Monitoring and Mitigation of *E. coli* O157:H7 in Commercial Dairies

T. A. McAllister[1], K. Stanford[2] and S. J. Bach[3]

[1]Agriculture and Agri-Food Canada Research Centre, Lethbridge AB T1J 4B1
E-mail: mcallister@em.agr.ca
[2]Alberta Agriculture, Food and Rural Development, Lethbridge AB T1J 4V6
[3]Agriculture and Agri-Food Canada Research Centre, Summerland BC V0H 1Z0

Take Home Message

- Oral access to ropes is not a suitable method for monitoring *E. coli* O157:H7 in dairy cattle.
- Prevalence of *E. coli* O157:H7 is highest during summer months, a trend that corresponds with similar seasonality of somatic cell counts.
- Strict hygienic practices in dairies may reduce the prevalence of *E. coli* O157:H7, as this pathogen was not detected in the cleanest of five dairies studied. Younger cattle are more likely to shed *E. coli* O157:H7 than are older cattle.
- Frequent mixing of animals may increase the prevalence and fecal shedding of *E. coli* O157:H7.
- *E. coli* O157:H7 isolates belonged to three main genetic clusters and genotypes tended to be dairy-specific. Dairies that mixed animals tended to yield isolates with greater genetic diversity.
- Strategies for direct control of *E. coli* O157:H7 should be commercially available within 3 to 5 years.

Introduction

Gradual progress is being made in implementing on-farm food safety protocols (Powell et al. 2002), but the feasibility and mechanics of monitoring and mitigating pathogens in commercial production systems is a huge challenge. Cattle are recognized reservoirs of the pathogen, *Escherichia coli* O157:H7 (Bach et al. 2002) and human disease outbreaks have been linked to contaminated meat (Bell et al. 1994), unpasteurized milk (Murinda et al. 2002) and the environment (Varma et al. 2003). The potential severity of human

infection (Bach et al. 2002) and the scale of disease outbreaks (Bender et al. 2004) has established *E. coli* O157:H7 as a pathogen worthy of surveillance and reduction in an on-farm food safety program.

Escherichia coli O157:H7 has previously been monitored in individual dairy cattle through rectally collected fecal samples (Fitzgerald et al. 2003) or fecal swabs (Rice et al. 1999). Because fecal shedding of this organism is transient and intermittent (Bach et al. 2002), reliable estimation of prevalence requires that samples be collected from all animals resident in a facility (Stanford et al. 2005). Collection of individual samples by rectal grab or fecal swab is feasible during milking, when cows are handled daily, but dry cows, heifers, and weaned calves are typically held in loose housing, penned in groups. Individual monitoring of these animals would require deliberate additional handling, with associated inconvenience and injury risk (to humans and livestock). For practical purposes, therefore, an economical method of monitoring cattle for this pathogen that does not require individual animals to be handled or farm staff to enter group pens would be desirable.

Collection of fecal pat samples has been used for monitoring groups of dairy cattle (Cobbold et al. 2004) in the United States, but this method may be less sensitive than rectally-collected fecal samples (Lahti et al. 2003), and requires that personnel enter the pen for sample collection. *Escherichia coli* O157:H7 is also harboured orally by cattle, and it has been isolated from manila ropes exposed to oral contact (chewing) by feedlot cattle (Smith et al. 2003, Stanford et al. 2005), but the suitability of this procedure for dairies has not been examined. In this paper, we describe a 12-month study conducted at five dairy farms in southern Alberta to examine factors influencing transmission and maintenance of *E. coli* O157:H7 and to assess the utility of manila ropes as an on-farm method of monitoring this organism in dairy cattle. Potential strategies currently under development for mitigating *E. coli* O157:H7 in livestock will also be discussed.

■ Description of Study

Collection and Analysis of Samples

Over a 1-year period, manila rope and fecal pat samples were collected monthly from all cattle pens at five commercial dairies in southern Alberta and assessed for the presence of *E. coli* O157:H7. Participating dairies were selected on the basis of geographic proximity (located within a 30-km radius) and diversity of management practices with regard to mixing of animals and pen reassignments. Numbers and age/type (e.g., cows dry, lactating, or calving; heifers; milk-fed and older calves) of cattle in each pen were recorded

on sampling days, and milk samples from bulk tanks were collected for estimation of somatic cell counts.

For rope sampling, a 120-cm length of manila rope was tied along the fence line of each pen in a location accessible to the cattle for oral contact. The rope was collected after 4 h and tested for the presence of *E. coli* O157:H7. During the same 4 h, a pooled fresh fecal pat (one for every 20 animals in the pen) was amassed, and processed for isolation of *E. coli* O157:H7 using specialized immunomagnetic beads. Genetic assay for the toxin genes associated with *E. coli* O157:H7 was used to confirm the identity of suspected isolates. Those confirmed as *E. coli* O157:H7 were further classified into subtypes by way of the DNA characterization technique, pulsed-field gel electrophoresis (PFGE). This technique allowed the degrees of relatedness among the isolates to be compared between and within dairies.

Description of Dairies

Herd size and composition varied substantially among the five dairies (Table 1). Dairies A and E had milking herds approximately twice as large as those at Dairies B and D. The milking herd at Dairy C was intermediate to the others in size, but nearly twice as many dry cows were maintained relative to the number of lactating cows, compared with the other operations. At the time of the study, Dairy D was in the process of expanding, thus the number of heifers exceeded the number of mature cows.

Management practices also differed widely among the participating dairies. During the study period, Dairy D purchased a number of animals from a distant (>500 km) source, whereas the other dairies obtained all replacements from within the herd. Dairies A, C and D marketed weaned steer calves and maintained mixed sex pens of older calves (3 to 9 months of age), whereas bull calves were sold shortly after birth from Dairies B and E. All calves monitored in this study were weaned, and milk-fed calves ranged in age from 1 to 12 wks. The outdoor pens at Dairies A, B, D, and E were well drained and generally dry, in contrast to those at Dairy C, where standing water and mud were commonly observed in the pens.

Table 1. Average monthly herd composition, by number of head and by number of pens, changes in pen density, and movement of animals among pens from October 2002 to September 2003 in the five commercial dairy farms in southern Alberta involved in the *E. coli* O157:H7 study

Animal type	Dairy									
	A		B		C		D		E	
	Head	Pens	Head	Pens	Head	Pens	Head	Pens	Head	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[a]	0	0[a]	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[b]	0	14	1	11	0	0[b]	0
Calves, milk-fed[c]	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/month[d]	84		50		100		65		33	
MAP/month[e]	1.3		0		0.2		0.4		0.2	

[a]Not ever present at times of sampling.
[b]Farm did not maintain steer calves.
[c]Milk-fed calves were 1 to 12 wks of age.
[d]CPD: Change in pen density, calculated as average number of pens increasing in population by at least 15% × average % of population increase in pens.
[e]MAP: Movement among pens, calculated as the average number of pens emptied, i.e., in which all animals moved to new pens.

■ Findings of the Dairy Study

Prevalence of *E. coli* O157:H7 and Efficacy of Ropes

Across dairies, overall prevalence of *E. coli* O157:H7 as assessed by fecal pats was 13.7% (Table 2), a level similar to the average value (18.8%) reported for cattle in southern Alberta feedlots (Stanford et al. 2005). No *E. coli* O157:H7 was detected at Dairy E, whereas 19.3% of samples from Dairy A and 29.2% from Dairy C tested positive. Poor pen drainage at Dairy C may have contributed to the propagation of *E. coli* O157:H7 in the environment and an increased rate of transmission among cattle. Similar prevalences of *E. coli* O157:H7 have been recorded in other studies with beef and dairy cattle (Hancock et al. 1998; Van Donkersgoed et al. 1999).

Table 2. Proportions of pens in five commercial dairies in southern Alberta positive for *Escherichia coli* O157:H7 as assessed by pooled fecal samples (PAT) or manila ropes over a one-year period[a]

Dairy ID	Sample type[b]	No. of pens sampled	2002			2003									Total
			O	N	D	J	F	M	A	M	J	J	A	S	
A	PAT	8	2	0[c]	0	2	0	0	0[c]	0	1	4	6	4	19/98
	ROP	8	0	0[c]	0	0	0	0	0[c]	0	0	1	0	1	2/98
B	PAT	6	0	0	0	0	0	0	0	0	0	5	4	3	12/72
	ROP	6	0	0	0	0	0	0	0	0	0	1	0	0	1/72
C	PAT	8	2	1	0	1	2	0	0	2	4	4	3	2	21/72
	ROP	8	0	0	0	0	1	0	0	1	0	0	0	0	2/72
D	PAT	9	0	0	0	0[c]	0[c]	0[c]	0	0	2	4	3	0	9/105
	ROP	9	0	0	0	0[c]	0[c]	0[c]	0	0	0	0	0	0	0/105
E	PAT	7	0	0	0	0	0	0	0	0	0	0	0	0	0/96
	ROP	7	0	0	0	0	0	0	0	0	0	0	0	0	0/96
Total	PAT	% positive	10.5	2.6	0	7.9	7.9	0	0	5.3	18.4	44.7	42.1	23.7	13.7
	ROP	% positive	0	0	0	0	2.6	0	0	2.6	0	4.8	0	2.6	1.1

[a]Values shown are numbers of pens testing positive

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

[c]In Nov 2002 and Apr 2003, nine pens were sampled at Dairy A. In Jan, Feb and March of 2003, only 8 pens were sampled at Dairy D.

Rates of isolation of *E. coli* O157:H7 from fecal pats (13.7%) was significantly higher than from manila ropes (1.1%; Table 2). This virtual failure of the ropes as a detection method for *E. coli* O157:H7 in dairy cattle is in stark contrast to the promising results that were obtained with this monitoring technique in feedlot cattle (Smith et al. 2003; Stanford et al. 2005). Possibly, the novelty of the ropes in the pens is decreased in dairies as compared with feedlots due to the higher level of environmental stimuli, and as a result, oral contact by mature dairy cattle was less. The ropes were vigorously chewed by milk-fed calves, yet a paradox existed in that even though *E. coli* O157:H7 was detected in18% of the fecal pat samples from these calves (Table 2), it was not recovered from the ropes. Milk-fed calves may have yet to develop the oral populations of *E. coli* O157:H7 that are commonly observed in mature cattle. Allowing longer periods of access to the rope may improve the utility of this monitoring technique. However, given that this technique was 13X less sensitive than fecal pat sampling, and that it failed to detect *E. coli* O157:H7 in younger animals, it does not appear to be a suitable method for monitoring pathogens in dairies.

Seasonality

Shedding of *E. coli* O157:H7 peaked in the summer (June to September), at which time collection of positive samples was found to be 15 times as likely as at other times of the year (Table 3). Seasonality was particularly evident at Dairies B and D, where *E. coli* O157:H7 was detected only during this 4-month high risk period. In contrast, the organism was detected at Dairies A and C at other times of the year. Other researchers have also observed peak shedding of *E. coli* O157:H7 in the summer or fall (Murinda et al. 2002; LeJeune et al. 2004). Somatic cell counts in bulk tank samples were also highest in July, August and September (Table 4), a result that coincides with the seasonality of mastitis (Norman et al. 2000). This summer seasonality may be indicative of enhanced growth of bacterial pathogens in the environment or of their transmission by vectors such as flies. Heightened levels of *E. coli* and other coliforms in bedding has been identified as a causative factor of mastitis (Schukken et al. 1990) and feces on the pen floor has been shown to be a primary source of *E. coli* O157:H7 in feedlot pens (Bach et al. 2005). Consequently, heightened growth of microorganisms in these environments during the summer may increase the risk of infection of the herd. Although a cause and effect relationship has clearly not been established, somatic cell counts may offer some insight with regard to the relative degree to which *E. coli* O157:H7 might be present in a dairy environment (Schukken et al. 1991).

Table 3. Patterns of detection[a] of *E. coli* O157:H7 in pooled fecal pats collected monthly from pens of animals in four of the five southern Alberta dairies surveyed in 2002-2003[b]

		Month of sampling											
		O	N	D	J	F	M	A	M	J	J	A	S
Dairy A													
	Milking herd										■	■	
	Dry cows				■							■	
	Heifers											■	
	Older[c] calves (group1)				■					■		■	■
	Older calves (group 2)										■		■
	Milk-fed[c] calves (group1)	■									■	■	■
	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											■	

[a]As determined by immunomagnetic separation.
[b]No *E. coli* O157:H7 was detected in fecal pats from Dairy E (see Table 2).
[c]Older calves were 3 to 9 mo. of age; milk-fed calves were 1 to 12 wk old.

Table 4. Bulk tank somatic cell counts (x 1000) by month at five dairies in southern Alberta

		Dairy					Mean by month	
Sampling date		A	B	C	D	E		SEM
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[d,e]	11.5
	February	125	137	161	270	161	170.8[b]	25.8
	March	155	133	157	270	124	167.8[b,c]	26.3
	April	127	95	135	259	143	151.8[d,e]	28.0
	May	123	104	145	191	151	142.8[e]	14.7
	June	190	133	94	226	171	162.8[c,d]	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 ($P < 0.05$).

Age of Animal/Stage of Production

Calves and heifers were 2.6 times more likely to shed *E. coli* O157:H7 than were mature cows (Table 5). The number of positive samples did not differ between milk-fed calves (1 to 12 wks of age) and weaned calves (3 to 9 months old), or among lactating, calving, and dry cows. Other researchers (Cobbold and Desmarchelier 2000) have also reported that *E. coli* O157:H7 is more prevalent in younger than in older dairy cattle.

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

		Dairy						
		A	B	C	D	E	Total	OR[b]
Lactating cows								
	No. of samples	12	12	24	24	12	84	
	% positive	16.7	0	25.0	4.2	0	10.7	
Dry cows								2.6X
	No. of samples	12	12	12	12	24	72	
	% positive	16.7	16.7	25.0	8.3	0	11.1	decr.
Calving cows								
	No. of samples	2	0	0	9	12	23	
	% positive	0	0	0	0	0	0	
Heifers								
	No. of samples	24	36	12	36	36	144	
	% positive	12.5	22.2	33.3	13.9	0	13.9	
Calves[c] (older)								2.6X
	No. of samples	24	0	12	12	0	48	
	% positive	25.0	0	33.3	8.3	0	22.9	incr.
Calves[c] (milk-fed)								
	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	

[a]As detected with enrichment and immunomagnetic separation (using Dynabeads™ anti-*E. coli* O157:H7).
[b]OR: odds ratio comparing the likelihood of shedding *E. coli* O157:H7.
[c]Older calves were 3 to 9 months of age; milk-fed calves were 1 to 12 weeks of age.

Animal Density and Movement among Pens

Density of animals within pens remained relatively stable at Dairy E, but varied considerably at Dairies A and C (Table 1). Dairy A also averaged 6.5 times more movement of animal groups among pens than the other four dairies. Increased shedding of *E. coli* O157:H7 associated with greater mixing of animals has been reported for dairy calves (Garber et al. 1995) and feedlot cattle (Stanford et al. 2005). In the present study, *E. coli* O157:H7 was not detected at Dairy E, whereas prevalence was highest at Dairies A and C, at which changes in pen density were greatest. Importing new breeding stock at Dairy D was not associated with elevated prevalence of *E. coli* O157:H7, presumably because the new animals were not carriers.

Genetic Relatedness of *E. coli* O157:H7 Isolates

Twenty three unique subtypes were identified among the *E. coli* isolates collected in the present study. Characterization of banding patterns determined that 92% of the isolates could be classified into three clusters of genotype relatedness (i.e., >90% similarity of banding patterns). The remaining isolates exhibited homologies ranging from 51 to 78% and were defined as diverse subtypes. All *E. coli* O157:H7 isolates obtained from the manila ropes were represented identically among the isolates obtained from feces.

The greatest diversity of *E. coli* O157:H7 genotypes was observed at Dairy B, from which isolates representative of all three restriction endonuclease pattern clusters (REPC), plus diverse subtypes, were obtained (Figure 1). In stark contrast, all of the isolates collected from Dairy C originated from a single cluster (REPC 1). The majority of isolates from Dairy A fell into REPC 2, whereas those from Dairy D originated primarily from REPC 3. It has been reported previously that specific genotype clusters of *E. coli* O157:H7 may be associated with specific farms (Murinda et al. 2002). The remarkable conservation of the single *E. coli* O157:H7 genotype at Dairy C suggests that conditions were particularly favorable for its establishment and persistence. Other researchers have also reported that certain herds of cattle exhibit little diversity among *E. coli* O157:H7 isolates (Rice et al. 1999). Despite the close geographical proximity of the dairies examined in this study, the observed strain diversity was similar to that reported over a much larger geographic area (Shere et al. 1998; Murinda et al. 2002). Davis et al. (2003) reported that strain diversity increases with geographic distance, but adjacent dairies (A and B) did not harbor the same *E. coli* O157:H7 genotypes. Thus, it appears that differences in farm management and environmental factors such as sanitation may exert greater influence on genetic diversity of *E. coli* O157:H7 at a given location than does simple geography.

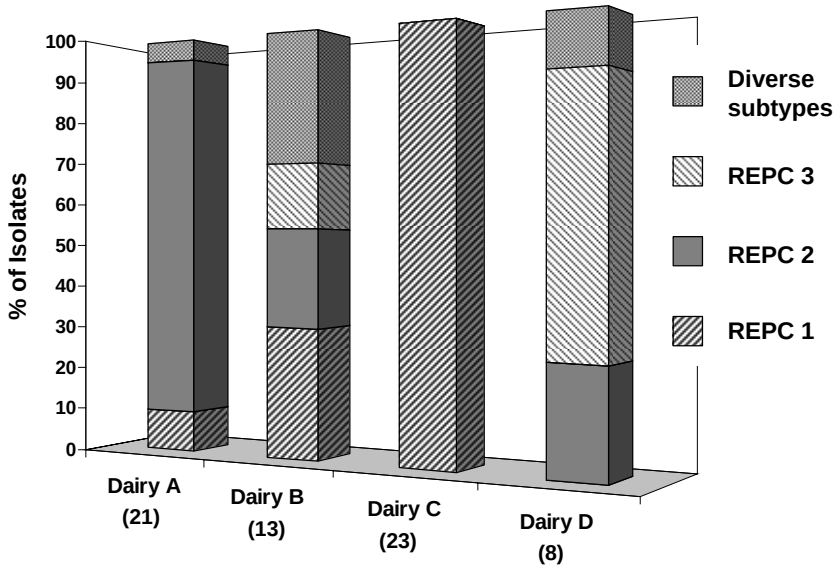

Figure 1. Distribution of *E. coli* O157:H7 isolates from four southern Alberta dairies among restriction endonuclease pattern clusters (REPC) identified following *Xba*I and *Spe*I digestion. Numbers of isolates from each dairy are shown in parentheses. Clustered isolates exhibited >90% banding similarity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

■ Mitigation Methods for *E. coli* O157:H7

No effective means for controlling fecal shedding of *E. coli* O157:H7 in dairy cattle have been described to date. However, several technologies directed at mitigating *E. coli* O157:H7 in ruminants are presently under development, and a select few direct-fed microbial products are included in feedlot diets in the United States for this purpose. Simulation models have shown that intervention strategies that reduce or eliminate *E. coli* O157:H7 in the live animal can help prevent contamination of carcasses and reduce the risk of food-borne illness. Control of *E. coli* O157:H7 in environmental sources such as water troughs, feed and manure is also critical in breaking the cycle of infection and re-infection of livestock. Several technologies with potential to mitigate this pathogen in Canadian dairy cattle should be available in the next 3 to 5 years.

Vaccination

Vaccination may be an effective preventative strategy for reducing carriage of *E. coli* O157:H7 by cattle. The goal of vaccination is either to reduce susceptibility of cattle to colonization by *E. coli* O157:H7, or to decrease the duration of such colonization. In order for *E. coli* O157:H7 to be shed intermittently in feces, it must first be able to survive as a resident of the gastrointestinal tract of cattle. Researchers believe that *E. coli* O157:H7 adheres to the large intestine wall by way of virulence factors secreted directly into host cells (Nataro and Kaper 1998). A vaccine that induces production of antibodies against these virulence factors could prevent adherence of the organism and result in its elimination from the gastrointestinal tract. Vaccination of cattle with proteins excreted by *E. coli* O157:H7 significantly reduced the number of *E. coli* O157:H7 shed in feces, the number of animals shedding, and the duration of shedding episodes in cattle experimentally challenged with this pathogen (Potter et al. 2004). Field trials from both Canada and the United States are presently being assessed and preliminary reports indicate that this approach is apparently also effective for reducing shedding of *E. coli* O157:H7 by cattle under commercial production conditions. Controlled studies required to enable registration of *E. coli* O157:H7 vaccine for use in Canada are presently underway.

Direct-Fed Microbials

Some direct-fed microbials have proven effective for reducing the duration and extent of shedding of *E. coli* O157:H7 by cattle. Eighteen bacterial isolates from cattle (one *Proteus mirabilis* and 17 strains of *E. coli*) were found to produce metabolites inhibitory to *E. coli* O157:H7. These were administered orally to calves 2 d prior to their inoculation with *E. coli* O157:H7. Calves that received the selected bacteria carried *E. coli* O157:H7 in their rumen for 9 to 17 days only, compared with 22 to 32 days in the control calves. The duration of fecal shedding was reduced from 25-32 d in the control group, to 14-19 d in calves that received the direct-fed microbial (Zhao et al. 1998). More recent study has shown that supplementing diets with selected strains of *Lactobacillus acidophilus* (Brashears et al. 2003) or mixtures of *Lactobacillus*- and *Propionibacterium*-based direct-fed microbials (Younts-Dahl et al. 2004) reduced shedding of *E. coli* O157:H7 by cattle. Many direct-fed microbials may claim to reduce the prevalence of pathogens in cattle, but unlike the aforementioned preparations, few have been specifically selected or evaluated for this purpose. Presently, there are no direct-fed microbials in Canada registered for their ability to reduce the shedding of *E. coli* O157:H7 in cattle.

Bacteriophage Therapy

Bacteriophage therapy, which was initially described in the early 1900's, has been used successfully to prevent and treat human bacterial infections with pathogens such as *Salmonella*, *Shigella,* and *Staphylococcus* spp. Treatment with bacteriophage has also been effective for controlling enteropathogenic *E. coli* infection in calves, piglets and lambs (Smith and Huggins 1983).

Our laboratory is presently involved in a collaborative project with Health Canada to develop a bacteriophage specific for *E. coli* O157:H7. Bacteriophage, or "bacterial viruses", attach to the outer surface of bacterial cells such as *E. coli* O157:H7 (Figure 2) and inject their genetic material into the bacterium. After many cycles of replication, the nascent phages cause lysis and death of the bacterial cell, and are released back into the environment. Waddell et al. (2000) determined that the duration of fecal shedding of *E. coli* O157:H7 by calves orally inoculated with 3×10^9 cfu (colony-forming units) of the bacterium was significantly reduced when 10^{11} pfu (plaque-forming units) of each of six O157-specific bacteriophages were administered to the calves 7, 6, 1 and 0 d prior to, and 1 d after, their inoculation with *E. coli* O157:H7. Shedding by the control calves (i.e., those inoculated with *E. coli* O157:H7 but given no phage) persisted for 6 to 14 d, whereas bacteriophage-treated calves shed the pathogen for only 6 to 8 d. Unlike vaccination therapy, bacteriophage also may serve as an effective biocontrol for *E. coli* O157:H7 residing in environmental reservoirs in animal facilities (e.g., feces, water, troughs, feed, etc.).

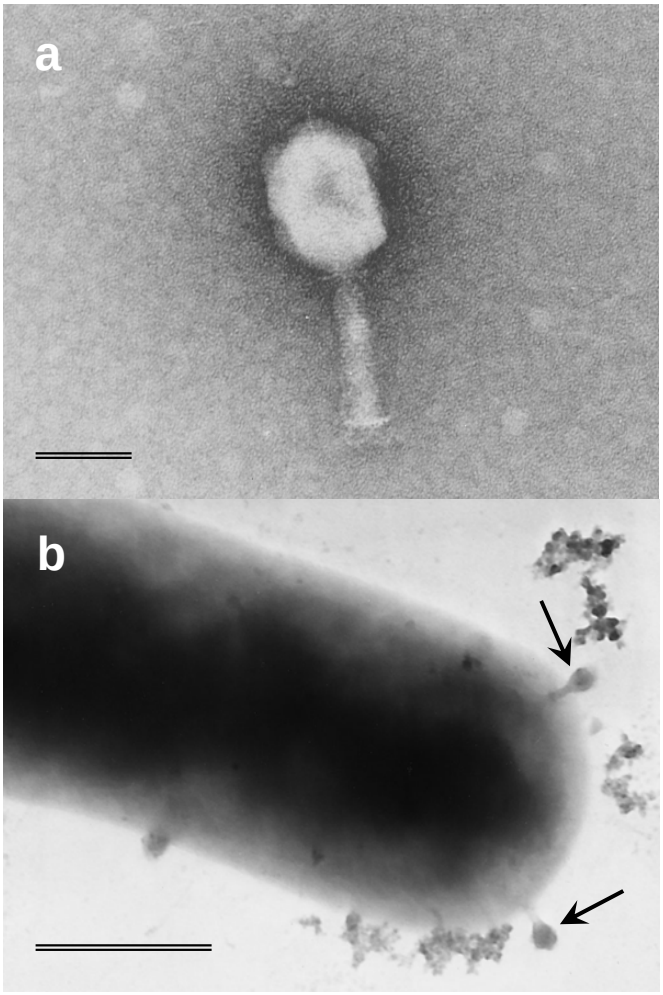

Figure 2. Electron micrographs showing a) bacteriophage DC22, which is specific for *Escherichia coli* O157:H7, and b) an *E. coli* O157:H7 cell with the bacteriophage (arrows) attached to its outer surface. The bacteriophage injects its genetic material into the bacterial cell where it replicates and 100 to 200 new bacteriophage are assembled. The new bacteriophage are released into the environment upon lysis of the bacterial cell. Bar in a) = 0.1 µm; in b) = 0.5 µm.

Antibody Therapy

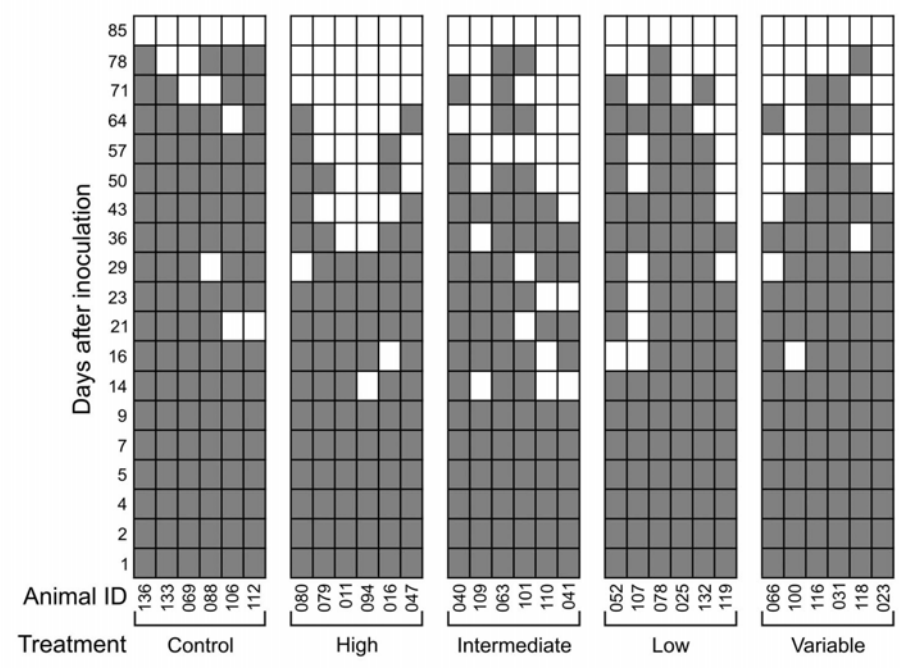

Figure 3. Patterns of fecal shedding of *E. coli* O157:H7 by experimentally inoculated sheep fed spray-dried whole egg from hens immunized or not against enterohemorrhagic *E. coli* (EHEC) antigen. The rams were dosed orally with *E. coli* O157:H7 on day 0, then on days 2, 3 and 4, were fed the control and/or antibody-containing egg preparations (100 g/d) in combinations of 100:0 (Control), 0:100 (High), 50:50 (Intermediate) or 25:75 (Low). Sampling days upon which fecal samples were culture-positive for *E. coli* O157:H7 are shown in grey; white boxes indicate days upon which samples were culture negative. The frequency of positive samples was lower (*P* = 0.002) in treatment group 'High' than in the Control group. From Cook et al. (2005).

Chicken egg yolks are a rapid, non-invasive, efficient and economical vehicle for production and delivery of specific antibodies (Tini et al. 2002). One laying hen can produce more than 20 g of egg yolk antibody (IgY) per year, with more than 100 mg of IgY in each egg yolk. Sunwoo and colleagues (2002) reported high antibacterial activity of specific chicken IgY antibodies against *E. coli* O157:H7 cells *in vitro*. Our research has demonstrated that feeding IgY antibodies (in spray-dried whole eggs from hens immunized against an enterohemorrhagic *E. coli* antigen) to lambs challenged with *E. coli* O157:H7 reduced their shedding of the bacterium (Figure 3, Cook et al. 2005). Work is

ongoing in our laboratory that focuses on developing IgY antibodies with even greater specificity for the primary *E. coli* O157:H7 antigens involved in colonization of the ruminant gastrointestinal tract.

Seaweed Extract

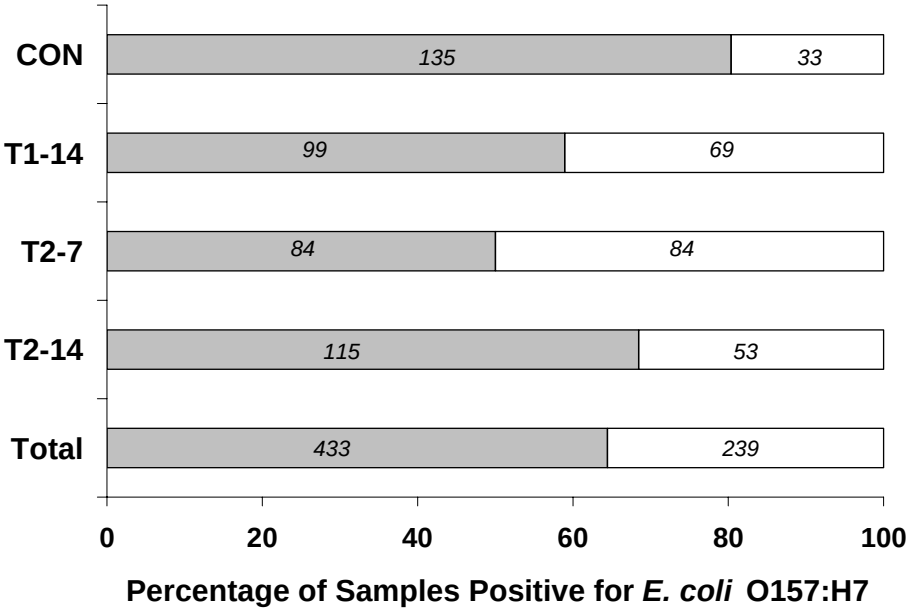

Figure 4. Effect of including a seaweed extract (Tasco®) in a barley-based finishing diet on fecal shedding of *E. coli* O157:H7 by experimentally inoculated steers. Thirty two steers were dosed orally with 10^{10} cfu of *E. coli* O157:H7 on day 0. Beginning on d 7, their diets were supplemented with Tasco® at 0% (control, CON); at 1% for 14 d (T1-14); at 2% for 7 d (T2-7); or at 2% for 14 d (T2-14). Fecal samples collected from each steer (21 samples over 14 wk) were assessed for the presence of *E. coli* O157:H7 by enrichment and immunomagnetic separation. Numbers of culture-positive and culture-negative samples observed in each treatment group are marked on the bars above in the grey and white areas, respectively.

Tasco® is a proprietary product processed from the seaweed, *Ascophyllum nodosum*, which grows along the coastline between high and low tides of the North Atlantic Ocean, extending from Nova Scotia to Norway. Research has shown that supplementing cattle diets with Tasco® has beneficial effects on growth performance, carcass characteristics and shelf life of the beef (Allen et al. 2001). At Texas Tech University, supplementing finishing diets with Tasco® (at 2% of DM) for two weeks prior to slaughter reduced the incidence of

naturally occurring *E. coli* O157:H7 in the feces and on the hides of the cattle (Braden et al. 2004). We have also found that Tasco decreased the prevalence of shedding in cattle challenged with *E. coli* O157:H7 (Figure 4). Studies to assess the effectiveness of Tasco® for reducing the prevalence of *E. coli* O157:H7 upon entry of cattle to the feedlot are presently in progress.

■ Conclusion

There are few absolutes concerning the ecology of *E. coli* O157:H7 in herds of cattle, because of the complexity of the interactive effects of farm management, animal behaviour, environment factors and climate. That the risk of shedding *E. coli* O157:H7 is influenced by season, and that shedding is increased in immature as compared to mature cattle, are largely undisputed, and the study described herein also supports these rules of thumb. The present study also suggests that poor sanitary conditions and frequent mixing of animals can lead to an increased prevalence of *E. coli* O157:H7. There is some evidence that seasonal peaks in somatic cell counts may correspond to the degree of pathogen prevalence across dairies. Adherence to standard practices for proper hygiene may reduce the prevalence of *E. coli* O157:H7 until such time that more directed methods for mitigation of this pathogen are commercially available.

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