-related factors on the incidence of *E. coli* O157:H7 on commercial dairy farms.

RESULTS AND DISCUSSION

Prevalence of *E. coli* O157:H7

Across dairy farms, overall prevalence of *E. coli* O157:H7 in FECAL samples was 13.5% (Table 1), similar to the 18.8% reported in a companion study of 4 commercial Alberta feedlots monitored over the same period (Stanford et al., 2005). Beef feedlots tend to have increased animal turnover, stocking density, and mixing of animals compared with dairies, with those factors elevating the risk of shedding *E. coli* O157:H7 (Garber et al., 1995; Dargatz et al., 1997; Stanford et al., 2005). However, dairy cattle in the present study were largely confined in barns, a factor also shown to increase the prevalence of shedding of the *E. coli* O157:H7 (Synge et al., 2003; Ogden et al., 2004). Consequently, the reported prevalence of *E. coli* O157:H7 in beef and dairy animals assessed concurrently has often been similar (Chapman et al., 1997; Hancock et al., 1998; Van Donkersgoed et al., 1999), although Cobbold et al. (2004) found a higher prevalence of a number of shiga-toxigenic serotypes of *E. coli* in dairy compared with beef herds.

Outside North America, the predominance of shiga toxin-producing serotypes of *E. coli* other than O157 (Robins-Browne et al., 1998; Widiashi et al., 2003) should be noted. From a dairy perspective, however, an understanding of all shiga toxin-producing *E. coli* including O157 is important to ensure the safety of milk and the meat produced from culled dairy cows. Comparing dairies, animals located on dairy C were 1.6 times more likely to shed *E. coli* O157:H7 ($P < 0.001$) than cattle located on the other dairies (Table 2), whereas animals on dairy E were only about half (53%) as likely ($P < 0.001$) to shed the organism than animals at other locations. In fact, dairy E was the dairy from which no positive samples were collected during the study.

Importation of Cattle

During the study period, dairy D purchased a number of animals from a distant (>500 km) source, in contrast to the other dairies, which were closed to outside stock during the study period. Importation of new breeding stock to dairy D did not influence shedding of *E. coli* O157:H7. Although dairy D imported breeding stock before the May sample collection, positive isolates from the pens containing outsourced stock (heifers and lactating cows) were not collected until July (Table 3). This lack of relationship would be in agreement with other studies where influx of animals into dairies did not affect shedding of *E. coli* O157:H7 (Hancock et al., 1997; Rice et al., 1999). Transport and commingling of animals is a source of stress (Bach et al., 2004) and, in the beef feedlot environment, increases the risk of shedding of *E. coli* O157:H7 (Dargatz et al., 1997; Stanford et al., 2005). However, Lahti et al. (2003) determined that dairy bull calves from multiple sources assembled at a finishing unit were colonized with *E. coli* O157:H7 originating at the finishing unit. Similarly, Rice et al. (1999) found no relationship between outsourcing stock in dairies and strain diversity and risk of shedding *E. coli* O157:H7.

Table 2. Prevalence[1] of *Escherichia coli* O157:H7 in fecal pats collected monthly from group pens at 5 southern Alberta dairies between October 2002 and September 2003.

	Dairy						
	A	B	C	D	E	Total	OR[2]
Lactating cows							0.385
No. of samples	12	12	24	24	12	84	
% positive	16.7	0	25.0	4.2	0	10.7	
Dry cows							
No. of samples	12	12	12	12	24	72	
% positive	16.7	16.7	25.0	8.3	0	11.1	
Calving cows							
No. of samples	2	0	0	9	12	23	
% positive	0	0	0	0	0	0	
Heifers							2.6
No. of samples	24	36	12	36	36	144	
% positive	12.5	22.2	33.3	13.9	0	13.9	
Calves (older)[3]							
No. of samples	24	0	12	12	0	48	
% positive	25.0	0	33.3	8.3	0	22.9	
Calves (milk-fed)[3]							
No. of samples	24	12	12	12	12	72	
% positive	25.0	16.7	33.3	8.3	0	18.1	
Total no. of samples	98	72	72	105	96		
Total % positive	19.4	16.7	29.2	8.6	0	13.7	
OR[4]	NS[5]	NS	1.6	NS	0.53		

[1]Detected by enrichment and immunomagnetic separation (using Dynabeads anti-*E. coli* O157).

[2]OR = Odds ratio comparing the likelihood of shedding *E. coli* O157:H7 for cow group vs. calf group ($P < 0.05$), when OR = 1.0 for the opposite group.

[3]Older calves were 3 to 9 mo of age; milk-fed calves were 1 to 12 wk of age.

[4]OR = Odds ratio comparing the likelihood of shedding *E. coli* O157:H7 ($P < 0.05$) on that farm compared with all other farms for which OR = 1.0 for each comparison.

[5]NS = Not significant ($P > 0.05$).

Animal Density Mixing and Movement Among Pens

Density of animals within pens remained relatively stable in dairy E, but showed considerable variation in dairies A and C (Table 4). Dairy A also averaged 6.5 times greater movement of animal groups among pens than did other dairies, which largely maintained specific classes of cattle at consistent pen locations on the farm. Animal density (number per pen) was not associated with shedding of *E. coli* O157:H7 in feedlot cattle (Dargatz et al., 1997; Van Donkersgoed et al., 2001), although changing animal density in pens was not evaluated in those studies. Garber et al. (1995) reported that crowding and mixing dairy calves increased the shedding of *E. coli* O157:H7; moreover, mixing or movement among pens increased shedding in feedlot cattle (Stanford et al., 2005). In the present study, it is intriguing that the dairy where no *E. coli* O157:H7 was detected had a relatively stable pen density and a low level of animal translocation, whereas the 2 dairies where the organism was most prevalent had greater movement of animals among pens or increases in pen density. Although results from the present study concerning animal density, mixing, and movement are interesting, they are largely anecdotal. Future large-scale controlled studies would be required to definitively establish the relationships between shedding *E. coli* O157:H7 and changing animal density in pens or their movement and mixing.

Calf Rearing

Dairies A, C, and D marketed weaned steer calves and maintained mixed-sex pens of older calves (3 to 9 mo of age), whereas other dairies sold the bull calves shortly after birth (Table 4). All calves monitored in the study had been separated from cows, and milk-fed calves were the youngest animals evaluated (age 1 to 12 wk). No effect of calf rearing on shedding of *E. coli* O157:H7 could be determined as shedding patterns differed markedly among dairies following similar calf-rearing strategies.

Pen Drainage and Manure Management

Outside pens of dairies A, D, and E were well-drained and generally dry and clean in contrast to those of dairy C where standing water, mud, and manure stockpiles were common. The increased likelihood of shedding *E. coli* O157:H7 by cattle from dairy C compared with

Table 3. Patterns of detection[1] of *Escherichia coli* O157:H7 in pooled fecal pats collected monthly from pens of cattle in 4 of the 5 southern Alberta dairies surveyed in 2002–2003.[2]

	Month of sampling											
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Dairy A												
Milking herd										*	*	
Dry cows				*							*	
Heifers											*	
Older[3] calves (group 1)				*					*		*	*
Older calves (group 2)										*		*
Milk-fed[3] calves (group 1)	*									*	*	*
Milk-fed calves (group 2)	*									*	*	*
Dairy B												
Milking herd												
Dry cows										*	*	
Heifers-large										*	*	*
Heifers-medium										*		*
Heifers-small										*	*	*
Milk-fed calves										*	*	
Dairy C												
Milking herd-bred									*		*	
Milking herd-open		*						*		*	*	
Dry cows	*								*		*	
Heifers	*								*	*		*
Mixed calves					*			*	*	*		
Milk-fed calves				*	*					*		*
Dairy D												
Milking herd										*		
Dry cows									*			
Heifers-large										*	*	
Heifers-small									*	*	*	
Older calves										*		
Milk-fed calves											*	

[1]As determined by immunomagnetic separation.

[2]No *E. coli* O157:H7 was detected in fecal pats from Dairy E (see Table 1).

[3]Older calves were 3 to 9 mo. of age; milk-fed calves were 1 to 12 wk old.

those of other farms was at least partially due to pen conditions on the farm. Wet and muddy pen conditions have been linked (Smith et al., 2001) to an increased risk of shedding *E. coli* O157:H7, and flushing alleys with water is thought to promote shedding of the organism by distributing fecal bacteria throughout the cow housing environment (Garber et al., 1999).

Dairy Herd Composition and Stage of Production

Herd composition differed widely among the dairy farms (Table 4). The milking herds of dairies A and E were approximately twice the size of those of B and D. Dairy C was intermediate in size, but maintained approximately twice as many dry cows as a proportion of the milking herd as compared with the other farms. Garber et al. (1999) determined that larger dairies (>100 lactating cows) had an elevated risk of shedding *E. coli* O157:H7. However, in the present study, 1 of the largest dairies had no positive samples and was only about half as likely ($P < 0.001$) to have cattle shedding *E. coli* O157:H7 than the other dairies. Clearly, the likelihood of cattle shedding *E. coli* O157:H7 is affected by variables other than herd size.

Because dairy D was undergoing expansion, the heifer population exceeded that of mature cows in contrast to the other dairies monitored. Although incidence of shedding *E. coli* O157:H7 was not elevated in cattle at dairy D, calves and heifers across all dairy farms were 2.6× more likely ($P < 0.05$) to shed the organism than were mature dairy cows (Table 2). Those results are similar to other reports (Garber et al., 1995; Mechie et al., 1997; Cobbold and Desmarchelier, 2000) in which immature animals were more at risk for shedding *E. coli* O157:H7 than mature animals.

Shedding rates did not differ among milk-fed (1 to 12 wk of age) and mixed-sex calves (3 to 9 mo age), as all calves were weaned and group-penned. For mature cattle, the stage of production (lactating, dry, or calving) did not affect ($P > 0.61$) the risk of shedding *E. coli* O157:H7, with animals classed as calving cows from 1 wk pre- to 1 wk postparturition. Although no FECAL samples positive for *E. coli* O157:H7 were collected in pens of calving cows, those pens contained a low number

Table 4. Average monthly herd composition, by number of cattle and number of pens, changes in pen density, and movement of cattle among pens from October 2002 to September 2003 in 5 commercial dairy farms in southern Alberta.

	Dairy									
	A		B		C		D		E	
Animal type	Cattle	Pens	Cattle	Pens	Cattle	Pens	Cattle	Pens	Cattle	Pens
Milking herd	125	1	66	1	83	2	52	2	100	1
Dry cows	27	1	19	1	33	2	9	2	24	1
Calving cows	2	1	0[1]	0	0[1]	0	1	1	5	1
Total mature	154	3	85	2	116	4	62	5	129	3
Heifers	23	1	50	3	30	2	93	3	84	3
Calves 3-9 mo.	23	2	0[2]	0	14	1	11	0	0[2]	0
Calves, milk-fed[3]	19	3	8	1	13	1	8	1	4	1
Total immature	65	6	58	4	57	4	112	4	88	4
Total herd	219	9	143	6	173	8	174	9	217	7
CPD/mo[4]	84		50		100		65		33	
MAP/mo[5]	1.3		0		0.2		0.4		0.2	

[1]Not ever present at times of sampling.

[2]Farm did not maintain steer calves.

[3]Milk-fed calves were 1 to 12 wk of age.

[4]CPD = Change in pen density, calculated as average number of pens increasing in population by at least 15% × average % of population increase in pens.

[5]MAP = Movement among pens, calculated as the average number of pens emptied, i.e., in which all animals moved to new pens.

of animals (maximum of 6 cows). Our results agree with those of Cobbold and Desmarchelier (2000), but contradict those of Mechie et al. (1997), in which dairy cows increased shedding of the organism for the first month after calving. In beef herds, Gannon et al. (2002) reported increased shedding of *E. coli* O157:H7 after calving in direct contrast to the results of Synge et al. (2003). Variations in dairy management and in sample collection time relative to actual calving date may be partially responsible for those inconsistent results.

Reports of the effect of lactation on shedding *E. coli* O157:H7 are contradictory. Compared with the present study, where lactation status had no effect on shedding *E. coli* O157:H7, Fitzgerald et al. (2003) reported a higher incidence of shedding in lactating compared with nonlactating animals, whereas Wilson et al. (1993) found increased shedding of *E. coli* O157:H7 in dry cows. As *E. coli* O157:H7 is shed sporadically (Bach et al., 2002), it is not surprising that the results of the present study, where lactating and dry cows were monitored year-round, differ from previous studies, where individual animals were sampled on a maximum of 2 d.

Seasonality

The shedding of *E. coli* O157:H7 peaked from June through September, with collection of positive isolates 15 times more likely ($P < 0.001$) in these months than in others (Table 3). Dairies B and D were strongly seasonal, with positive isolates only present during the 4-mo "peak" period, whereas farms A and C also had positive isolates in other months of the year. These results agree with the bulk of studies conducted in the northern hemisphere in which peak shedding for the organism occurs in summer or fall (Hancock et al., 1997; Mechie et al., 1997; Murinda et al., 2002; LeJeune et al., 2004). In contrast, Ogden et al. (2004) recently determined that the peak period of shedding *E. coli* O157:H7 in Scottish cattle was most influenced by the seasonal confinement of the cattle in barns during the winter. Management factors such as moving and mixing the animals undoubtedly have an effect on shedding *E. coli* O157:H7, although studies to quantify the influence of these factors would be difficult to conduct under commercial dairy conditions.

Based on the SCC of bulk tank samples collected from the farms (Table 5), seasonality appeared to affect more than the shedding of *E. coli* O157:H7. Across dairies, SCC was higher in the months of July, August, and September ($P < 0.01$) than in all other months except November. Seasonal peaks of clinical mastitis occurring in the summer or fall have been noted previously (Schukken et al., 1992; Norman et al., 2000), although factors leading to seasonal increase in SCC have never been adequately explained (Cook et al., 2002). Although it is extremely unlikely that *E. coli* O157:H7 directly influences SCC, heightened levels of *E. coli* and other coliforms in bedding have been identified as a causative factor of mastitis (Hogan et al., 1989; Schukken et al., 1990). As shedding patterns of *E. coli* O157:H7 (Garber

Table 5. Bulk tank SCC (×1000/mL) by month at 5 dairies in southern Alberta.

Sampling date		Dairy					Mean by month	SEM
		A	B	C	D	E		
2002	October	159	114	232	249	131	177.0[b]	27.0
	November	187	184	223	264	100	191.6[a]	27.1
	December	105	139	217	241	123	165.0[d]	26.9
2003	January	142	155	144	195	127	152.6[de]	11.5
	February	125	137	161	270	161	170.8[b]	25.8
	March	155	133	157	270	124	167.8[bc]	26.3
	April	127	95	135	259	143	151.8[de]	28.0
	May	123	104	145	191	151	142.8[e]	14.7
	June	190	133	94	226	171	162.8[cd]	22.8
	July	227	234	111	322	191	217.0[a]	34.1
	August	186	171	161	287	170	195.0[a]	23.3
	September	251	176	150	311	187	215.0[a]	29.2
Mean by dairy		164.8[A]	148.0[A]	160.8[A]	257.1[B]	148.2[A]	175.8	10.1

[a–e]Means (by month) lacking a common superscript differ ($P < 0.05$).
[A,B]Means (by dairy) lacking a common superscript differ ($P < 0.05$).

et al., 1995; Murinda et al., 2002; Bach et al., 2004) and somatic cells (Cook et al., 2002) have both been linked to increased animal stress, unidentified environmental stressors such as flies or heat might be responsible for heightened seasonal shedding. Recent work by Edrington et al. (2004) raises the possibility of physiological mechanisms triggered by day length leading to seasonal shedding of *E. coli* O157:H7. Alternatively, increased environmental temperatures may promote faster bacterial growth within feces on the pen floor (Hogan et al., 1989) or greater proliferation of bacteria within the environment in general (Russell, 1999). As feces on the pen floor have clearly been shown to be the primary source of *E. coli* O157:H7 in penned cattle (Bach et al., 2005), heightened bacterial populations in the environment may lead to an increased incidence of fecal-oral inoculation. A greater bacterial load in dairy barns may lead to the simultaneous peaks in shedding *E. coli* O157:H7 and SCC that were observed. Similarly, a recent study in 10 European countries (Kovats et al., 2004) found that once environmental temperatures exceeded 6°C, a linear association existed between temperature and reported cases of food poisoning.

Efficacy of Ropes and Fecal Pats for Monitoring *E. coli* O157:H7

Escherichia coli O157:H7 was isolated from a higher ($P < 0.001$) proportion of FECAL samples (13.5%) than ROPE samples (1.1%; Table 1). The virtual failure of ROPE to detect *E. coli* O157:H7 in dairy animals was unexpected, due to the promising results for this monitoring technique reported for feedlot cattle (Irwin et al., 2002; Smith et al., 2003; Stanford et al., 2005). Perhaps chewing or licking the rope was reduced in mature dairy cattle compared with yearling feedlot animals, as was reported by Lanier et al. (2000), in which sensitivity of cattle to some environmental stimuli was found to decline with age. Conversely, the increased stimulation and human interaction in dairies may have reduced the degree of novelty of ropes within the pen environment and the subsequent degree of oral contact by dairy cattle. Alternatively, as high-producing dairy cattle spend up to 4 h/d actively eating (Morita et al., 1996), 4-h access may have been insufficient to ensure adequate use of the ropes by dairy cattle. This possibility is supported by the fact that the ropes available to mature animals often appeared untouched after a 4-h period of access.

In contrast, ropes were vigorously chewed by milk-fed calves to the point that ropes were largely consumed after 4 h. Paradoxically, *E. coli* O157:H7 was detected in 18% of FECAL samples from milk-fed calves (Table 2), but never detected in ROPE samples (data not shown). The intense salivation and chewing by the calf ultimately consuming the rope may have resulted in the sampling of only 1 calf per pen. Alternatively, milk-fed calves may not have developed oral populations of *E. coli* O157:H7, although further monitoring would be required to verify these suppositions.

Compared with commercial feedlot pens containing up to 300 animals (Smith et al., 2001), average pen size of weaned calves/heifers in the 5 dairies monitored was low (ranging from 6 to 71 cattle (data not shown). In small pens of animals, animals colonized with *E. coli* O157:H7 may have not accessed the rope. Less than one-half of feedlot cattle in pens averaging 128 cattle orally accessed ropes in a 2-h period (Irwin et al., 2002). Perhaps the greater stimuli in the environment of dairy cattle compared with that of beef cattle (Le Neindre, 1989) reduced the overall utility of ropes for detection of *E. coli* O157:H7 in dairy cattle.

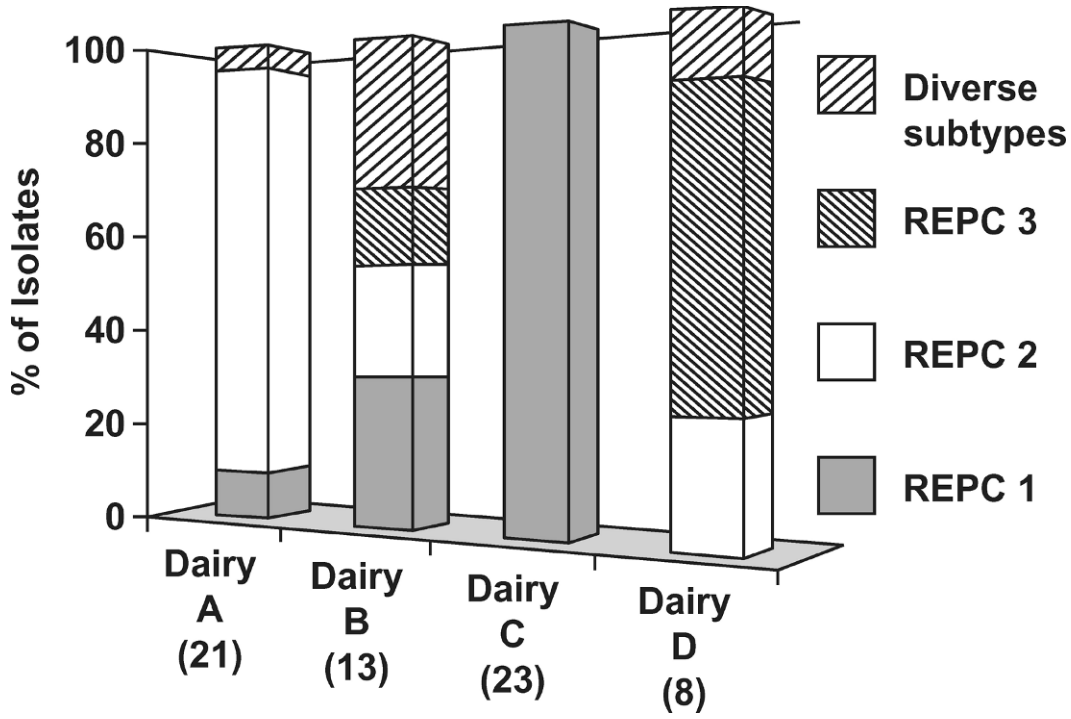


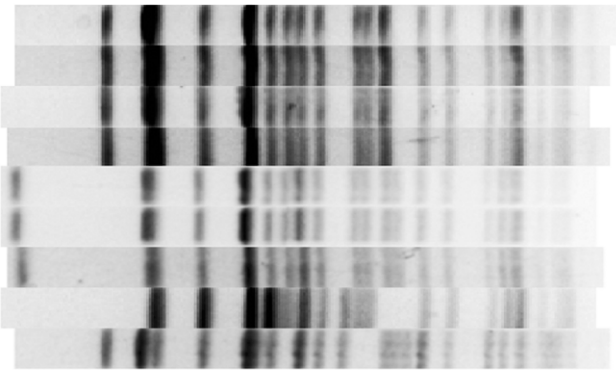
Figure 1. Distribution of *Escherichia coli* O157:H7 isolates (n = 65) from 4 southern Alberta dairies among restriction endonuclease pattern clusters (REPC) identified following *Xba*I and *Spe*I digestion. Numbers of isolates from each dairy are shown in parentheses. Clustered isolates exhibited >90% banding similarity after restriction digest whereas diverse subtypes shared 39.1 to 85.1% similarity with any of the other isolates.

Subtype Diversity as Determined by Pulsed-Field Gel Electrophoresis

Digestion of DNA from 65 *E. coli* O157:H7 isolates with *Xba*I and *Spe*I produced 23 unique subtypes. Subtypes showing >90% homology were grouped into REPC (Figure 1), with 92% of isolates belonging to 3 REPC. The remaining 8% diverse isolates showed 51 to 78% homology. Although few isolates were collected from ROPE samples, rope isolates were identical to fecally derived subtypes (Figure 2).

The 5 dairies monitored in this study were within a 30-km radius of each other, with dairies A and B adjoining. Close geographic proximity may increase the degree of homology of subtypes of *E. coli* O157:H7 present on farms (Davis et al., 2003), although previous surveys (Faith et al., 1996; Shere et al., 1998; Rice et al., 1999) reported that dairies each maintained a distinct set of closely related *E. coli* O157:H7 subtypes. Dairies A and D showed a degree of diversity of *E. coli* O157:H7 isolates similar to that found in previous studies (Faith et al., 1996; Shere et al., 1998; Murinda et al., 2002). The majority of isolates from each of dairies A and D (18/21 and 5/8, respectively) belonged to 2 distinct REPC, although isolates from other clusters were also present (Figure 1). In contrast, dairy C showed remarkable conservation of a single REPC, despite collection of isolates over a 12-mo period. Perhaps the normally wet and muddy pen environment of this dairy increased the fitness of only 1 strain of *E. coli* O157:H7. Inadequate manure management on dairy C could have also selected for a single subtype and increased shedding of *E. coli* O157:H7 (Synge et al., 2003). A combination of environmental and management factors were likely responsible for the increased ($P < 0.001$) shedding of *E. coli* O157:H7 by cattle from dairy C compared with other monitored animals, and the maintenance of isolates from a single REPC. Similar to the present study, Rice et al. (1999) reported little diversity in *E. coli* O157:H7 in 1 of 41 cattle herds monitored, although reasons for this lack of genetic diversity were not identified.

In contrast to dairy C, where isolates were exclusively of 1 REPC, isolates from dairy B were almost evenly split across clusters and showed the highest proportion of subtypes not belonging to 1 of the 3 primary REPC. Cattle from this dairy shed *E. coli* O157:H7 only over a 3-mo period, but isolate diversity was maintained over the entire shedding period. Maintenance of diverse subtypes of *E. coli* O157:H7 may be due to repeated introduction of new subtypes to a farm (Rice et al.,



Month	Dairy	Pen	Type	REPC
Feb	C	5	rope	1
Feb	C	5	fecal	1
Feb	C	5	fecal	1
May	C	5	rope	1
July	A	2	rope	2
July	B	5	rope	2
Jan	A	6	fecal	2
June	D	6	fecal	3
Sep	A	9	fecal	NA

Figure 2. Restriction endonuclease pattern cluster (REPC) by month, dairy, pen, and sample type (from manila ropes (ROPE) or pooled fecal samples (FECAL). NA = Not applicable, isolate did not share 90% homology with any other isolate.

1999), potentially from human travel (Davis et al., 2003), or from wild birds (Synge et al., 2003). Alternatively, the environmental and management stressors present on this farm may support the maintenance of a variety of *E. coli* O157:H7 subtypes.

Although all dairies in the present study were located within 30 km of each other and shared essentially the same climate, genetic diversity of isolates was equivalent to that reported for studies that covered much larger regions (Shere et al., 1998; Murinda et al., 2002). Strain diversity has been reported to increase with geographic distance (Davis et al., 2003), but the closest farms geographically did not maintain isolates from the same REPC. Consequently, differences in farm management and environmental factors such as sanitation may affect the genetic diversity of *E. coli* O157:H7 subtypes to a greater extent than does simple geography.

CONCLUSIONS

Absolutes concerning the ecology of *E. coli* O157:H7 in herds of cattle are few due to the complexity of the interactions among farm management, environment, and climate in relation to protocols used to monitor the organism. That the risk of shedding *E. coli* O157:H7 is seasonal and increased in immature compared with mature cattle is largely undisputed and supported by the results of the current study. However, each of the 5 dairies monitored was unique with respect to the degree of seasonality of shedding, prevalence of shedding, and the genetic diversity of *E. coli* O157:H7 subtypes maintained. Dairy management factors including increasing animal density in pens, translocation of pens of animals, and poor hygiene (wet, muddy, dirty pens) may affect the shedding of *E. coli* O157:H7. Regardless of their desirability, controlled studies to clearly delineate the relative contribution of these factors are almost impossible to conduct under commercial production conditions. Determination of management and environmental factors leading to concurrent increases in SCC and fecal shedding of *E. coli* O157:H7 require further investigation. As a means of monitoring pens of dairy cattle for colonization with *E. coli* O157:H7, collection of fecal pat samples would be recommended over the oral sampling technique using ropes.

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