Escherichia coli O157:H7 infection in cows and calves in a beef cattle herd in Alberta, Canada

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SUMMARY

Escherichia coli O157:H7 infection of cows and calves in a naturally-infected beef cattle herd in Alberta, Canada, was investigated over 2 years, encompassing two calf production cycles. In both years of the study, *E. coli* O157:H7 was isolated from the faeces of cows shortly after but not before parturition in late winter: 6/38 (16%) in 1996 and 13/50 (26%) in 1997. At < 1 week post-partum, 13/52 (25%) calves born in 1997 were shedding the organism. Faecal shedding of *E. coli* O157:H7 by cows and calves continued over the 7 weeks that they were in the calving pens, with the organism being isolated from the faeces of 2–18% of cows and 23–26% of calves during this period. Five weeks after they were moved onto a native grass pasture, all the calves and all but one cow in 1997 had ceased shedding the organism. When the calves were weaned in the fall, *E. coli* O157:H7 was isolated from the faeces of 0–1·5% of the calves 1 week prior to weaning and from 6–14% of the calves within 2 weeks after weaning. Parturition, calving pens and weaning appear to be important factors in maintaining *E. coli* O157:H7 infections in this beef cattle herd. Isolates from cows and calves during the immediate post-partum period were mostly of the same pulsed-field gel electrophoresis (PFGE) type of *E. coli* O157:H7. Similarly, at weaning a common PFGE type of *E. coli* O157:H7, which differed slightly from the post-partum PFGE type, was isolated from the calves. These typing data suggest a common source of infection for the animals as well as demonstrate clonal turnover of resident populations of this pathogen.

INTRODUCTION

Escherichia coli O157:H7 and other enterohemorrhagic *E. coli* (EHEC) are associated with hemorrhagic colitis, the hemolytic uremic syndrome and thrombotic thrombocytopenic purpura in humans [1]. Human infections with *E. coli* O157:H7 are frequently associated with consumption of undercooked beef and raw milk [2–5], however, an increasing number of cases of infection with this pathogen have also been

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linked to fruits, vegetables and water thought to have been contaminated with bovine faeces [6, 7].

A substantial proportion of the Canadian beef rearing and packing industries are concentrated in western Canada, specifically in the province of Alberta. Pai and colleagues [8] reported a high incidence of haemorrhagic colitis in the Calgary area in southern Alberta in 1984 and Doyle and Schoeni [9] subsequently reported the isolation of *E. coli* O157:H7 from 5 of 17 ground beef samples from retail outlets in this city. Also, between 1987 and 1991, the

incidence of human infections with VT-producing *E. coli* (VTEC, mostly *E. coli* O157:H7) was 12·1/100 000 for the province of Alberta compared with 2·0/100 000 for Scotland during this same period [10].

Recent studies in Europe [11–13] and North America [14, 15] employing improved detection methods, have revealed much higher rates of infection than was previously estimated, and have confirmed that *E. coli* O157:H7 is widely distributed among young beef cattle. Although weaning, diet, contaminated feed and water and management have been identified as risk factors for cattle herd infections with *E. coli* O157:H7 [16–24], there is little information on the factors influencing early infection in beef breeding herds. Laegreid et al. [25] have reported that the majority of range-fed beef calves from herds in the mid-western USA are infected with *E. coli* O157:H7 at weaning. It is likely that some of these calves will continue to shed the organism in their faeces at slaughter and act as an important source of beef carcass contamination.

The objective of this study was to investigate the epidemiology of *E. coli* O157:H7 in a naturally infected beef herd at potentially important points in the cow-calf production cycle. Faecal excretion of *E. coli* O157:H7 was monitored from before calving until shortly after weaning over two breeding and rearing cycles. Parturition, the early post-partum period, confinement, weaning and the age of bred cows were identified as factors potentially influencing the occurrence and rates of shedding of *E. coli* O157:H7. The pulsed-field gel electrophoresis (PFGE) typing information on isolates of the organism from the cattle point to a common source of herd infection as well as suggest clonal turnover of resident populations of *E. coli* O157:H7.

MATERIALS AND METHODS

Animals

Angus-Herford cross cattle from the Animal Diseases Research Institute (Lethbridge, Alberta) research herd were studied over a 2-year period. Cattle from this closed beef herd were first shown to be shedding *E. coli* O157:H7 in their faeces in 1995. The herd could be conveniently sampled and has a size (ca. 100 breeding females) and management system typical of many beef cow-calf herds in western Canada and the mid-western USA [25]. Cows are artificially inseminated in June of each year. In 1996, 38 of 81 pregnant dams were available for study. Their faeces was

cultured for *E. coli* O157:H7 prior to parturition on 12 March, 1996 and then again the day following parturition from 26 March through 16 April, 1996. In 1997, the first 50 of 87 cows that gave birth and their 52 calves (including 2 sets of twins) were studied. In 1997, dams were sampled prior to parturition (10 February) and during the first week after parturition (26 March) and then three times again at approximately 2-week intervals (4 April, 21 April and 6 May) and then once again on 5 June. During the calving period, groups of up to 30 pregnant females were maintained in two pens of 780 m² each and fed an alfalfa silage ration. After calving, the cows and their calves were placed into a pen of equal size which was immediately adjacent to the birthing pen. The pre- and post-calving pens have their own feed and water supplies and are separated by a 10 m alleyway from other cow-calf pens. Cows and calves were moved from pens onto a 16 ha pasture on 2 June, 1996 and 1 May, 1997, where their diet was supplement with hay silage. In the second week of June in both years, cows and calves were moved onto a 81 ha native grass pasture and then rotated through four similar sized pastures over the summer months. In the fall (October to November) of 1995, 1996 and 1997, calves and cows were first placed together in pens for 1 week before the calves were weaned. The cows and weaned calves were maintained in separate 780 m² pens through the fall and winter months. Approximately 20 weaned heifer calves were selected each year as replacements for dams culled from the herd. The other weaned calves were fed in adjacent feedlot pens until late in the following year and then were slaughtered.

Bacteriology

Faecal samples (ca. 10 g) were collected from the rectum of the cattle by hand using a sterile latex glove. The samples were placed into 15 ml of Cary-Blair transport media, placed in a cooler containing ice and transported to the laboratory.

For detection and quantification of *E. coli* 0157:H7, faecal suspensions were; (1) plated directly on cefixime tellurite sorbitol MacConkey agar (CT-SMAC [26], consisting of sorbitol MacConkey agar containing 2·5 mg/l of potassium tellurite and 0·05 mg/l of cefixime) and (2) placed into modified trypticase soy broth (mTSB;[27]) and incubated at 42 °C for 6 h. Immuno-magnetic separation (IMS) was performed using magnetic polystyrene beads

coated with O157-antibody (Dynabeads anti-*E. coli*, Dynal, Oslo, Norway) according to the manufacturer's instructions. Following IMS a portion of the bead suspension was plated onto CT-SMAC agar and incubated at 37 °C overnight. Up to five sorbitol-negative colonies were selected at random and tested in the slide agglutination assay using anti-O157 antisera (Difco). Agglutination positive colonies were tested using an *E. coli* O157:H7-specific polymerase chain reaction assay which detects VT (*vt*), attaching and effacing (*eae*) and flagellar (*fli*C) gene targets of the organism [28]. *E. coli* 0157:H7 were also characterized by biochemical testing and H antigen serotyping [29].

Bacterial sub-typing

Bacteriophage typing was carried out as described by Ahmed and colleagues [30] and extended by Khakharia and colleagues [31].

Pulsed field gel electrophoresis (PFGE) was performed according to the Center for Diseases Control (CDC) manual standard 1-day protocol [32]. Briefly, a single colony of each strain was inoculated onto Veal Infusion Yeast Extract agar (Difco) and grown at 37 °C for 18 h. Bacteria were harvested using a sterile cotton swab and suspended in Cell Suspension Buffer (100 mM Tris, 100 mM EDTA, pH 8·0). Next, proteinase K was added to the cell suspension to a final concentration of 0·1 mg/mL and the suspension was embedded in 1·2% SeaKem Gold agarose (FMC BioProducts, Rockland, Maine) prepared using TE buffer (10 mM Tris, pH 8·0, 1 mM EDTA) containing 1% sodium dodecyl sulfate. Once solidified, the agarose plugs were transferred to 5·0 ml of lysis buffer (50 mM Tris, pH 8·0, 50 mM EDTA, 1% sarcosine, 0·1 mg/ml proteinase K) and incubated at 54 °C with shaking for 2 h. The agarose plugs were washed with distilled water four times for 15 min each with TE (10 mM Tris, pH 8·0; 1 mM EDTA, pH 8·0) at 50 °C. Agarose plugs were then sliced to 1mm thickness and the slices were equilibrated with $1 \times$ M buffer (Amersham Pharmacia, Baie d'Urf, Quebec) at room temperature for 15 min and then digested at 37 °C overnight with 40 U of *Xba*I (Amersham Pharmacia) according to the manufacturer's instructions. Agarose slices were next placed in $0·5 \times$ TBE (0·89 M Tris, 0·89 M boric acid, 0·02 M disodium EDTA, pH 8·0) for 5 min at room temperature. Electrophoresis was performed with a CHEF DR III electrophoresis unit (Bio–Rad, Mississauga, Ontario) with 1% Seakem

Gold agarose at 14 °C. Initial and final switch times were 2·2 and 54·2 sec, respectively, and the total run time was 21 h. After electrophoresis, the gels were stained with ethidium bromide (0·5 μg/ml) and rinsed with distilled water, then illuminated with a UV lamp and photographed using the Geldoc 1000 System (Bio–Rad). Interpretation of the PFGE patterns was aided by use of the Molecular Analyst Software Fingerprinting PLUS, v. 1.6 (Bio–Rad). Patterns were categorized according to the criteria set out by Tenover et al. [33]. The *E. coli* O157:H7 strain G5244, obtained from the CDC, was used as the standard in PFGE comparisons.

Statistical methods

The McNemar test was used to compare proportions of cows shedding the organism in their faeces before and after parturition. The Fisher's exact test was used to compare the proportion of cows with *E. coli* O157:H7 in their faeces that also had faecal positive calves to the proportion of cows with *E. coli* O157:H7 in their faeces that had faecal negative calves. Statistical tests were conducted using EpiCalc 2000, Version 1.0 (http://www.mvatt.demon.co.uk/epicalc.htm).

RESULTS

Parturition

E. coli O157:H7 was not isolated from faecal samples of any of the cows prior to parturition in late winter of 1996 or 1997 (Table 1). In contrast, the organism was recovered from the faeces of 6 of 38 (15·8%) of these dams within 1 week of parturition in 1996 and from 13 of 50 (26%) dams within 1 week of parturition in 1997. The difference in the isolation rate of the organism from the faeces of the dams before parturition and 1 week after parturition was significant ($P < 0·05$, $P < 0·01$) in both years. At 12 weeks postpartum, the organism was also isolated from the faeces of 13·2% of the cows in 1996 and at 7 weeks post-partum from 18% of the cows in 1997. *E. coli* O157:H7 was isolated from the faeces of 13 of 52 (25%) of the calves within the first week of parturition in 1997. At 12 weeks postpartum, *E. coli* O157:H7 was isolated from faecal samples from 7 of 38 (18·4%) of the calves in 1996 and at 7 weeks postpartum, from 21 of 52 (41·2%) calves in 1997.

Five weeks after the cows and calves were put onto native grass pasture (ca. 12–16 weeks postpartum), *E. coli* O157:H7 was not isolated from the faeces of

Table 1. *Faecal shedding of* E. coli *O157:H7 by dams and calves*

Location and time	1996		1997	
	Cows*	Calves*†	Cows*	Calves*†
Drylot pens				
Pre-partum				
-2 to -4 weeks	0 (0·0%)	NA	0 (0·0%)	NA
Post-partum				
< 1 week	6 (15·8%)	ND	13 (26%)	13 (25%)
3 weeks	ND	ND	8 (16%)	14 (26·9%)
5 weeks	ND	ND	1 (2%)	12 (23·1%)
7 weeks	5 (13·2%)	7 (18·4%)	9 (18%)	21 (41·2%)
Pasture				
5 weeks	0 (0%)	0 (0%)	1 (2%)	0 (0%)
Drylot pens				
> 1 week pre-weaning	NA	1 (1·5%)	NA	1 (1·2%)
2 weeks post-weaning	NA	4 (6·0%)	NA	12 (14·1%)

NA, Not applicable, ND, Not done.
* In 1996, 38 cow-calf pairs and in 1997, 48 cows-calf pairs and 2 cows with twins were tested.
† In 1996, 67 calves and in 1997, 85 calves were tested 1 week pre- and 2 weeks post-weaning.

either dams or calves in 1996 and in 1997 was isolated from the faeces of 1 of 50 dams but not from the faeces of the 52 calves.

E. coli O157:H7 was isolated from the faeces of 5 cow-calf pairs the first sampling date after parturition during the spring of 1997 (data not shown). However, 8 cows were culture positive but their calves were culture negative; and 8 calves were culture positive but their dams were culture negative. While a greater percentage of cows from which *E. coli* O157:H7 was isolated on this sampling date had calves which were also culture positive compared with cows which were faecal culture negative (5/13 or 38·5% versus 8/35 or 22·8%), this difference was not significant ($P > 0.05$).
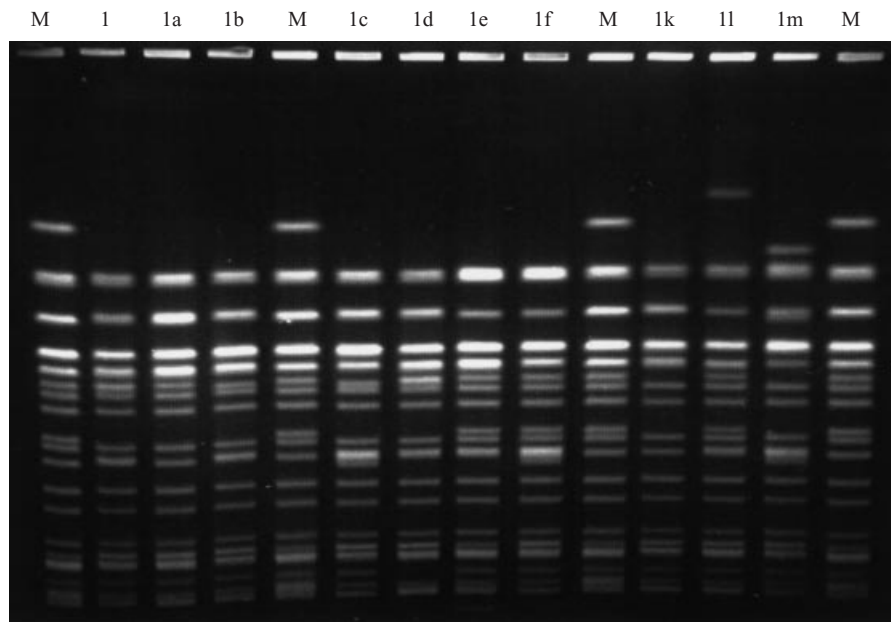
Over the first 12 weeks post-partum in 1997, in 4 cow-calf pairs the organism was not isolated from either the dam or its calf (data not shown). In 20 cases, the organism was isolated from the faeces of the calves but not their dams', in 18 cases it was isolated from the faeces of both the dam and at least 1 of the calves (2 of the dams had twins), and in 7 cases the organism was isolated from the faeces of the dam but not its calf. Nine calves had the organism isolated from their faeces in 2 consecutive samples, 3 in 3 consecutive samples and 1 in 4 consecutive samples. In contrast, only 3 dams had 2 consecutive positive samples and 1 dam had 3 consecutive positive samples.

In 1997, *E. coli* O157:H7 was isolated from the faeces of 14 of 21 (66·7%) cows less than 5 years of age and from 11 of 29 (37·9%) cows greater than 5 years of age, with 7 of 9 of the first calf heifers culture positive (data not shown). In addition, the dams < 5 years of age had more faecal samples positive than older dams from the 5 sampling periods during the first 12 weeks post-partum (22/105 or 19·0% versus 12/145 or 8·3%). Calves of young dams also had higher faecal isolation rates for the organism than calves from older dams, with 8/21 or 38·1% and 5/31 or 16·1% of the first post-partum faecal samples culture positive, respectively. This was also the case when the total number of faecal samples culture positive for the organism from the 5 postpartum samples were considered. Calves from dams > 5 years of age had 34/155 or 21·9% of faecal samples positive while calves from dams < 5 years of age had 26/105 or 24·81% of faecal samples positive.

Direct CT-SMAC *E. coli* O157:H7 plating results for cows and calves tended to parallel enrichment-IMS culture results (data not shown). There were more calves positive on direct plating 33/52 (63·5%) than cows 12/50 (24%) and 5 calves had 2 consecutive direct plating positive faecal cultures while none of the cows had 2 consecutive direct plating positive faecal cultures.

During the post-partum period in the spring of 1997, a total of 201 of 260 faecal samples from these 52 calves (77·3%) and 216 of 250 samples from the 50 cows (86·4%) were negative on both direct and IMS-

Fig. 1. PFGE analysis of *E. coli* O157:H7 isolates from cows and calves. *E. coli* O157:H7 total DNA was digested with *Xba*I. Lanes with PFGE types 1, 1a, 1b, 1c, 1d, 1e, 1f, 1k, 1l and 1m are identified in the photograph. Lanes labeled M are *E. coli* O157:H7 G5244 total DNA digested with *Xba*I and run on PFGE.

enrichment culture. *E. coli* O157:H7 was isolated from 32 of these samples from calves on both direct and IMS-enrichment culture and from 13 samples from cows using these 2 cultural methods. The organism was isolated from 27 calf samples and from 13 cow samples on IMS-enrichment culture alone. In all cases faecal samples positive on direct plating were also positive by enrichment-IMS culture.

Weaning

Among the 1997 cow-calf pairs, *E. coli* O157:H7 was isolated from the faeces of 1 calf prior to weaning and 7 calves post-weaning (Table 1). Six of these calves also had the organism isolated from their faeces in the first 7 weeks post partum but 2 did not. Only 3 of the 8 calves which were culture positive during weaning had dams which were culture positive during the early post partum period.

E. coli O157:H7 was not isolated from the faeces of any of 74 calves sampled 1 week prior to weaning in October and November of 1995, however, the organism was isolated from the faeces of 6/74 (8·1%) of these calves 2 weeks after weaning (data not shown). In 1996, *E. coli* O157:H7 was isolated from the faeces of 1 of 67 (1·5%) calves prior to weaning and from the faeces of 4 of 67 calves (6·0%) 2 weeks after weaning and in 1997, from the faeces of 1 of 85 (1·2%) calves prior to weaning and from the faeces of 12 of 85 (14·1%) 2 weeks after weaning (Table 1).

PFGE types of *E. coli* O157:H7

All *E. coli* O157:H7 isolates from the faeces of cows and calves were phage type 87 and could be assigned to 1 of 10 *Xba*I PFGE types; 1, 1a, 1b, 1c, 1d, 1e, 1f, 1k, 1l and 1m (Fig. 1). These PGFE types did not differ from type 1 by more than 1 or 2 bands.

The majority of *E. coli* O157:H7 isolates from the faeces of cows and calves during the first 2 months following parturition in 1997 belonged to PFGE type 1 (Table 2). *E. coli* O157:H7 type 1 isolates only, were obtained one or more times from 24 of 27 cows and 20 of 22 calves during the post partum period (26 March to 5 May, 1997). One cow initially had *E. coli* O157:H7 of type 1 isolated from the faeces and subsequently type 1d was isolated and *E. coli* O157:H7 type 1a and 1b were isolated from the faeces of one cow each. One calf initially had *E. coli* O157:H7 type1a isolated from its faeces and then type 1 was isolated and one calf had *E. coli* O157:H7 type 1b isolated from its faeces.

While 8 cow-calf pairs had the same *E. coli* O157:H7 PFGE type, type 1, isolated from the cow and the calf on the same day, in 3 other cases the cow and the matching calf's *E. coli* O157:H7 PFGE types differed. On two occasions an *E. coli* O157:H7 type 1 and type 1a were isolated together (once from the cow and calf and once from the calf and cow, respectively). *E. coli* O157:H7 PFGE type 1 and type 1b were also isolated together from a cow-calf pair.

At weaning in November of 1997, *E. coli* O157:H7

Table 2. *Pulsed-field gel electrophoresis (PFGE) types of selected* E. coli
O157:H7 isolates from cows and calves (1997)

PFGE type (s)	Post partum (March–May)			Weaning (November)
	Cows*	Calves*	Cow-calf pairs†	Calves*
1 only	24	20	8	0
1a only	1	0	0	0
1b only	1	1	0	0
1c only	0	0	0	6
1d only	0	0	0	0
1e only	0	0	0	1
1/1a	0	1	2	0
1/1b	0	0	1	0
1/1c	0	0	0	0
1/1d	1	0	0	0

* Number of animals with this *E. coli* O157:H7 PFGE type(s) isolated from the faeces on one or more occasion.

† Number of cow-calf pairs with this *E. coli* O157:H7 PFGE type or combination of PFGE types isolated from their faeces.

isolates examined from six calves were of PFGE type 1c and one was of PFGE type 1e (Table 2). PFGE type 1c was also isolated from 6/7 cows in late spring of 1998 (see below). For 5 of the 6 calves with type 1c, *E. coli* O157:H7 PFGE type 1 was isolated from their faeces in the spring post partum period. The calf with PFGE type 1e and one calf with type 1c did not have *E. coli* O157:H7 isolated from their faeces in the spring (data not shown).

In contrast to isolates from the spring of 1997, *E. coli* O157:H7 PFGE type 1 was not isolated from cow samples in 1996 or 1998. In 1996 (12 March–3 June), 6 of 9 *E. coli* O157:H7 isolates from cows were of *E. coli* O157:H7 type 1e and the 3 other isolates belonged to PFGE types 1f, 1k and 1l. Six of 7 *E. coli* O157:H7 strains isolated from cows 15 June, 1998 were of PFGE type 1c and one was PFGE type 1m. *E. coli* O157:H7 was isolated from the same cows in different years in two cases. One cow had PFGE type 1l in 1996 and type 1c in 1998, and the other cow had PFGE types 1e and 1 in 1996 and 1997, respectively.

DISCUSSION

In this study, points in the cow-calf production cycle were identified as important in the transmission of *E. coli* O157:H7. Infection of calves appears to occur shortly after parturition with the most likely source of infection being their dams. Mechie and colleagues [34] also noted that excretion rates of *E. coli* O157:H7 by

dairy cattle was highest after calving. The cause of this post-partum increase in faecal shedding of the organism is unknown but may be related to hormonal influences, other metabolic changes related to parturition or to dietary stress. While Mechie and colleagues [34] suggested that the increase in feeding of concentrate following parturition may be responsible for this effect, the diet of the dams in the present study was not altered following parturition. They were fed alfalfa silage throughout the pre-and post-partum periods with no concentrate supplementation. Recently, Laegreid and colleagues [25] noted that 100% of beef cattle dams were sero-positive in a *E. coli* O157:H7 LPS blocking ELISA assay at the time of parturition. However, no information was provided by these workers with respect to isolation of the organism from the faeces of these cattle. Further study is required to more precisely characterize the factors responsible for the post-parturient faecal shedding of *E. coli* O157:H7 by dams observed in this study.

E. coli O157:H7 was isolated from many more faecal samples with enrichment-IMS than by direct plating on CT-SMAC. Enrichment-IMS followed by plating CT-SMAC is reported to detect as few as 100 colony forming units of the organism per gram of faeces [35]. Direct plating, in contrast, is reported to be at least 10- to 100-fold less sensitive and only detects samples with between 10^4 and 10^5 organisms per ml of faecal suspension [26, 36]. The relatively

large number of positive faecal samples where the organism was isolated from direct plating on CT-SMAC suggests that substantial numbers of the organism are present in the faeces of these cattle.

More dams and their calves shed *E. coli* O157:H7 in their faeces in pens compared to the pasture environment. Kudva and colleagues [37] also reported that sheep orally inoculated with *E. coli* O157:H7 eventually stopped shedding the organism when allowed to graze on a Sage brush-bunch grass pasture. It is, however, unclear if diet or the feedlot environment or both were important variables in maintaining these infections. Hancock et al. [38] reported that the median within-herd prevalence of faecal shedding of *E. coli* O157:H7 by weaned dairy heifers fed in drylots or allowed to graze on manured and non-manured pastures was 1·6, 0·83 and 0·42 %, respectively. While these percentage difference in prevalence were not significant over a 6-month period, it was noted that the overall prevalence of faecal shedding of the organism by the heifers in July was much higher than in December. It is possible that greater differences in prevalence of faecal shedding of this pathogen in these environments may have emerged if the analysis had been confined to a time of the year with high prevalence. In addition, differences in age class of the animals, management system, herd type (dairy versus beef), sizes of pastures and regional differences in vegetation and climate must also be taken into account when comparing studies.

Water has been shown to be a source of the pathogen for cattle [21] and it is also clear that the organism can survive in certain feeds [39] and in manure [40, 41] for considerable periods of time. This bacterial pathogen has also been isolated from cattle hides [15], milk [34] and from the mouths of cattle [21, 42]. Clearly, cattle distributed in a large pasture would be expected to have decreased exposure to this human pathogen from manure, feed and possibly water. However, the change in diet could also play a significant role in reducing the number of cattle shedding and numbers of organisms shed into the environment. A grass diet would certainly be expected to cause a change in the intestinal microflora as well as parameters such as volatile fatty acid species and concentrations and the pH of the digesta. A recent study has shown that grain feeding selects for acid-resistant *E. coli* strains and that feeding Timothy hay rapidly reduces the numbers of these organisms shed in the faeces [23]. While this appears to be the case, the authors of this somewhat controversial study failed to demonstrate that *E. coli* O157:H7 was one of the acid-resistant *E. coli* strains selected for by grain feeding and reduced by hay feeding. Recent studies by Hovde and colleagues [24] showed that hay feeding increases rather than decreases faecal shedding of *E. coli* O157:H7 by beef cattle which were orally inoculated with the organism. Another possibility is that factors other than energy content of the feed may be involved in the hay or grain effects observed. For example, we have observed increased faecal shedding of *E. coli* O157:H7 by cattle fed a barley diet compared to those fed a corn diet [42]. In addition, naturally occurring antimicrobial substances in certain plants may play a role in faecal shedding of the organism by cattle e.g. Duncan and colleagues have shown that certain coumarins derived from plants inhibit growth of *E. coli* O157:H7 [43].

E. coli O157:H7 was isolated from very few calves immediately prior to weaning, however, once the calves were weaned, increased faecal shedding of the organism was again observed. Weaned beef and dairy calves have been reported to have a higher prevalence of infection than unweaned calves [22, 25, 44]. Cobbold and Desmarchelier [44] reported a high prevalence of faecal shedding of *E. coli* O157:H7 on a dairy farms where weaned dairy calves were in contact with the rest of the herd. Beef calves are weaned and reared in very large groups which provide an excellent opportunity for them to be exposed to a variety of pathogens. While isolation of weaned beef calves may decrease the risk of spreading *E. coli* O157:H7 to other calves in feedlots, it is unlikely that this strategy would be widely adopted by industry because of the costs involved in implementing this control measure.

While it is possible that the organism was acquired from sources in pens, it is also likely that certain of these calves act as carriers of *E. coli* O157:H7 and only excrete the pathogen in their faeces under certain physiological conditions. Long-term faecal shedding of this pathogen by certain cattle within herds has been noted in several studies [21, 34, 45]. These 'carrier' animals are likely to act as an important source of infection for others in the group. It would be helpful to more precisely define the factors which trigger faecal excretion of the organism by these cattle and contribute to long-term carriage of this pathogen in the intestine. Changes in livestock density, social stress, feed and withdrawal of milk may all play a role in the increase in post-weaning faecal shedding of the organism.

A greater number of dams less than 5 years compared with dams greater than 5 years of age shed the organism in their faeces following parturition. Mechie and colleagues [34] also noted a decrease in shedding of the organism in dairy cattle with increasing age, and in several other studies, young cattle shed the organism more frequently and for longer periods of time than older cattle [15, 19, 45–47]. It is tempting to suggest that this age-related difference in faecal shedding of the organism is related to the maturity of the intestinal immune response of these animals. However, other explanations have also been advanced, including changes in the anatomy and physiology and the microflora of the digestive tract with age and changes in feeding regimes for older animals. In this study, the feeding regimes for the cattle were the same regardless of age. Further study is required to define age-related changes in the gastrointestinal tract that influence faecal shedding of *E. coli* O157:H7 by cattle.

The PFGE typing data suggest that three distinct types of *E. coli* O157:H7 predominated in cows in 1996 (type 1e), cows and calves in the spring of 1997 (type 1) and weaned calves in the fall of 1997 and cows in the spring of 1998 (type 1c). A greater variety of *E. coli* O157:H7 types would be expected if faecal shedding were to spontaneously occur in several animals at the same time. The data, therefore, suggest a common source of the organism such as a contaminated feed or water or an individual carrier animal such as described above. Other groups of workers have also noted the predominance of single PFGE types in specific dairy herds [21, 46, 48].

While these *E. coli* O157:H7 PFGE types predominated in the faeces of cows and calves at specific times, variants of these main types were also occasionally encountered (1f, 1k and 1l in 1996, 1a, 1b and 1d in 1997 and 1 m in 1998). Presumably this reflects genetic variability in the *E. coli* O157:H7 population and it is possible that this pool of new types provides a means for the emergence of a new dominant clone. The preponderance of *E. coli* O157:H7 type 1c among weaned calves, appears to reflect a group shift in the PFGE type shed, particularly since 5 of 6 of the calves with type 1c at weaning shed type 1 shortly after birth. This group shift not only suggests a point source but also that selective pressures exist to bring about an *E. coli* O157:H7 type shift. Clonal turnover or shift in *E. coli* O157:H7 type has been noted in individual humans and cattle that shed the organism for long periods of time [49–51]. Changes in the preponderance

of a particular *E. coli* O157:H7 type within groups of cattle over time have also been previously noted [21].

In summary, the results of this study indicate that post-partum shedding of *E. coli* O157:H7 and cow-calf or calf-calf transmission under confined conditions in the post-partum period are potentially important factors in initial infection of beef calves. Control of infection at this early point in the production cycle could reduce the rate of infection of calves and later dissemination of the organism at weaning. Further study is required to determine if the dynamics of *E. coli* O157:H7 infection of cows and calves in this single beef cattle herd in Alberta are generally applicable.

REFERENCES

1. Tarr PI. *Escherichia coli* O157:H7: clinical, diagnostic, and epidemiological aspects of human infection. Clin Infect Dis 1995; **20**: 1–8.
2. Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev 1991; **13**: 60–98.
3. Karmali MA. Infection by verocytotoxin-producing *Escherichia coli*. Clin Microbiol Rev 1989; **2**: 15–38.
4. Sharp JC, Ritchie LD, Curnow J, Reid TM. High incidence of haemorrhagic colitis due to *Escherichia coli* 0157 in one Scottish town: clinical and epidemiological features. J Infect 1994; **29**: 343–50.
5. Sharp JCM, Coia JE, Curnow J, Reilly WJ. *Escherichia coli* O157 infections in Scotland. J Med Microbiol 1994; **40**: 3–9.
6. Tauxe RV. Emerging foodborne diseases: an evolving public health challenge. Emerg Infect Dis 1997; **3**: 425–34.

7. Chalmers RM, Aird H, Bolton FJ. Waterborne *Escherichia coli* O157. J Appl Microbiol 2000; **88** Suppl: 124S–132S.

8. Pai CH, Gordon R, Sims HV, Bryan LE. Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7; Clinical, epidemiological and bacteriological features. Ann Intern Med 1984; **101**: 738–42.

9. Doyle MP, Schoeni JL. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl Environ Microbiol 1987; **53**: 2394–6.

10. Waters JR, Sharp JC, Dev VJ. Infection caused by *Escherichia coli* O157:H7 in Alberta, Canada, and in Scotland:a five-year review, 1987–1991. Clin Infect Dis 1994; **19**: 834–43.

11. Bonardi S, Maggi E, Bottarelli A, et al. Isolation of verocytotoxin-producing *Escherichia coli* O157:H7 from cattle at slaughter in Italy. Vet Microbiol 1999; **67**: 203–11.

12. Chapman PA, Siddons CA, Malo AT, Harkin MA. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. Epidemiol Infect 1997; **119**: 245–50.

13. Chapman PA, Wright DJ, Norman P, Fox J, Crick E. Cattle as a possible source of verocytotoxin-producing *Escherichia coli*-O157 infections in man. Epidemiol Infect 1993; **111**: 439–47.

14. Heuvelink AE, van den Biggelaar FL, de Boer E, et al. Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 strains from Dutch cattle and sheep. J Clin Microbiol 1998; **36**: 878–82.

15. Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher GA, Koohmaraie M, Laegreid WW. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc Natl Acad Sci USA 2000; **97**: 2999–3003.

16. Van Donkersgoed J, Graham T, Gannon V. The prevalence of verotoxins, *Escherichia coli* 0157:H7, and *Salmonella* in the feces and rumen of cattle at processing. Can Vet J 1999; **40**: 332–8.

17. Dargatz DA, Wells SJ, Thomas LA, Hancock DD, Garber L. Factors associated with the presence of *Escherichia coli* O157 in feces of feedlot cattle. J Food Prot 1997; **60**: 466–70.

18. Hancock DD, Besser TE, Rice DH, Herriott DE, Tarr PI. A longitudinal study of *Escherichia coli* O157 in 14 cattle herds. Epidemiol Infect 1997; **118**: 193–5.

19. Herriott DE, Hancock DD, Ebel ED, Carpenter LV, Rice DH, Besser TE. Association of herd management factors with colonization of dairy cattle by Shiga toxin-positive *Escherichia coli* O157. J Food Prot 1998; **61**: 802–7.

20. Harmon BG, Brown CA, Tkalcic S, et al. Fecal shedding and rumen growth of *Escherichia coli* O157:H7 in fasted calves. J Food Prot 1999; **62**: 574–9.

21. Shere JA, Bartlett KJ, Kaspar CW. Longitudinal study of *Escherichia coli* O157:H7 dissemination on four dairy farms in Wisconsin. Appl Environ Microbiol 1998; **64**: 1390–9.

22. Zhao T, Doyle MP, Shere J, Garber L. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. Appl Environ Microbiol 1995; **61**: 1290–3.

23. Diez-Gonzalez F, Callaway TR, Kizoulis MG, Russell JB. Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. Science 1998; **281**: 1666–8.

24. Hovde CJ, Austin PR, Cloud KA, Williams CJ, Hunt CW. Effect of cattle diet on *Escherichia coli* O157:H7 acid resistance. Appl Environ Microbiol 1999; **65**: 3233–5.

25. Laegreid WW, Elder RO, Keen JE. Prevalence of *Escherichia coli* O157:H7 in range beef calves at weaning. Epidemiol Infect 1999; **123**: 291–8.

26. Chapman PA, Wright DJ, Siddons CA. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces. J Med Microbiol 1994; **40**: 424–7.

27. Padhye N, Doyle M. Rapid procedure for detecting enterohemorrhagic *Escherichia coli* O157:H7 in food. Appl Environ Microbiol 1991; **57**: 2693–8.

28. Gannon VP, D'Souza S, Graham T, King RK, Rahn K, Read S. Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains. J Clin Microbiol 1997; **35**: 656–62.

29. Sowers EG, Wells JG, Strockbine NA. Evaluation of commercial latex reagents for identification of O157 and H7 antigens of *Escherichia coli*. J Clin Microbiol 1996; **34**: 1286–9.

30. Ahmed R, Bopp C, Borczyk A, Kasatiya S. Phage-typing scheme for *Escherichia coli* O157:H7. J Infect Dis 1987; **155**: 806–9.

31. Khakhria R, Duck D, Lior H. Extended phage-typing scheme for *Escherichia coli* 0157:H7. Epidemiol Infect 1990; **105**: 511–20.

32. Centers for Disease Control and Prevention. Standardized molecular subtyping of foodborne bacterial pathogens by pulsed-field gel electrophoresis. Atlanta: CDC, 1998.

33. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; **33**: 2233–9.

34. Mechie SC, Chapman PA, Siddons CA. A 15 month study of *Escherichia coli* O157:H7 in a dairy herd. Epidemiol Infect 1997; **118**: 17–25.

35. Sanderson MW, Gay JM, Hancock DD, Gay CC, Fox LK, Besser TE. Sensitivity of bacteriologic culture for detection of *Escherichia coli* O157:H7 in bovine feces. J Clin Microbiol 1995; **33**: 2616–9.

36. Chapman PA, Malo AT, Siddons CA, Harkin M. Use of commercial enzyme immunoassays and immunomagnetic separation systems for detecting *Escherichia coli* O157 in bovine fecal samples. Appl Environ Microbiol 1997; **63**: 2549–53.

37. Kudva IT, Hatfield PG, Hovde CJ. Effect of diet on the shedding of *Escherichia coli* O157:H7 in a sheep model. Appl Environ Microbiol 1995; **61**: 1363–70.

38. Hancock DD, Besser TE, Kinsel ML, Tarr PI, Rice DH, Paros MG. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiol Infect 1994; **116**: 199–207.

39. Lynn TV, Hancock DD, Besser TE, et al. The occurrence and replication of *Escherichia coli* in cattle feeds. J Dairy Sci 1998; **81**: 1102–8.

40. Wang G, Zhao T, Doyle MP. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. Appl Environ Microbiol 1996; **62**: 2567–70.

41. Kudva IT, Blanch K, Hovde CJ. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. Appl Environ Microbiol 1998; **64**: 3166–74.

42. Buchko SW, Holley RA, Olson WO, Gannon VPJ, Veira DM. The effect of different grain diets on fecal shedding of *Escherichia coli* O157:H7 by steers. J. Food Prot 2000; **63**: 1467–74.

43. Duncan SH, Flint HJ, Stewart CS. Inhibitory activity of gut bacteria against *Escherichia coli* O157 mediated by dietary plant metabolites. FEMS Microbiol Lett 1998; **164**: 283–8.

44. Cobbold R, Desmarchelier P. A longitudinal study of Shiga-toxigenic *Escherichia coli* (STEC) prevalence in three Australian diary herds. Vet Microbiol 2000; **71**: 125–37.

45. Cray WCJ, Moon HW. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. Appl Environ Microbiol 1995; **61**: 1586–90.

46. Heuvelink AE, van den Biggelaar FL, Zwartkruis-Nahuis J, et al. Occurrence of verocytotoxin-producing *Escherichia coli* O157 on Dutch dairy farms. J Clin Microbiol 1998; **36**: 3480–7.

47. Hancock DD, Rice DH, Thomas LA, Dargatz DA, Besser TE. Epidemiology of *Escherichia coli* O157 in feedlot cattle. J Food Prot 1997; **60**: 462–5.

48. Rice DH, McMenamin KM, Pritchett LC, Hancock DD, Besser TE. Genetic subtyping of *Escherichia coli* O157 isolates from 41 Pacific Northwest USA cattle farms. Epidemiol Infect 1999; **122**: 479–84.

49. Karch H, Russmann H, Schmidit H, Schwarzkopf A, Heeseman J. Long-term shedding and clonal turnover of enterohemorrhagic *Escherichia coli* O157 in diarrheal diseases. J Clin Microbiol 1995; **33**: 1602–5.

50. Faith NG, Shere JA, Brosch R, et al. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. Appl Environ Microbiol 1996; **62**: 1519–25.

51. Akiba M, Sameshima T, Nakazawa M. The shift of genetic subtypes of *Escherichia coli* O157:H7 isolates from cattle. Epidemiol Infect 1999; **122**: 343–6.