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VETERINARY CONTINUING EDUCATION

ST. PAUL, MINNESOTA
UNITED STATES OF MINNESOTA

Journal of Food Protection, Vol. 69, No. 2, 2006, Pages 252–259
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Prevalence and Characterization of *Escherichia coli* O157 Isolates from Minnesota Dairy Farms and County Fairs

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MS 05-312: Received 30 June 2005/Accepted 19 September 2005

ABSTRACT

Samples were collected from 26 organic and conventional farms and 12 county fairs in Minnesota during 2001 and 2002 to identify the presence of *Escherichia coli* O157. Immunomagnetic separation was used for isolation of *E. coli* O157. Isolates were further characterized by the presence of virulence marker genes (stx_1, stx_2, *eaeA, E-hly, katP, etpD,* and *espP*), antimicrobial susceptibility profiles, and genotypes. During 2001, *E. coli* O157 was isolated from 16 (5.2%) of 305 fecal samples and from 7 (36.8%) of 19 farms. During 2002, *E. coli* O157 was isolated from 6 (4.5%) of 132 fecal samples from weaned calves at 4 (23.5%) of 17 farms. During 2001 and 2002, cattle manure samples were collected from 12 county fairs, and *E. coli* O157 was isolated from 19 (11%) of 178 samples and 9 (75%) of 12 county fairs. Among 40 *E. coli* O157 isolates, 17 isolates (43%) had both the stx_1 and stx_2 genes, and 21 strains (53%) had the stx_2 gene only. Thirteen percent of O157 isolates were resistant to tetracycline, and 25% were resistant to sulfadimethoxine. Heterogeneity of *E. coli* O157 strains was demonstrated by the presence of 22 different pulsed-field gel electrophoresis (PFGE) patterns. Four PFGE patterns matched those of isolates previously found in humans. The presence of *E. coli* O157 at county fairs suggests the potential for transmission to the public, who may have contact with cattle or their environment.

Domestic ruminants, especially cattle, have been implicated as the major reservoir of *Escherichia coli* O157:H7, which can cause severe hemorrhagic diarrhea and acute kidney failure in children *(1, 7, 8)*. *E. coli* O157:H7 was first recognized as a cause of illness during an outbreak in 1982 traced to contaminated beef patties *(42)*. Since then, undercooked ground beef is considered to be a common source of infection *(13, 49, 50)*. Ground beef, however, is not the only source of infection. Outbreaks associated with other contaminated foods (e.g., vegetables or unpasteurized milk or apple cider), contaminated water, or direct contact with animals have been reported *(6, 9, 25, 28, 51)*.

The Centers for Disease Control and Prevention estimates that *E. coli* O157 causes 73,000 illnesses and 61 deaths annually in the United States *(33)*. Minnesota consistently has one of the highest reported rates of human *E. coli* O157 infection. There also appear to be regional differences in human O157 incidence, which may be due to site-specific physician and laboratory practices or other regional factors *(5)*. One regional factor may be the number of cattle that can serve as reservoirs of human infection through direct cattle-human contact (e.g., petting zoos, farms, and county fairs) or environmental contamination (e.g., water or crops). Minnesota has the fifth largest number of dairy cows in the United States *(53)*.

Some researchers have speculated that products from organic farms may be at greater risk of microbial contamination than those from conventional farms because of differing farming practices; however, organic farms theoretically should have lower prevalences of antimicrobial-resistant organisms and pesticide residues *(35, 43)*. The U.S. organic food industry grew 20% in 2003, with estimated consumer sales of $10.8 billion. According to the Organic Trade Association 2004 manufacturer survey, the sale of organic dairy products increased 20% during 2003 and accounted for 13% of the organic foods sold *(40)*. Clearly, this increase in consumer demand makes it important to evaluate the safety of organic products.

Recent outbreaks associated with animal exhibits have documented the potential risk to the public who may have contact with ruminants or their environment *(4, 30)*. Because of this concern, public health authorities have devised guidelines to prevent such infections *(37)*. These outbreaks are often associated with *E. coli* O157 because of its low infectious dose and its ability to survive in the environment. Investigators recently highlighted the association between county fair attendance and *E. coli* O157 infection and suggested that attendance at agricultural fairs may contribute to the seasonal summer peak in disease incidence in the United States *(14)*.

To date, little work has been done in Minnesota to document the occurrence of *E. coli* O157 on farms or other areas where animals are kept. The objectives of this study were to determine the prevalence of *E. coli* O157 at some

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TABLE 1. *PCR primers and conditions*

			PCR conditions[a]				
Primer	Nucleotide sequences (5′ to 3′)	Gene	Denature time at 94°C (s)	Annealing	Extension time at 72°C (s)	Product size (bp)	Reference
LP30	CAGTTAATGTGGTGGCGAAGG	*sltI*	60	60°C, 60 s	60	348	*12*
LP31	CACCAGACAATGTAACCGCTG						
LP43	ATCCTATTCCCGGGAGTTTACG	*sltII*	60	60°C, 60 s	60	584	*12*
LP44	GCGTCATCGTATACACAGGAGC						
AE19	CAGGTCGTCGTGTCTGCTAAA	*eaeA*	60	60°C, 60 s	60	1,087	*21*
AE20	TCAGCGTGGTTGGATCAACCT						
Hly A1	GGTGCAGCAGAAAAAGTTGTAG	*hlyA*	40	55°C, 90 s	100	1,551	*44*
Hly A4	TCTCGCCTGATAGTGTTTGGTA						
wkat-B	CTTCCTGTTCTGATTCTTCTGG	*katP*	40	55°C, 90 s	150	2,125	*10*
wkat-F	AACTTATTTCTCGCATCATCC						
D1	CGTCAGGAGGATGTTCAG	*etpD*	40	52°C, 90 s	80	1,062	*45*
D13R	CGACTGCACCTGTTCCTGATTA						
esp-A	AAACAGCAGGCACTTGAACG	*espP*	40	56°C, 60 s	150	1,830	*11*
esp-B	GGAGTCGTCAGTCAGTAGAT						

[a] Final extension was carried out at 72°C for 10 min.

of Minnesota's organic and conventional dairy farms and county fairs and to characterize *E. coli* O157 isolates based on the distribution of virulence factor genes, antimicrobial resistance profiles, and molecular subtypes.

MATERIALS AND METHODS

Sample collection. Samples were collected from 26 Minnesota dairy farms (19 farms in 2001 and 17 farms in 2002). Enrolled farms were part of a separate multistate study, which has been previously described *(19)*. Farms in the present study were limited to those from the larger multistate study that were located in Minnesota and from which samples were collected on or after April 2001. Of the 26 farms, 8 were organic and 18 were conventional. Organic farms had to be certified as organic by a recognized certification agency. A subset of 8 to 16 fecal samples was randomly selected for *E. coli* O157 testing from 25 to 55 samples collected per visit from herds during April through October 2001. Fecal samples were collected by rectal retrieval from cows and calves using separate gloves for each sample. In addition, 5 to 10 fecal samples from weaned calf pens and milk filters were also collected in 2002. Demographic information (i.e., animal inventory, rolling herd average, somatic cell count, average bacterial count, housing facility, and participation in the Dairy Herd Improvement Association) from each dairy herd was collected through the use of pretested questionnaires filled out during personal interviews. An initial questionnaire collected detailed information about herd management practices, and at each sampling visit a shorter questionnaire was administered to capture information more likely to change over time, such as herd additions or changes in rations or feeding practices.

Fecal samples were collected from cattle manure piles at 12 county fairs in Minnesota during the summers of 2001 and 2002. County Fair Boards were contacted to seek permission and to identify days when cattle were being shown. Five to 10 samples were collected from areas designated for manure disposal using a grid system to allow for random sample collection.

Isolation of *E. coli* O157 by immunomagnetic separation. One gram of each fecal sample collected was diluted and mixed in tryptic soy broth containing novobiocin (20 μg/ml). After 6 h of incubation at 37°C, 500 μl of culture was mixed with 10 μl of magnetic beads coated with antibodies against *E. coli* O157 (Dynal, Oslo, Norway) for 20 min *(16)*. The samples were placed into a magnetic rack and washed three times with a washing solution (10 g/liter peptone, 5 g/liter NaCl, 3.5 g/liter Na_2HPO_4, and 1.5 g/liter NaH_2PO_4, pH 7.2). The immunomagnetic beads were suspended in 100 μl of the washing solution and spread plated onto CHROMagar O157 (CHROMagar Microbiology, Paris, France), supplemented with tellurite (2.5 mg/liter), and incubated at 37°C overnight. Typical colonies were tested with a O157:H7 latex agglutination assay (RIM *E. coli* O157:H7 latex test, Remel, Lenexa, Ks.). API 20E kits (bioMérieux, Hazelwood, Mo.) were used to confirm *E. coli* strains. All positive agglutination test isolates were tested for O157 confirmation by PCR based on the presence of specific genes, including the *uidA*, rfb_{O157}, and $fliC_{H7}$ genes.

Identification of virulence factor genes. For each agglutination-positive isolate, a PCR using a Robocycler thermal system (Stratagene, Inc., La Jolla, Calif.) was performed to detect virulence marker genes using specific primers for Shiga-like toxin (stx_1 and stx_2), *E. coli* attaching and effacing (*eae*), enterohemorrhagic *E. coli* (*hlyA*), catalase peroxidase (*katP*), type II secretion system (*etpD*), and serine protease (*espP*). The PCR primers and conditions are described in Table 1.

Chromosome-encoded virulence genes (stx_1, stx_2, and *eae*) and plasmid encoded virulence genes (*hlyA, katP, etpP,* and *espP*) were detected in a total 50-μl reaction volume containing a 2.0 mM concentration of deoxynucleoside triphosphates, a 1 μM concentration of each primer, 5 μl of 10× PCR buffer, and 1.25 U of *Taq* DNA polymerase (Promega, Madison, Wis.) with addition of 2 μl of DNA extracts from the supernatant of bacterial cell suspension after centrifugation.

Antimicrobial susceptibility testing. All isolates were submitted to the Minnesota Veterinary Diagnostic Laboratory at the University of Minnesota for antimicrobial susceptibility testing. MICs were measured using the Sensititre Susceptibility System (Trek Diagnostic Systems Inc., Cleveland, Ohio). The following 18 antibiotics were included on the antimicrobial susceptibility panel: amikacin, amoxicillin–clavulanic acid, ampicillin, cefazolin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, enrofloxacin, gentamicin, imipenem, orbifloxacin, spectinomycin, sulfadimethoxine, tetracycline, ticarcillin, ticarcillin–clavulanic acid, and

TABLE 2. *Demographic characteristics of farms*

Factors		Organic		Conventional		Total	
		No.	%	No.	%	No.	%
Herds		8	30.8	18	69.2	26	100
Average number of milking cows per herd		36.9		129.2		100.8	
Rolling herd average (lb)[a]	Mean	14,722 (6,684)		20,853 (9,467)		18,967 (8,611)	
	Minimum	12,000 (5,448)		16,000 (7,264)		12,000 (5,448)	
	Maximum	16,311 (7,405)		26,271 (11,927)		26,271 (11,927)	
Somatic cell count (CFU/ml) (average from bulk tank during the last 6 mo)	100,000–199,999	0	0.0	2	11.1	2	7.7
	200,000–299,999	3	37.5	8	44.4	11	42.3
	300,000–399,999	2	25.0	4	22.2	6	23.1
	400,000–499,999	3	37.5	3	16.7	6	23.1
	≥500,000	0	0.0	1	5.6	1	3.8
Average bacterial count (CFU/ml) (milk shipped during the last 6 mo)	<5,000	2	25.0	6	33.3	8	30.8
	5,000–9,999	3	37.5	4	22.2	7	26.9
	10,000–19,999	3	37.5	5	27.8	8	30.8
	20,000–29,999	0	0.0	2	11.1	2	7.7
	30,000–39,999	0	0.0	1	5.6	1	3.8
Barn type	Tie stall only	8	100.0	8	44.4	16	61.5
	Free stall only	0	0.0	9	50.0	9	34.6
	Both tie or free stall	0	0.0	1	5.6	1	3.8
Participation in the Dairy Herd Improvement Association	Yes	5	62.5	18	100.0	23	88.5
	No	3	37.5	0	0	3	11.5

[a] Kilogram values are given in parentheses.

trimethoprim-sulfamethoxazole. This susceptibility panel mirrors the human and animal susceptibility panel used by the National Antimicrobial Resistance Monitoring System *(36)*. Established antimicrobial susceptibility cutoffs followed breakpoints recommended by the NCCLS *(38)*. However, no NCCLS cutoff was available for ceftiofur and sulfadimethoxine. Cultures with MIC values of ≥8 and ≥128 were considered resistant to ceftiofur and sulfadimethoxine, respectively.

PFGE. PulseNet's 1-day (24- to 48-h) standardized laboratory protocol for pulsed-field gel electrophoresis (PFGE) was used with slight modifications as previously described *(3)*. Bacterial strains were grown overnight on tryptic soy agar plates at 37°C. Bacterial colonies were suspended in cell suspension buffer (100 mM Tris and 100 mM EDTA, pH 8.0) and adjusted to an optical density of 1.3 to 1.4 using a spectrophotometer (Bio-Rad Laboratories, Hercules, Calif.). A 400-μl aliquot of adjusted cell suspension was mixed with 20 μl of proteinase K (20 mg/ml stock) and an equal volume (400 μl) of melted 1% SeaKem Gold agarose (BioWhittaker, Rockland, Maine) containing 1% sodium dodecyl sulfate, and the mixture was carefully dispensed into appropriate wells of a reusable plug mold (Bio-Rad). After solidification, the plugs were transferred to a round-bottom tube containing 1.5 ml of cell lysis buffer (50 mM Tris HCl, 50 mM EDTA, pH 8.0, and 1% sarcosine) and 0.5 mg/ml proteinase K. Cells were lysed in a 54°C water bath for 2 h with constant and vigorous agitation at 175 to 200 rpm. After lysis, the plugs were washed twice with preheated sterile distilled water and four times with preheated TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) for 10 to 15 min per wash at 50°C with the same agitation. Plugs were stored in 2 ml of TE buffer at 4°C until restriction digestion of DNA. DNA in agarose plugs was digested with a 50 U per sample concentration of *Xba*I (Promega) for at least 2 h in a 37°C water bath. The plugs were loaded into the wells in a 1% pulsed-field certified agarose gel. DNA restriction fragments were separated with a CHEF Mapper (Bio-Rad) with pulse times of 5 to 50 s at 14°C for 22 h in 0.5× Tris-borate-EDTA buffer. The gel was stained with ethidium bromide (Sigma-Aldrich, St. Louis, Mo.), and restriction fragment patterns were photographed with a UV transilluminator. *E. coli* G5244 strains were used as a standard control.

Data analysis. The PROC FREQ chi-square analysis (or Fisher's exact test) and PROC TTEST were used to identify significant differences in herd characteristics and prevalence of *E. coli* O157 between organic and conventional dairy farms. The PC SAS system for Windows was used for all comparisons (SAS version 8.2, SAS Institute Inc., Cary, N.C.).

RESULTS

Farm demographics. Organic farms had significantly fewer milking cows (average of 37 cows) than did conventional operations (129 animals) ($P < 0.01$). The mean rolling-herd average was 14,722 and 20,853 lb per cow per year (6,684 and 9,467 kg per cow per year) on organic and conventional farms, respectively ($P < 0.01$). Five (62.5%) of eight organic farms and 8 (44.5%) of 18 conventional farms had somatic cell counts higher than 300,000 cells per ml (Fisher's exact test, $P = 0.34$). All eight organic farms and 9 (50%) of 18 conventional farms had tie-stall housing (Fisher's exact test, $P = 0.02$). All conventional herds were affiliated with the Dairy Herd Improvement Association, but only five (62.5%) of eight organic herds were affiliated (Fisher's exact test, $P = 0.02$) (Table 2).

TABLE 3. *Prevalence of* E. coli *O157 on organic and conventional dairy farms*

	2001				2002[a]			
Farm type	No. of farms	No. (%) of positive farms	No. of samples	No. (%) of positive samples	No. of farms	No. (%) of positive farms	No. of samples	No. (%) of positive samples
Organic	7	4 (57.1)	136	10 (7.4)	5	2 (40.0)	30	4 (13.3)
Conventional	12	3 (25.0)	169	6 (3.6)	12	2 (16.7)	102	2 (2.0)
Total	19	7 (36.8)	305	16 (5.2)	17	4 (23.5)	132	6 (4.5)

[a] Calf pen samples.

Detection of *E. coli* O157 on dairy farms. During 2001, *E. coli* O157 strains were isolated from 16 (5.2%) of 305 fecal samples from 7 (36.8%) of 19 dairy farms (Table 3). During 2002, *E. coli* O157 was isolated from 6 (4.5%) of 132 pen samples from 4 (23.5%) of 17 dairy farms. No *E. coli* O157 was isolated from 22 milk filters collected from 15 dairy farms. Overall, 14 *E. coli* O157 isolates (8.4%) were recovered from 166 samples from organic farms versus 8 (3.0%) of 271 samples from conventional farms. *E. coli* O157 was found on four (50%) of eight organic farms and 3 (16.7%) of 18 conventional farms (Fisher's exact test, $P = 0.15$).

Detection of *E. coli* O157 at county fairs. Fifteen county fairs were visited during 2001 ($n = 4$) and 2002 ($n = 11$), representing 12 separate county fairs. Three county fairs were visited in both 2001 and 2002. *E. coli* O157 was isolated from 19 (10.7%) of 178 cattle manure pile samples and 9 (75%) of 12 county fairs. Of the 19 isolates, 3 were isolated during summer 2001 and 16 were from summer 2002.

Molecular and antimicrobial susceptibility characteristics of *E. coli* O157 isolates. Twenty-one (53%) of 40 *E. coli* O157 isolates possessed only the stx_2 gene, and 17 isolates (43%) had both stx_1 and stx_2 (Fig. 1). One isolate possessed only the stx_1 gene. All isolates were serotype O157:H7, with the exception of one O157:H12. The genes *eaeA, hlyA,* and *etpD* were detected from all *E. coli* O157:H7 isolates. No virulence factors were detected from the *E. coli* O157:H12 isolate. There was no difference in Shiga toxin type of isolates obtained from county fairs and organic and conventional farms.

Ten (25%) of 40 *E. coli* O157 isolates were resistant

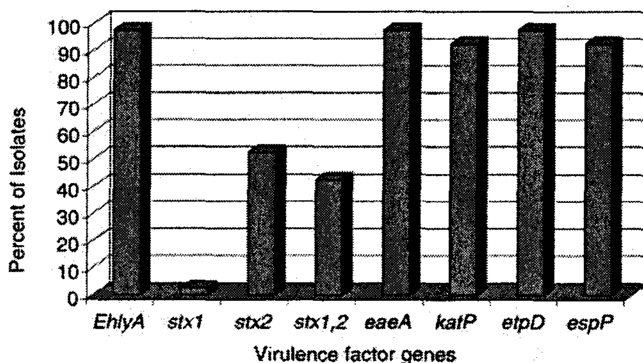

FIGURE 1. *Percentage of virulence factor genes from* E. coli *O157 isolates (*n *= 40).*

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to sulfadimethoxine, including five (13%) isolates that were also resistant to tetracycline. Intermediate resistances were observed for sulfadimethoxine (25 isolates), spectinomycin (6), chloramphenicol (2), cefoxitin (2), and cephalothin (1). All isolates were susceptible to the remaining 12 antimicrobials on the panel. There were no observed differences between organic and conventional farms in the resistance profiles of the isolates.

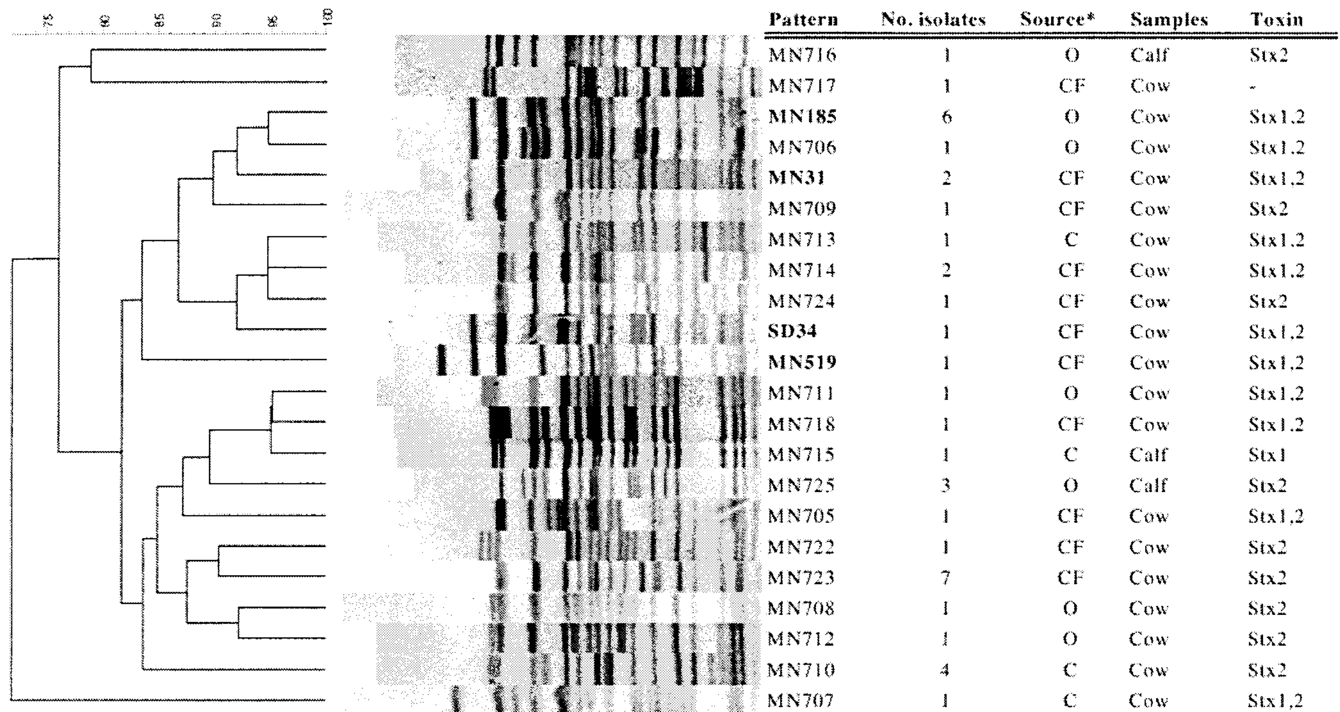
PFGE pattern analysis. From the 40 *E. coli* O157 isolates, 22 different subtype patterns were identified (Fig. 2). Four subtype patterns of *E. coli* O157:H7 were indistinguishable from patterns of isolates from human *E. coli* O157:H7 infection cases previously identified by the Minnesota Department of Health. Eleven different subtypes were identified from the 21 isolates from eight dairy herds. Four of the eight dairy herds harbored multiple isolates (range, two to eight isolates per farm). For those dairy herds with multiple isolates, two or three different subtype patterns were found. An indistinguishable subtype pattern was identified from two different farms.

Eleven different subtypes were identified from the 19 isolates from nine county fairs. Multiple isolates were identified at five of nine county fairs. The number of isolates ranged from two to seven, with one or two different patterns identified per county fair. An indistinguishable subtype pattern was identified from two different county fairs. No indistinguishable PFGE patterns were observed between county fairs and dairy farms.

DISCUSSION

This study was unique in that the occurrence of *E. coli* O157 on organic versus conventional dairy farms in Minnesota was compared. To date, little information has been available on the prevalence of *E. coli* O157 on organic dairy farms. Among our 26 enrolled dairy farms, organic herds were smaller, were less likely to be affiliated with the Dairy Herd Improvement Association, tended to house cattle in tie stalls, and had a lower rolling herd average compared with conventional herds. The enrolled herds were a subset of herds from a larger multistate study in which similar differences between organic and conventional farms were found *(57)*. Farm management practices, such as those employed in organic farming, may have an impact on fecal shedding of *E. coli* O157. More work, however, is needed to identify farm-specific factors associated with shedding of these bacteria.

Our findings document the presence of *E. coli* O157 in



Pattern	No. isolates	Source*	Samples	Toxin
MN716	1	O	Calf	Stx2
MN717	1	CF	Cow	-
MN185	6	O	Cow	Stx1,2
MN706	1	O	Cow	Stx1,2
MN31	2	CF	Cow	Stx1,2
MN709	1	CF	Cow	Stx2
MN713	1	C	Cow	Stx1,2
MN714	2	CF	Cow	Stx1,2
MN724	1	CF	Cow	Stx2
SD34	1	CF	Cow	Stx1,2
MN519	1	CF	Cow	Stx1,2
MN711	1	O	Cow	Stx1,2
MN718	1	CF	Cow	Stx1,2
MN715	1	C	Calf	Stx1
MN725	3	O	Calf	Stx2
MN705	1	CF	Cow	Stx1,2
MN722	1	CF	Cow	Stx2
MN723	7	CF	Cow	Stx2
MN708	1	O	Cow	Stx2
MN712	1	O	Cow	Stx2
MN710	4	C	Cow	Stx2
MN707	1	C	Cow	Stx1,2

FIGURE 2. *Dendrogram of* E. coli *O157 isolates based on pulsed-field gel electrophoresis (PFGE). Bold type represents PFGE patterns previously identified in human isolates. * O, organic farm; C, conventional farm; CF, Minnesota county fair.*

cattle manure piles at county fairs. A similar finding was previously reported in a multistate study of 32 agricultural fairs in the United States, in which *E. coli* O157 was isolated from 13.8% of beef cattle, 5.9% of dairy cattle, 5.2% of sheep, and 7.1% of the pest fly pools *(27)*. The presence of *E. coli* O157 from areas of likely public contact in an agricultural fair environment highlights the importance for county and state fair officials to take preventive measures during fair exhibitions through appropriate signage, handwashing facilities, education, and other measures as recommended by the National Association of State and Public Health Veterinarians *(37)*.

Herd prevalence of *E. coli* O157:H7 in dairy cattle appears to be highly variable, and this variability may be associated with region, season, and detection methods. The herd prevalence in the present study was 37% in 2001 and 24% in 2002. In 1996, before the advent of current culture methods (i.e., immunomagnetic separation), a national dairy survey identified *E. coli* O157 on 24% of the dairy operations *(22)*. Using immunomagnetic separation, LeJeune and Kauffman *(31)* recently reported that the herd prevalence of dairy farms in Ohio was highly variable at different sampling periods for the same group of farms, ranging from 0 to 60% per sampling period. In studies conducted in Europe and North America using immunomagnetic separation, herd prevalences ranging from 7 to 38% have been reported *(15, 17, 39, 47)*.

PFGE subtyping patterns, antibiotic susceptibility profiles, and virulence gene profiles can be used to characterize the clonal diversity of *E. coli* O157 isolates. However, there is limited information about the genetic diversity of *E. coli* O157:H7 in various animal and farm environments *(23, 24, 54)*. In these few studies, genotypic diversity of *E. coli* O157:H7 isolates was demonstrated *(2, 32)*. We also observed diverse and heterogenous strains of *E. coli* O157, as indicated by PFGE subtypes. No common strains between farms and county fairs in the same county were detected. This result supports the hypothesis that there are many strains, some of which may be farm specific. However, some strains appear to be more widespread, as indicated by indistinguishable isolates from human and animal sources. PFGE subtyping has been used to assist in the identification of outbreak sources and transmission routes and in the development of intervention strategies for the prevention of infection by foodborne pathogens. Subtyping methods, however, have not yet been fully integrated or utilized to understand the movement of enteric pathogens from farm to table. To improve surveillance, more comprehensive databases need to be established to include animal, human, food, and environmental sources *(32)*. More linking of databases may clarify the contribution of different animal, food, and environment sources to human illness.

All *E. coli* O157 isolates had the H7 antigen, except one strain that had the H12 antigen. Unlike the typical sorbitol-negative O157:H7 isolates, this unique H12 serotype was sorbitol positive and had no identified virulence factors. The commonality of *E. coli* O157 strains with no virulence factors from cattle feces is largely unknown. It is unclear whether these strains are evolutionary precursors or mutations of the predominant O157:H7 strains.

Antibiotic-resistant *E. coli* O157 strains were not common in our study. An increase in the antimicrobial resistance of *E. coli* O157 has been reported in various geographic areas of the world *(18, 20, 29, 34, 46, 48, 56)*. Antibiotics have been used for disease prevention and growth promotion in animal production and for treatment of sick animals. Antibiotic use can result in development of antibiotic resistance, especially among certain bacterial species in the intestinal tracts of food animals. Resistant bacteria from food animals might enter human populations through the food chain and pose a risk to the public *(48, 55)*.

In the present study, 13 and 25% of *E. coli* O157 isolates from cattle were resistant to tetracycline and sulfadimethoxine, respectively. These findings are similar to those reported in previous studies *(34, 48)*. No multidrug-resistant strains (resistance to five or more antimicrobials) were identified. Meng et al. *(34)* noted that 24% of *E. coli* O157 isolates from animal, foods, and humans were resistant to at least one antibiotic and 19% were resistant to three or more antibiotics. More than 70% of the resistant strains were resistant to streptomycin, sulfasoxazole, and tetracycline *(34)*. In a National Antimicrobial Resistance Monitoring System study, 7% of *E. coli* O157 isolates were resistant to only one drug and 6% were resistant to multiple drugs *(26)*. Resistance to sulfamethoxazole (10%), tetracycline (4%), or streptomycin (2%) were most common *(26)*. Because food animals such as ruminants are considered the primary reservoir of *E. coli* O157, these bacteria can serve as important indicators for the development of antimicrobial-resistant strains that can be spread in meat products.

Data from this study provide initial information on the antimicrobial resistance profiles of *E. coli* O157 isolates in Minnesota. Periodic sampling is recommended to monitor changes over time. We hypothesized that isolates from farms with different management practices (i.e., organic versus conventional farms) have different antimicrobial resistance patterns. This hypothesis was not supported by the data collected, but these findings may reflect the small sample size or other multifactorial causes for antimicrobial resistance, including pathogen-specific factors.

Our findings document the prevalence of *E. coli* O157 on Minnesota dairy farms and county fairs. However, these findings still do not elucidate the reasons for the high reported incidence rates of human *E. coli* O157 illness in Minnesota *(5)*. The majority of human illnesses are currently attributed to consumption of undercooked ground beef *(41)*. Cull dairy cows account for approximately 17% of the ground beef in the United States *(52)*, and because Minnesota currently ranks fifth in milk production, many cull dairy cattle are processed as ground beef. *E. coli* O157 was also isolated more frequently from organic farms than from conventional farms. Some currently unexplored management factors may play a role in pathogen shedding, exposure, or reexposure on organic farms. Other findings include the identification of common virulence genes among isolates, the lack of antibiotic-resistant isolates, and the genetic heterogeneity of isolates from participating farms.

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ACKNOWLEDGMENTS

This study was supported by grants from the Academic Health Center Faculty Research and Department Program (FRD 00-07) at the University of Minnesota and from the U.S. Department of Agriculture Cooperative State Research Education and Extension Service National Research Initiative (Epidemiological Approaches of Food Safety, award 99-35212-8563). We extend special thanks to Vivian Mai and Peter Gillis (Department of Food Science and Nutrition, University of Minnesota, St. Paul) and Fe Leano and Dave Boxrud (Minnesota Department of Health) for their technical assistance.

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