SUPPLEMENT

Verotoxin-Producing *Escherichia coli* in Sheep Grazing an Irrigated Pasture or Arid Rangeland Forages

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Worldwide, verotoxin-producing *Escherichia coli* (VTEC) have been recognized as the cause of many sporadic cases or major outbreaks of human illnesses involving consumption of contaminated meat, especially beef. Although sheep products have not been linked to reported human illnesses, their role as a food safety risk factor should not be ignored. The objective of this study was to assess VTEC prevalence in two groups of ewes (20 each) grazing an irrigated pasture or arid range in a western United States environment (Nevada) over 1 year (summer of 1999 to summer of 2000). A random sample ($n = 504$) of potential VTEC isolates were tested for verotoxicity and were screened for the presence (polymerase chain reaction [PCR]) and expression (VTEC-reversed passive latex agglutination assay) of the toxin genes (i.e., VT1 and VT2). Forty-one VTEC isolates (16 having only the VT1 gene and 25 having both VT1 And VT2 genes) were detected in both groups of ewes. Except for seven isolates, the genotype and phenotype data matched. All the isolates (nonmotile [H^-]) were non-O157:H7 VTEC (i.e., O91:H^- [$n = 25$], O128:H^- [$n = 9$], and untypeable ones [$n = 7$]). More infected ewes (nine versus three) and different VTEC strains were found in the irrigated pasture than in the arid range. Because our ewes were shedding two VTEC serotypes known to cause human illnesses, it is beneficial to identify VTEC-positive sheep before slaughter as an initial control point before entering the food chain. Exp Biol Med 228:358–364, 2003

Key words: *Escherichia coli*; grazing sheep; verotoxins; food-borne pathogens; food safety

In recent years, the *Escherichia coli* O157:H7 serotype has gained attention in the United States because of its contamination of ground beef and causation of human illness outbreaks (1–3). This serotype was first recognized as a pathogen in 1982 after two human illness outbreaks in Oregon and Michigan (4). In the past two decades, *E. coli* O157:H7 also caused major human illness outbreaks worldwide, including one affecting 600 people (two deaths) in the western United States (5) and another affecting 10,000 people (11 deaths) in Japan (6). *E. coli* O157:H7 is one of over 60 serotypes of verotoxin-producing *E. coli* (VTEC) that cause a variety of human illnesses (2, 7, 8) such as mild diarrhea, hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS). These illnesses result from verotoxins (VT1 and VT2) similar to those produced by *Shigella dysentariae* (9) and are known for their toxic effects on African-Green Monkey kidney (Vero) cells in cultures (10). These toxins are different proteins, are encoded by different genes, and have similar toxic effects (11).

Several non-O157:H7 VTEC (e.g., O6:H31, O26:H^- [nonmotile], O26:H11, O48:H21, O91:H^-, O104:H21, O113:H21, and O128:H2) have been associated with human illness outbreaks in the United States (11, 12) and in other countries (11, 13–15). Because many of these outbreaks were traced to consumption of undercooked beef that had been contaminated with bovine feces, cattle have been considered reservoirs of VTEC (16). Recent evidence indicated that sheep harbor O157:H7 (17–20) and non-O157:H7 (21, 22) VTEC at rates similar to or higher than those reported for cattle and suggested their potential role as a food safety risk factor (23). Prevalence rates of 67% and 45% were reported for sheep in Germany (21) and Australia (24), respectively.

Worldwide, sheep have been shown to shed several non-O157:H7 VTEC in their feces. For example, serotypes

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O5:H⁻, O69:H8, O75:H⁻, O75:H40, O91:H⁻, O123:H⁻, and O163:H19 were detected in Australia (24). In Germany, serotypes O5:H⁻, O6:H10, O30:H12, O71:H12, O77:H4, O90:H21, O90:H24, O91:H⁻, O119:H25, O123:H10, O125:H14, O125:H26, O128:H2, O136:H20, O146:H8, and O146:H21 also were reported (21, 25). In the United States, non-O157:H7 VTEC (i.e., O5:H⁻, O6:H10, O6:H49, O88:H⁻, O91:H⁻, O128:H⁻, O128:H2, and O146:H21) were also detected (20, 26, 27). Several of these VTEC serotypes (e.g., O5:H⁻, O88:H⁻, O91:H⁻, O128:H⁻, O128:H2, O146:H21, and O163:H19) have been associated with sporadic cases or major outbreaks of human illnesses (28). Therefore, lamb, mutton, and their products share a food safety risk similar to that of beef.

Because the importance of food safety has increased dramatically in recent years, it is important to understand the types and trends of VTEC shedding by meat animals, including sheep. Therefore, the objective of this study was to assess VTEC prevalence in two groups of sheep that were managed differently in the western United States over 1 year. The sheep were allowed to graze an irrigated pasture or arid rangeland forages.

Materials and Methods

Animals and Sample Collection. Two groups of ewes (20 each) were selected at random from the yearling ewe population of a large sheep flock (>1000 ewes) at Rafter 7 Ranch (Yerington, NV). Only healthy ewes were selected for the study, and all ewes were similar in age (15 months old) and genetics (7/8 Merino and 1/8 Rambouillet). The first group of ewes was allowed to graze an irrigated pasture (Group P) containing tall fescue (*Festula arundinacea*) and white clover (*Trifolium repens*). The ewes were supplemented with alfalfa (*Medicago sativa*) hay only during winter. The second group of ewes was allowed to graze arid rangeland forages (Group R) such as Indian ricegrass (*Oryzopsis hymenoides*) and various shrubs, including white sage (*Ceratoides lanata*), fourwing saltbush (*Atriplex canescens*), and big sagebrush (*Artemisia tridentata*). The ewes continued to be healthy throughout the study and only one ewe from Group P was lost to predation after the first sample collection. The study was initiated in the summer of 1999 and continued to the summer of 2000. During this 1-year investigation, fresh feces from both groups of ewes were sampled at the same time. The ewes were sampled twice in 1999 (late August and early December) and twice in 2000 (late March and early July). Therefore, the sampling schedule allowed for assessing VTEC prevalence in three seasons (i.e., spring, summer, and winter). Two sampling times were devoted to the summer months (i.e., late August 1999 and early July, 2000) that were associated with the highest prevalence rates of O157:H7 and non-O157:H7 VTEC in sheep (23) and cattle (16). On each collection day, one fecal sample was obtained from the rectum of each ewe, placed in a sterile Whirl-pak bag (Nasco, Modesto, CA), and transferred on ice within 2 hr to our laboratory for analysis. Processing of the fecal samples began immediately upon their arrival.

Bacteriological Analysis. Initial selection of *E. coli* isolates to be screened for VTEC was conducted by adding 1 g of fresh feces to 25 ml of enrichment medium (i.e., tryptic soy broth [TSB]; Hardy Diagnostics, Santa Maria, CA] containing 20 μg/ml novobiocin (Sigma, St. Louis, MO) and 40 μg/ml vancomycin (Sigma). The feces and medium were mixed vigorously and the suspension then was incubated at 25°C for 20 min to allow for antibiotic selection. The optimal dilutions for plating were established by preliminary experiments. Fecal suspensions were serially diluted to 10^{-2}, plated in duplicate onto sorbitol-MacConkey (SMAC) agar (Hardy Diagnostics) containing novobiocin (20 μg/ml) and vancomycin (40 μg/ml), and incubated at 37°C for 18 hr. Fecal suspensions also were incubated at 37°C for 18 hr with continuous shaking for enrichment. The enriched suspensions were serially diluted to 10^{-7}, and the 10^{-5}, 10^{-6}, and 10^{-7} dilutions were plated in duplicate onto SMAC agar containing novobiocin (20 μg/ml) and vancomycin (40 μg/ml). The plates were incubated at 37°C for 18 hr. All colonies were enumerated by using a Bantex Model 900A illuminated colony counter (American Bantex Corp., Burlingame, CA).

Nonsorbitol-fermenting bacteria (i.e., white colonies) on SMAC plates were subcultured to 4-methylumbelliferyl-β-D-glucuronide (MUG) MacConkey (MMUG; Hardy Diagnostics) agar grid plates. If a SMAC plate contained 10 or less sorbitol-negative colonies, all were transferred to MMUG plates. However, if the SMAC plate had greater than 10 white colonies, a subset (29) of them (i.e., 10%) was randomly selected and transferred to MMUG plates. MMUG plates were incubated at 37°C for 18 hr and were observed on a UV light box (Fotodyne, New Berlin, WI). Results were recorded for MUG positive and MUG negative (i.e., blue fluorescence or no fluorescence under UV light, respectively) and lactose fermentation (29–31).

Verotoxin Production. The isolates collected (i.e., sorbitol negative/MUG negative or sorbitol negative/MUG positive) from the ewes in Group P (n = 232) and Group R (n = 272) were tested for verotoxicity. The isolates were grown in 5 ml of TSB at 37°C for 18 hr. The cultures were centrifuged (3000g for 10 min) and the supernatants were filtered through 0.2-μm sterile syringe filters (ISC BioExpress, Kaysville, UT). The sterile supernatant was used for testing verotoxicity (10, 31). Negative controls of Eagle's minimal essential medium (Mediatech, Herndon, VA), TSB medium, and non-VTEC O157:H7 (ATCC 43888) were analyzed with each set of plates. In addition, each set of plates contained a positive control panel of supernatants from three *E. coli* O157:H7 isolates known to produce only VT1 (ATCC 43890), only VT2 (ATCC 43889), or both toxins (ATCC 43895). VTEC-positive and VTEC-negative isolates were determined by the absence or presence of a confluent monolayer, respectively. Results were recorded after 24 and 48 hr of incubation.

Characterization of Isolates. The isolates from ewes in Group P (n = 28) and Group R (n = 13) that were toxic to Vero cells were confirmed as *E. coli* by biochemical tests using the API 20E Identification System (bioMerieux, Inc., Hazelwood, MO).

Detection of the Verotoxin Genes. The verotoxin genotype of the 504 isolates collected (i.e., 232 from Group P and 272 from Group R) was confirmed by polymerase chain reaction (PCR). In a single tube, a 50-µl PCR reaction mixture was prepared using MasterTaq kit (Eppendorf Scientific, Westbury, NY) following the manufacturer's instructions (5 × TaqMaster PCR enhancer, 10 × Taq buffer with 0.15 mM $MgCl_2$, 0.1 µl each of 100 m*M* nucleotide, and 1.0 units of Taq polymerase). One hundred nanograms of both forward and reverse primers were used for each reaction. Whole cells from each bacterial culture (1 µl of liquid culture) were added to the PCR mixture instead of purified nucleic acids. Oligonucleotide primers were commercially prepared (Life Technologies/GIBCO-BRL, Rockville, MD). The sequence of the primers and the length of their predicted products are published previously for VT1 (32) and VT2 (33). The PCR was performed using a Mastercycler Gradient thermocycler (Eppendorf Scientific) following the temperature program previously reported (33). Amplified DNA fragments were visualized by gel electrophoresis on 1% agarose-containing ethidium bromide (10 mg/ml).

Expression of the Verotoxin Genes. The VTEC-Reversed Passive Latex Agglutination (VTEC-RPLA) assay (Denka Seiken Co., Tokyo, Japan) was performed according to the manufacturer's instructions. Isolates were grown in 5 ml of TSB at 37°C for 18 hr without shaking. From this culture, 1 ml was placed in a 1.5-ml Eppendorf tube and was centrifuged at 3000*g* for 20 min. In 96-well V-bottom microtiter plates (Costar, Corning, NY), culture supernatants were mixed 1:1 (25 µl) with the supplied diluent. An equal volume of latex particles sensitized with rabbit polyclonal anti-VT1 or anti-VT2 immunoglobulin G antibody was mixed in appropriate wells. Plates were covered, incubated at room temperature, and examined for latex agglutination after 18 hr. The positive and negative control toxins supplied with the kit were run with each assay. A positive result was recorded when agglutination in the sample well was two levels above the control well.

Serotyping of VTEC Isolates. The VTEC isolates were serotyped at the *Escherichia coli* Reference Center (Pennsylvania State University, University Park, PA).

Results

Characterization of the VTEC Isolates. Based on the microbiological methods (i.e., sorbitol fermentation and β-glucuronidase activity), initial isolates (n = 232) representing fecal samples from the ewes in Group P were selected and screened for verotoxicity. Twenty-eight of these isolates (i.e., 12% of total) were confirmed to be verotoxic (Table I). Similarly, isolates (n = 272) representing fecal samples from the ewes in Group R were selected and screened for verotoxicity. Thirteen of these isolates (i.e., 5% of total) were also confirmed to be verotoxic (Table II). Based on the API identification system, the 41 verotoxic isolates were confirmed as *E. coli*. Due to the large numbers of bacteria present in ovine feces, we limited initial selection and screening of potential VTEC isolates to sorbitol-negative colonies because classic O157:H7 demonstrate these characteristics. To increase the probability of detecting sorbitol-negative non-O157:H7 VTEC, we did not limit

Table I. Characteristics of VTEC Isolates Detected in Feces of Sheep Grazing an Irrigated Pasture[a]

Collection	Number of isolates	Ewe	Characteristics							
			Biochemical		Toxicology	Genotype		Phenotype[b]		Serotype
			Sorb[c]	MUG[d]	Vero[e]	VT1	VT2	VT1	VT2	
Late August, 1999	2	A	–	+	+	–	+	–	–	Untypeable
	2	B	–	+	+	–	+	–	+	O91:H–[f]
Early December	3	C	–	+	+	–	+	–	+	O91:H–
	2	B	–	+	+	–	+	–	+	O91:H–
	2	D	–	+	+	–	+	–	+	O91:H–
	3	E	–	+	+	–	+	–	+	O91:H–
Late March, 2000	3	F	–	+	+	+	+	–	+	O128:H–
	2	G	–	+	+	+	+	+	+	O91:H–
	4	B	–	+	+	+	+	+	+	O91:H–
	1	H	–	+	+	–	+	+	+	O128:H–
	1	H	–	+	+	–	+	+	+	Untypeable
	3	I	–	+	+	+	+	+	+	O91:H–

[a] Of the 20 ewes tested, nine shed VTEC isolates in their feces during three collection periods and all ewes tested negative for VTEC during the fourth collection period (i.e., early July, 2000).
[b] Expression of the toxin genes determined by the RPLA-VTEC kit (Denka Seiken Co.).
[c] Sorbitol fermentation.
[d] 4-Methylumbelliferyl-β-D-glucuronide.
[e] Toxicity to vero cells (10).
[f] Nonmotile.

Table II. Characteristics of VTEC Isolates Detected in Feces of Sheep Grazing Arid Rangeland[a]

Collection	Number of isolates	Ewe	Characteristics							
			Biochemical		Toxicology	Genotype		Phenotype[b]		Serotype
			Sorb[c]	MUG[d]	Vero[e]	VT1	VT2	VT1	VT2	
Early December, 1999	4	J	–	+	+	+	+	+	+	O91:H^{-f}
	5	K	–	+	+	+	+	+	+	O128:H$^-$
	4	L	–	+	+	+	+	+	+	Untypeable

[a] Of the 20 ewes tested, three shed VTEC isolates in their feces during the second collection period (i.e., early December, 1999) and all ewes tested negative for VTEC during the remaining three collection periods (i.e., late August, 1999, early March, 2000, and early July, 2000).
[b] Expression of the toxin genes determined by the RPLA-VTEC kit (Denka Seiken Co.).
[c] Sorbitol fermentation.
[d] 4-Methylumbelliferyl-β-D-glucuronide.
[e] Toxicity to vero cells (10).
[f] Nonmotile.

our selection to MUG-negative isolates (a second unique characteristic of O157:H7). Because of our selection procedure, it is assumed that if there were sorbitol-positive VTEC serotypes present in the feces of our ewes, they would not have been detected in this study. Our results (Tables I and II) showed that all the VTEC isolates were sorbitol negative and MUG positive.

Detection of the Verotoxin Genes. Determination of the genotype of the 28 VTEC isolates from Group P and the 13 isolates from Group R by PCR revealed distinct variations. With regard to VTEC isolates from Group P (Table I), 16 had only the VT2 gene, whereas the remaining 12 had both VT1 and VT2 genes. The 13 VTEC isolates from Group R (Table II) had both VT1 and VT2 genes.

Expression of the Verotoxin Genes. The VTEC-RPLA kit (Denka Seiken Co.) was used to examine expression of the toxin genes in the VTEC isolates from the ewes. With the exception of seven isolates from Group P (Table I), the PCR results (i.e., presence of the toxin genes) for both groups corresponded with the RPLA data (i.e., expression of the toxin genes). Of these seven isolates (Table I), two from Ewe A had, but did not express, the VT2 gene and three from Ewe F had, and did not express, the VT1 gene. Two isolates (i.e., O128:H$^-$ and an untypeable one) from Ewe H reacted with the VT1-latex beads, but did not have the VT1 gene. Except for two isolates (from Ewe A; Table I), the gene expression data from the VTEC-RPLA assay matched that from the Vero cell assay. These two isolates were toxic to Vero cells, had only the VT2 gene, and did not express that gene when examined by the VTEC-RPLA assay.

Serotyping of VTEC Isolates. Serotyping the VTEC isolates from the ewes in Group P (Table I) revealed that 21 (from six ewes) were O91:H$^-$ and four (from two ewes) were O128:H$^-$. The remaining three VTEC isolates (from two ewes) could not be serotyped (i.e., unreactive with the "O" and "H" monovalent antisera available). Table I indicates that Ewe B was shedding the same VTEC serotype (i.e., O91:H$^-$) consistently in the first three collection periods. However, it appears that this ewe was shedding one VTEC strain during the first two collection periods because of the similarity of presence and expression of the VT2 gene. This ewe also appeared to start shedding a different VTEC strain during the third collection period. This is because the isolates detected at that time had and expressed both VT1 and VT2 genes. In general, no VTEC isolates were detected in feces of the ewes in Group P during the fourth collection period.

Serotyping the VTEC isolates from the ewes in Group R (Table II) revealed a unique pattern with regard to VTEC type and its carrier. Four isolates from Ewe J were O91:H$^-$, five isolates from Ewe K were 128:H$^-$, and four isolates from Ewe L were untypeable. These three ewes were shedding different VTEC serotypes during the second collection period and tested negative for VTEC in a manner similar to that of the remaining ewes during the three other collection periods.

Discussion

Different patterns of fecal shedding of VTEC were detected for the two groups of ewes examined in this study. For example, ewes in Group P shed VTEC during the first three collection periods (i.e., early August 1999, early December 1999, and late March 2000) with prevalence rates of 10%, 21%, and 26%, respectively. However, ewes in Group R shed VTEC only during the second collection period (i.e., early December 1999) with a prevalence rate of 15%. The fact that more ewes were shedding VTEC in Group P (n = 9) than in Group R (n = 3) suggests that the different management systems and/or forage types may have influenced infection, carriage, and shedding of these pathogens. Ewes in Group P grazed an irrigated pasture (i.e., dense in edible vegetation and nutrients) that allowed their rotation within a limited area and, therefore, increased potential reinfection or infection through horizontal transmission. Ewes in Group R, however, grazed rangeland forages (i.e., less dense in edible vegetation and nutrients) that forced the ewes to travel continuously to new areas that may have reduced the risk of infection. With regard to the diet, ewes in Group R consumed edible parts of shrubs known (34) to contain various chemicals (e.g., volatile oils, tannins, and coumarins) that may have reduced carriage and shedding of VTEC. For example, *in vitro* studies with coumarins (de-

rivatives of benzo-α-pyrone commonly occur in plants in the free state or as glycosides; Ref. 35) suggested their role in reducing growth and survival of *E. coli* O157 in the gut (36).

Long-term carriers were not found among sheep that were naturally (18, 20) or artificially (37, 38) infected with *E. coli* O157:H7. Sheep have been shown to be colonized naturally by *E. coli* O157:H7 in a transient and seasonal manner (18, 20) similar to that of cattle (39–41). Kudva *et al.* (18) reported that prevalence of *E. coli* O157:H7 in Idaho was highest (31.4%) in June and lowest (0%) in November. In a 16-month study covering two summers, Kudva *et al.* (20) also illustrated that their sheep tested positive for *E. coli* O157:H7 only in the summer months. A similar trend was found in the United Kingdom when sheep were tested for *E. coli* O157 over a 1-year period (19). Our results (Tables I and II) showed that *E. coli* O157:H7 was not the predominant VTEC serotype in our sheep that carried strains belonging to the O91:H$^-$ and O128:H$^-$ serotypes. Our data also emphasized the transient pattern of VTEC shedding by sheep. However, prevalence of non-O157:H7 VTEC in our ewes did not follow the seasonal trend previously reported for *E. coli* O157:H7 (18–20). The ewes grazing arid rangeland forages tested positive for VTEC only during winter (Table II), whereas those grazing an irrigated pasture had the highest prevalence rates (21% and 26%) during winter and spring, respectively (Table I). Our previous studies (1 year each) with dairy (42) and beef (43) heifers grazing irrigated pastures also showed trends of VTEC prevalence similar to those in Tables I and II. In the first study (42), VTEC isolates (O26:H$^-$ or untypeable ones) were only found during winter. In the second study (43), VTEC isolates (O3:H$^-$, O6:H$^-$, O6:H49, O113:H$^-$, O157:H7, or untypeable) were more prevalent in winter than in spring or fall. The difference in VTEC prevalence in our studies and in those of others (18–20) may have been due to the high elevation and low rainfall in Nevada.

Because of the similar serotype (O91:H$^-$), genotype, and phenotype of the VTEC isolates shed by Ewe B during the first collection period and by Ewes B, C, D, and E during the second collection period (Table I), it appears that these ewes were carrying and shedding the same VTEC strain. Despite the separation between the two groups of ewes, our results suggest that Ewes B, G, and I were shedding during the third collection period the same VTEC strain that was shed by Ewe J during the second collection period. This VTEC strain belonged to the serotype O91:H$^-$ and had and expressed both toxin genes. Results (Tables I and II) showed that three different VTEC strains (belonging to the serotype O128:H$^-$) were shed by three ewes (two in Group P and one in Group R). The first strain (from Ewe F) had both toxin genes but expressed only VT2 gene, the second (from Ewe H) had and expressed only VT2 gene, and the third (from Ewe K) had and expressed both toxin genes. The finding of more than one VTEC serotype with different toxin characteristics on a farm has been reported previously (25, 44). Therefore, isolation of two VTEC serotypes (i.e., O91:H$^-$ and O128:H$^-$) with different verotoxin production characteristics from one flock of sheep is not unexpected.

VTEC serotypes other than O157:H7 have been isolated more frequently and in high numbers in sheep feces (21, 22). Our ewes shed only non-O157:H7 VTEC (i.e., O91:H$^-$ and O128:H$^-$) in their feces. These VTEC serotypes have been isolated from healthy sheep in the United States (26) and in Germany (21, 22, 25). Kudva *et al.* (20) detected O157:H7 and non-O157:H7 VTEC in feces of ewes and lambs grazing Idaho rangeland forages, and reported that O91:H$^-$ and O128:H$^-$ were the predominant non-O157:H7 serotypes. Our results and those of Kudva *et al.* (20) suggest that these VTEC serotypes are common in the western region of the United States. Interestingly, McCluskey *et al.* (27) detected *E. coli* O91:H$^-$ in seven of 882 lambs at a slaughter facility in the midwestern United States. Detection of this VTEC serotype in sheep in the midwestern and western regions of the United States suggests its wide distribution. Bettelheim *et al.* (24) detected *E. coli* O91:H$^-$ in 45% of the sheep tested in an Australian flock. Another member of the O128 VTEC serogroup (i.e., *E. coli* O128:H2) has been detected in feces of sheep from various regions in Germany (21, 25). *E. coli* O128:H2 also was detected in one of 882 lambs at slaughter in the United States. (27).

In general, members of the VTEC serogroups O91 and O128, including those isolated from our ewes (i.e., O91:H$^-$ and O128:H$^-$), have been associated with either sporadic or outbreaks of human illnesses worldwide. *E. coli* O91:H$^-$ has been associated with HC and HUS in the United Kingdom (45). Other O91 serotypes (i.e., *E. coli* O91:H14 and *E. coli* O91:H21) were associated with outbreaks of HUS in Germany (46) and Spain (47), respectively. A case of HUS in Victoria (Australia) was attributed to *E. coli* O91:H10 (48). Other members of the O91 serogroup were found to be the cause of a major outbreak of human illnesses in South Australia (24). Similarly, *E. coli* O91:H$^-$ was responsible for outbreaks of diarrhea, HC, and HUS in the United Kingdom (45, 49) and in other countries (50, 51). *E. coli* O128:H$^-$ also has been reported as the cause of outbreaks of human illnesses (28). Other members of the O128 serogroup were found to be responsible for outbreaks of diarrhea in different Asian countries (52). The VTEC serotype O128:H2 was the cause of HC and HUS in the United Kingdom (45) and in other countries (50, 51). *E. coli* O128:H$^-$ and *E. coli* O128:H2 also were isolated from patients with HUS in Spain (47).

Although sheep products have never been implicated in food-borne illness outbreaks, research has shown that they harbor both O157:H7 and non-O157:H7 VTEC at rates similar to or higher than those reported for cattle (21, 53). Prevalence of O157:H7 and non-O157:H7 VTEC in sheep feces at slaughter facilities in the United Kingdom (17), United States (27), and Australia (24) suggests potential

contamination of their products with such pathogens. These findings may explain detection of *E. coli* O157:H7 in retail lamb in the United States (2, 54, 55). Therefore, it can be concluded that lamb, mutton, and their products share a food safety risk similar to that of beef. Food safety and quality assurance are and will continue to be major concerns for producers, processors, retailers, and consumers. Although most outbreaks of human illnesses caused by VTEC were traced to beef or dairy cattle, sheep should not be excluded as a potential risk factor. Therefore, the sheep industry should develop measures that assure safety of lamb, mutton, and their products. Applying potential on-farm management practices that minimize carriage and fecal shedding of such pathogens in combination with proper handling during slaughter, processing, and packaging would support a competitive sheep industry.

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