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Characterization of *Escherichia coli* O157:H7 from Downer and Healthy Dairy Cattle in the Upper Midwest Region of the United States

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While cattle in general have been identified as a reservoir of *Escherichia coli* O157:H7, there are limited data regarding the prevalence and clonality of this pathogen in downer dairy cattle and the potential impact to human health that may occur following consumption of meat derived from downer dairy cattle. In the present study, conducted at two slaughter facilities in Wisconsin between May and October of 2001, we established a higher prevalence of *E. coli* O157:H7 in fecal and/or tissue samples obtained aseptically from intact colons of downer dairy cattle (10 of 203, 4.9%) than in those from healthy dairy cattle (3 of 201, 1.5%). Analyses of 57 isolates, representing these 13 positive samples (one to five isolates per sample), by pulsed-field gel electrophoresis, revealed 13 distinct *Xba*I restriction endonuclease digestion profiles (REDP). Typically, isolates from different animals displayed distinct REDP and isolates from the same fecal or colon sample displayed indistinguishable REDP. However, in one sample, two different, but highly related, REDP were displayed by the isolates recovered. Antimicrobial susceptibility testing indicated that 10 of the 57 isolates, recovered from 2 (1 downer and 1 healthy animal) of the 13 positive samples, were resistant to at least 1 of 18 antimicrobials tested. However, there was no appreciable difference in the frequency of resistance of isolates recovered from downer and healthy dairy cattle, and not all isolates with the same REDP displayed the same antimicrobial susceptibility profile. Lastly, it was not possible to distinguish between isolates recovered from downer and healthy cattle based on their *Xba*I REDP or antimicrobial susceptibility. These results indicate that downer cattle had a 3.3-fold-higher prevalence of *E. coli* O157:H7 than healthy cattle within the time frame and geographic scope of this study.

Over the past 20 years, *Escherichia coli* O157:H7 has emerged as a pathogen of significant public health concern in the United States, causing an estimated 73,000 cases of infection and 61 deaths per year (24, 35). Although person-to-person transmission has been documented, transmission of *E. coli* O157:H7, in most cases, occurs through contaminated food or water, with the consumption of raw or undercooked foods of bovine origin being the most common route of food-borne infection (25, 30, 39). Indeed, cattle are a primary reservoir of this organism worldwide (7). Numerous studies (2, 4, 7, 11, 16) have revealed that the organism is common in both dairy and beef herds, with a prevalence of up to 75% in dairy herds (17) and 63% in beef herds (18). The prevalence for individual animals within herds in North America and Europe is estimated at 1.8 to 16%, with levels as high as 36% being reported (2, 8, 32). Due to the ubiquity of *E. coli* O157:H7 among cattle, as well as its low infective dose and the severity of the resultant illness in humans, effective control of the pathogen may be possible only by eliminating this microorganism at its source rather than by relying on proper food handling and cooking thereafter. The development of intervention strategies at the farm level is largely dependent on acquiring a better understanding of the ecology of *E. coli* O157:H7 strains and the factors that facilitate their survival and dissemination. Ecological data may also help determine whether the extent of colonization of cattle is related to any specific farming or husbandry practices. If such relationships were identified, modifications could be made to reduce the herd and/or animal prevalence of serotype O157:H7 strains of *E. coli* associated with cattle.

Despite numerous investigations of cattle (2, 8, 17, 18, 32), there are limited data regarding the prevalence, clonality, and ecology of *E. coli* O157:H7 in dairy cattle with downer cow syndrome, or downer cattle. The term “downer cattle” refers to cattle that are suffering from assorted maladies, such as mastitis, calving paralysis, and milk fever, and/or injuries incurred during transport which render them immobile to various degrees (3, 9) and which may require the administration of antimicrobials as treatment (1). If their condition does not improve, these cattle are removed, or culled, from the production herd (11, 34, 37). Culled dairy cows account for approximately 17% of the ground beef produced in the United States (37, 38). Therefore, downer dairy cattle harboring *E. coli* O157:H7 at slaughter may be an important source of contamination and

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may contribute appreciably to the health risk associated with ground beef. Given the frequent association of *E. coli* O157:H7 with cattle and ground beef, as well as the use of downer and dairy cattle for ground beef production, the present study was conducted to gain insight on the comparative prevalence and clonality of this pathogen in downer compared to healthy dairy cattle in the upper Midwestern region of the United States. Also, since downer dairy cattle may receive antimicrobials as treatment for various maladies, and since it is generally accepted that the frequent use of antimicrobials in animal husbandry is implicated in accelerating the development of pathogens that are resistant to such antimicrobials, another objective of the present study was to establish the comparative susceptibility of isolates recovered from downer and healthy animals to a panel of antimicrobials.

(Portions of this research were presented at the Annual Meeting of the International Association for Food Protection, 30 June to 3 July 2002, San Diego, Calif. [C. M. Byrne, I. Erol, J. E. Call, D. Buege, C. W. Kaspar, C. Hiemke, P. Fedorka-Cray, J. Hermosillo, T. Ball, A. K. Benson, F. M. Wallace, M. Handy, and J. B. Luchansky, Abstr. Annu. Meet. Int. Assoc. Food Prot., abstr. 136, p. 92, 2002].)

MATERIALS AND METHODS

Sample design and collection. This study was conducted at two beef processing facilities, designated plant A and plant B, in Wisconsin between May and October of 2001. Plant A (four visits) primarily processed healthy dairy cattle, that is, cattle with no obvious maladies, while plant B (seven visits) primarily processed dairy cattle with downer cow syndrome. At plant A, attempts were made to collect samples from healthy cows rather than from steers, whereas at plant B all samples collected from downer cattle were from cows. Both plants received cattle primarily from three states (Illinois, Iowa, and Wisconsin) in the upper Midwest region of the United States. For a given visit, the downer cattle included animals, usually obtained by "collectors," from several farms. A collector is a business that transports animals for a farmer to slaughter for a fee. In contrast, for a given visit, healthy animals likely came from a common feedlot. At each visit to a plant, 21 to 65 samples, mostly fecal material but also some tissue material, were aseptically collected directly from intact colons for analyses, for a total of 201 and 203 fecal or tissue samples from downer and healthy cattle, respectively. Fecal or tissue samples (10 to 50 g) were obtained aseptically from intact colons by using sterile knives to open the colon. A sterile tongue depressor was then used to remove the colon contents and transfer them to a sterile receptacle. Samples were stored on ice before being transported to the laboratory and analyzed within 6 h of collection.

Microbiological analyses. Fecal and tissue samples were tested for *E. coli* O157:H7 by enrichment and immunomagnetic separation, according to the procedure of Chapman et al. (6), with the following modifications. Briefly, a 10-g sample of feces and/or colon tissue was homogenized and enriched in 90 ml of EC broth (Fisher Scientific, Pittsburgh, Pa.) containing novobiocin (20 μg/liter; Sigma Chemical Co., St. Louis, Mo.). The suspension was incubated at 37°C for 18 h, followed by immunomagnetic separation as follows: a 20-μl volume of anti-O157 immunomagnetic beads (anti-*E. coli* O157 Dynabeads; Dynal, Lake Success, N.Y.) was added to a microcentrifuge tube containing 0.5 ml of EC broth and 0.5 ml of phosphate-buffered saline (pH 7.2; Fisher) containing Tween 20 (0.05%; Sigma) (PBS-Tween 20), incubated at 25°C for 10 min on a platform rocker (60 cycles per min), and separated in a magnetic particle concentrator (MPC-S; Dynal). The immunomagnetic beads were washed three times in 1 ml of PBS-Tween 20. Fifty microliters of the bead suspension was then spread plated onto sorbitol MacConkey (Fisher) agar plates containing cefixime (0.5 mg/liter; Sigma) and potassium tellurite (2.5 mg/liter; Difco). After an 18-h incubation at 37°C, sorbitol-negative colonies were tested for the O157 and H7 antigens by latex agglutination with a RIM *E. coli* O157:H7 test kit (Remel, Lenexa, Kans.). Sorbitol-negative, latex-positive colonies were biochemically confirmed as *E. coli* by using API 20E biochemical identification strips as directed by the manufacturer (bioMérieux Vitek Inc., Hazelwood, Mo.). Up to five confirmed serotype O157:H7 isolates from each positive sample were retained for further characterization.

Molecular characterization via the PCR. Each isolate was further characterized for the presence of sequences encoding the somatic O157 antigen (rfb_{O157}), the H7 flagellar antigen ($fliC_{H7}$), Shiga toxin 1 (stx_1), Shiga toxin 2 (stx_2), the intimin protein (*eaeA*), and hemolysin (hly_{933}) by the PCR method essentially as described by Fratamico et al. (12). *E. coli* O157:H7 strain 933 was used as a positive control, while sterile, distilled, water was used as a negative control.

Molecular subtyping via PFGE. Each *E. coli* O157:H7 isolate was subtyped by the pulsed-field gel electrophoresis (PFGE) technique of contour-clamped homogeneous electric field (CHEF) electrophoresis as previously described (19). Genomic DNA was digested in agarose plugs with *Xba*I (Promega Corp., Madison, Wis.) as recommended by the manufacturer. The resulting DNA fragments were resolved by CHEF-PFGE with a CHEF-DRII apparatus (Bio-Rad Laboratories, Richmond, Calif.) at 200 V for 19 h at 14°C and switch times from 1 to 60 s. Low-range lambda concatemers (Promega) were used as DNA size standards. The fragments were visualized by using ethidium bromide (10 μg/ml; Bio-Rad) and short-wave UV light, and the image was captured by a Gel Doc 1000 system (Bio-Rad). The presence, absence, and similarity of restriction fragments were ascertained visually.

Susceptibility testing with Sensititre. All *E. coli* O157:H7 isolates were tested for susceptibility to 18 antimicrobials by using a custom-made panel on a semi-automated broth microdilution system (Sensititre; Trek Diagnostics, Westlake, Ohio) as per the manufacturer's instructions. The 18 antimicrobials used were amikacin, amoxicillin/clavulanic acid, ampicillin, apramycin, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, imipenem, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim/sulfamethoxazole. National Committee for Clinical Laboratory Standards guidelines (27, 28) were followed, and the antimicrobials were used in concentrations previously described by the National Antimicrobial Resistance Monitoring System (http://www.arru.saa.ars.usda.gov). The only exception was imipenem, which was used at 0.25 to 8.0 μg, with breakpoints of $\leq$4 μg/ml for sensitivity and $\geq$16 μg/ml for resistance.

Statistical analyses. Statistical differences between the prevalence of *E. coli* O157:H7 in downer and healthy cattle were determined with Student's t test. The percentages of downer and healthy cattle positive for *E. coli* O157:H7 were compared by calculating z values for proportions in independent samples (33).

RESULTS

Analyses of 404 fecal samples from dairy cattle at slaughter facilities in Wisconsin revealed that 13 samples contained *E. coli* O157:H7 and established a 3.3-fold-higher prevalence of this pathogen in downer cattle (10 of 203; 4.9%) than in healthy cattle (3 of 201; 1.5%) (Table 1). The observed difference was not statistically significant at a P value of 0.06. Regardless, a total of 57 isolates, 15 from healthy animals and 42 from downer animals, were retained for further analyses by selecting up to 5 isolates at random from each of the 13 positive samples. For the purposes of this study, isolates described herein with indistinguishable PFGE patterns, virulence genotypes, and antimicrobial susceptibility profiles recovered from the same sample were considered a single clone.

Analyses of these 57 isolates by PFGE revealed 13 distinct *Xba*I restriction endonuclease digestion profiles (REDP) (Table 1). With one exception, i.e., fecal samples 27 and 31 from 1 May 2001 that both displayed REDP 5, isolates from different animals displayed distinct REDP. In addition, isolates from the same fecal sample or animal typically displayed indistinguishable REDP, with the exception of isolates from sample 45, which displayed two different but highly related *Xba*I REDP. Highly related isolates showed only a one- or two-band difference among approximately 20 total *Xba*I fragments.

Characterization of the 57 *E. coli* O157 isolates by PCR revealed that all were positive for $fliC_{H7}$ and displayed four profiles based on the presence or absence of the virulence sequences tested as follows: (i) 38 of the 57 isolates (67%) had sequences for all four virulence genes tested, namely, Shiga

TABLE 1. Characteristics of *E. coli* O157:H7 isolates found in fecal and colon samples of cattle at slaughter

Plant (cattle type)	Collection date (mo/day/yr)	No. of *E. coli* O157:H7-positive fecal samples/ total (%)	Positive fecal sample no.	No. of isolates from positive sample[a]	MFS isolate no.[b]	PCR profile (virulence genes present)[c]	PFGE profile[d] (no. of isolates)	Antimicrobial susceptibility profile[e]
A (healthy)	05/08/01	2/33	19	5	724–728	stx_1, stx_2, *eae*, *hly*	1 (5)	Sulfa, Tet (MFS 724) Strep Sulfa, Tet (MFS 725–MFS 728)
			21	5	729–733	stx_1, stx_2, *eae*, *hly*	2 (5)	Pan-sensitive
	06/12/01	0/53						
	07/03/01	1/65	45	5	739–743	stx_1, stx_2, *eae*, *hly*	3 (4), 4 (1)	Pan-sensitive
	07/24/01	0/50						
Total		3/201 (1.5)						
B (downer)	05/01/01	2/25	27	1	744	stx_1, *eae*, *hly*	5 (1)	Pan-sensitive
			31	1	745	stx_1, stx_2, *eae*, *hly*	5 (1)	Pan-sensitive
	06/05/01	1/30	23	5	746–750	stx_1, *eae*, *hly*	6 (5)	Sulfa, Tet (MFS 746, MFS 747, and MFS 749) Strep, Sulfa, Tet (MFS 748 and MFS 750)
	06/19/01	1/21	20	5	751–755	stx_1, stx_2, *eae*, *hly*	7 (5)	Pan-sensitive
	07/17/01	1/33	28	5	761–763	stx_1, stx_2, *eae*	8 (5)	Pan-sensitive
					764–765	stx_1, stx_2, *eae*, *hly*		
	09/11/01	3/28	7	5	766–770	stx_1, stx_2, *eae*, *hly*	9 (5)	Pan-sensitive
			18	5	771–775	stx_1, stx_2, *eae*, *hly*	10 (5)	Pan-sensitive
			22	5	776–780	stx_1, stx_2, *eae*, *hly*	11 (5)	Pan-sensitive
	10/02/01	2/24	8	5	781–785	stx_2, *eae*, *hly*	12 (5)	Pan-sensitive
	10/09/01	0/42	11	5	786–790	stx_1, *eae*, *hly*	13 (5)	Pan-sensitive
Total		10/203 (4.9)						

[a] Number of isolates from one positive fecal/colon sample, following enrichment, plating, and analysis by PFGE.
[b] Internal isolate numbers from the Microbial Food Safety (MFS) Research Unit collection.
[c] Virulence factors tested for were Shiga toxin 1 and 2 (stx_1 and stx_2), intimin protein (*eae*), and hemolysin (*hly*).
[d] PFGE profile generated with *Xba*I.
[e] Strep, streptomycin; Sulfa, sulfamethoxazole; Tet, tetracycline.

toxin 1 (stx_1), Shiga toxin 2 (stx_2), intimin (*eaeA*) and hemolysin (*hly*); (ii) 11 of the 57 isolates (19%) had stx_1, *eaeA*, and *hly* sequences but not stx_2 sequences; (iii) 5 of the 57 isolates (9%) were missing stx_1 sequences but had the sequences for all of the other virulence factors tested; and (iv) 3 of the 57 isolates (5%) had all of the virulence sequences tested for, with the exception of *hly*. In general, isolates with the same REDP had the same virulence genes. There was one exception: two of the five isolates displaying REDP 8 (isolates MFS 764 and MFS 765) had the gene sequence for hemolysin, while the other three isolates (MFS 761, MFS 762, and MFS 763) did not.

The majority (47 of 57; 81%) of the isolates tested were sensitive to all 18 antimicrobials tested. However, 10 of 57 (18%) of these *E. coli* O157:H7 isolates (5 isolates from healthy cattle and 5 from downer cattle), representing two distinct *Xba*I REDP (one from healthy cattle and one from downer cattle), were resistant to at least 1 of the 18 antimicrobials tested. Indeed, 6 of these 11 isolates were resistant to three antimicrobials, sulfamethoxazole, streptomycin, and tetracycline. Not all isolates displaying a distinct *Xba*I REDP displayed the same antimicrobial resistance profile. For example, of the five isolates displaying REDP 1, four were resistant to sulfamethoxazole, streptomycin, and tetracycline while one was resistant to only sulfamethoxazole and tetracycline.

DISCUSSION

Serotype O157:H7 strains of *E. coli* remain a serious threat to public health and an appreciable economic burden to the food industry. Numerous studies have shown that cattle and food produced therefrom, particularly ground beef, are significant sources and vehicles of transmission of this pathogen. However, relatively little has been published on the recovery and clonality of *E. coli* O157:H7 strains from downer dairy cattle (8, 22, 35). Although precise numbers are not available, downer animals constitute about 1.5% of dairy cattle (15) and 20 U.S. Department of Agriculture-inspected plants slaughter at least 100 cows per day, most of which are downer animals (Robert E. Brewer, personal communication). The present study established an approximate 3.3-fold-higher prevalence of *E. coli* O157:H7 in downer dairy cattle (4.9%) than in healthy dairy cattle (1.5%) processed at two slaughter facilities during our sampling period. To our knowledge, this is the first investigation of the recovery and clonality of this pathogen in downer dairy cattle. In a similar study investigating *E. coli* O157:H7 in fecal pats in home pens of healthy cattle (0.24% positive samples among 12,718 total samples) versus hospital pens of sick cattle (0.29% positive samples among 3,144 total samples), Galland et al. (13) reported no significant difference in the prevalence of the pathogen between these two groups. Differences in experimental design and methodologies employed may be responsible for the differences in isolation frequency reported in the present study and the study by Galland and colleagues.

The recovery rate of about 1.5% for *E. coli* O157:H7 in healthy dairy cattle in the present study is in agreement with results reported for healthy dairy cattle in other studies conducted in the United States (2, 11, 13, 22, 38). For example, the

U.S. Department of Agriculture National Animal Health Monitoring System Dairy 1996 study reported an *E. coli* O157:H7 prevalence of 1.8% among 2,200 culled dairy cows at 97 markets across the United States (38). Similarly, McDonough et al. (22) reported a prevalence of 1.3% in culled dairy cows in New York State. However, other studies have reported much higher recovery rates of the pathogen in both beef and dairy cattle. For example, Chapman (8) reported a prevalence rate of 16% among 1,661 culled dairy cattle over a 1-year period. Similarly, Elder et al. (10) demonstrated an overall prevalence of enterohemorrhagic *E. coli* O157 (*E. coli* O157:H7 and O157:H$^-$/nonmotile) of 28% from feces (91 of 327 samples across 29 lots of animals) and 11% on hides (38 of 355 samples across 29 lots of animals) from fed cattle from 29 single sources (lots) presented for slaughter at processing plants in the Midwestern United States in July and August of 1999. The latter studies indicate that the overall prevalence of *E. coli* O157 (enterohemorrhagic *E. coli* O157:H7 and O157:H$^-$) in cattle may be significantly higher than prior estimates. This may be the result of improved enrichment and isolation procedures, notably immunomagnetic separation. Variations among studies may also be due to differences in the numbers of cattle sampled, the type of sample and how it was collected, and the age of the animals sampled, as well as in the patterns of *E. coli* O157:H7 shedding by animals, which are known to be intermittent (5). It may also be attributed, in part, to the experimental design and to seasonality (14). Fecal shedding of *E. coli* O157:H7 is more common in the warmer months of the year, namely, June to September (5, 7). Therefore, sampling plans that include only these times of the year will result in higher recovery rates than studies reporting average recovery rates, which may include results for colder months (14). Furthermore, single-time-point samples, such as those used in other studies, provide only limited information regarding shedding of organisms. The present study was conducted from May to October of 2001, when bovine fecal shedding of *E. coli* O157:H7 is probably highest, and it employed the most sensitive isolation techniques available; therefore, seasonality and methodology alone cannot be used to explain the difference between the recovery rate reported herein and those reported by other investigators. It should also be noted that the results herein refer to recovery of serotype O157:H7 strains. However, serotype O157:H$^-$ isolates were also recovered from at least two samples in this study, one each from downer and healthy cattle (data not shown). Therefore, if the present study had included methods for the detection of serotype O157:H$^-$ strains in addition to serotype O157:H7 strains, the overall prevalence, that is, the total number of both O157:H$^-$ and O157:H7 isolates, might have been slightly higher.

Molecular subtyping techniques, such as PFGE, have been widely used to investigate the ecology of *E. coli* O157:H7 at various points along the food chain. In the present study, analyses of multiple colonies from each of the 13 *E. coli* O157:H7-positive samples revealed 13 distinct *Xba*I REDP. In general, isolates from different fecal samples had different REDP. However, in one instance, isolates from two separate fecal samples (from downer cattle) had the same REDP and were recovered from samples 27 and 31 on 1 May 2001. Although it is not possible to match each cow with a source (farm), the two cows harboring O157:H7 strains with a common REDP may have been from a common farm, as truckers usually transported 3 to 10 downer cattle, collected from various farms, to the slaughter plant. Analyses of multiple isolates from a positive animal revealed that some animals harbored *E. coli* O157:H7 strains that had different, although similar, REDP. The levels of similarity among strains found in this study indicate that there may have been genetic changes, such as the loss of a plasmid or phage, which resulted in minor changes in the REDP, as reported by other investigators (11, 13, 21, 26, 32). In general, isolates displaying a distinct *Xba*I REDP had the same antimicrobial susceptibility profile. However, there were several exceptions to this, raising the possibility that although the REDP data suggest clonality of isolates, as evidenced by differences in antimicrobial susceptibility patterns, mixed populations exist within the same animal. The results of the present study are in agreement with the results of Meng et al. (26), who, while investigating isolates from the 1993 outbreak of *E. coli* infection from ground beef in the western United States, found that although all nine isolates had indistinguishable REDP, only two were resistant to streptomycin, sulfamethoxazole, and tetracycline. The researchers attributed this to the fact that genes encoding antimicrobial resistance are often carried on plasmids and genomic DNA fingerprinting methods, such as PFGE, are not sufficiently discriminatory to recognize subtle differences on plasmids.

Researchers have speculated that the use of antimicrobials to promote animal performance and well-being has played a role in accelerating the development of antimicrobial-resistant pathogens (20, 29, 32, 40). Therefore, we anticipated a difference in the susceptibility of isolates recovered from healthy versus downer dairy cattle, hypothesizing that bacteria from downer cattle might be more resistant due to the probability of increased antimicrobial use. In contrast, healthy cows are not routinely treated with antimicrobials while producing milk (23). However, there was no appreciable difference in the frequency of antimicrobial resistance of isolates recovered from healthy and downer cattle, suggesting that antimicrobial use practices between the two groups had no significant effect on the development of resistance. It is possible that farmers are not administering antimicrobials to downer dairy cattle because of cost, declining profits, larger numbers of animals per farm, and/or more rapid turnover of milking cows. The latter scenario would explain, at least in part, why there was not a statistically significant difference in antibiotic resistance between isolates from healthy and downer animals. Regardless, the results of the present study concur with those of a previous study by Galland et al. (13), who demonstrated that there was no difference in the antimicrobial resistance profiles between cattle in home pens and those in hospital pens, where antimicrobial use was presumably higher.

Of the 57 *E. coli* O157:H7 isolates tested, 10 (18%) were resistant to at least 1 of the 18 antimicrobials tested. Streptomycin, sulfamethoxazole, and tetracycline were the three antimicrobials to which resistance was displayed, with resistance to tetracycline and sulfamethoxazole occurring more often. The results of the present study are in agreement with those of Meng et al. (26), who reported that 24% of the *E. coli* O157:H7 isolates tested were resistant to at least one antimicrobial, with greatest resistance to streptomycin and tetracycline. Schroeder et al. (31) also reported that among 189 *E. coli* O157:H7

isolates recovered from various sources between 1985 and 2000, 19 (10%) were resistant to sulfamethoxazole and 16 (8%) were resistant to tetracycline. These two antimicrobials are commonly used to treat respiratory infections, diarrhea, mastitis, and other infections in beef and dairy cattle (29). Use of these or any antimicrobial for therapeutic or nontherapeutic indications may exert selective pressure and result in the development and maintenance of serotype O157:H7 strains of *E. coli* that are resistant to these and related antimicrobials. Further, these three antimicrobials have been used in veterinary and human medicine for decades and the genes which encode these antimicrobials are commonly found on plasmids, thus making it possible for the organism to more readily acquire resistance to these antimicrobials. Other classes of antimicrobials, including penicillins and cephalosporins, are also used to treat disease in cattle (29). Interestingly, the majority of *E. coli* O157:H7 isolates, including those in the present study, remain susceptible to these antimicrobials. Comparison with generic *E. coli* indicates greater resistance among non-O157:H7 *E. coli* strains, suggesting that *E. coli* O157:H7 is refractory to developing resistance. The use of antimicrobials to treat *E. coli* O157:H7 is a contentious area in the management of infection and is currently contraindicated, as it may exacerbate the disease (36).

The present study provided further insight into the ecology of *E. coli* O157:H7 in downer dairy cattle. The influence of geography and seasonality notwithstanding, we observed a 3.3-fold-higher prevalence of serotype O157:H7 strains of *E. coli* in downer than in otherwise healthy dairy cattle. However, there was no appreciable difference in the prevalence or frequency of antimicrobial resistance among *E. coli* O157:H7 isolates recovered from downer and healthy dairy cattle. Regarding the former, although not statistically significant, our study demonstrated a higher prevalence of serotype O157:H7 strains of *E. coli* in downer than in healthy dairy cattle. Considering the *P* value (0.06), additional studies that include a larger number of animals, a broader geographic scope, and an extended sampling period are needed to determine if the difference we observed in our initial study is indeed significant. If further studies show that downer dairy cattle have a significantly higher prevalence of this pathogen than healthy cattle, perhaps some consideration should be given to exclude downer or suspect animals from the meat supply or to channel such animals into cooking operations. Also, studies to ascertain if the numbers and duration of shedding of this pathogen in downer dairy cattle differ from those of healthy dairy cattle would be highly beneficial. The validation of such a relationship would provide a potential intervention target for the control of *E. coli* O157:H7 on farms.

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