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Serotypes and Virulence Gene Profiles of Shiga Toxin-Producing *Escherichia coli* Strains Isolated from Feces of Pasture-Fed and Lot-Fed Sheep

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Shiga toxin-producing *Escherichia coli* (STEC) strains possessing genes for enterohemolysin (*ehxA*) and/or intimin (*eae*), referred to here as complex STEC (cSTEC), are more commonly recovered from the feces of humans with hemolytic uremic syndrome and hemorrhagic colitis than STEC strains that do not possess these accessory virulence genes. Ruminants, particularly cattle and sheep, are recognized reservoirs of STEC populations that may contaminate foods destined for human consumption. We isolated cSTEC strains from the feces of longitudinally sampled pasture-fed sheep, lot-fed sheep maintained on diets comprising various combinations of silage and grain, and sheep simultaneously grazing pastures with cattle to explore the diversity of cSTEC serotypes capable of colonizing healthy sheep. A total of 67 cSTEC serotypes were isolated, of which 21 (31.3%), mainly isolated from lambs, have not been reported. Of the total isolations, 58 (86.6%) were different from cSTEC serotypes isolated from a recent study of longitudinally sampled healthy Australian cattle (M. Hornitzky, B. A. Vanselow, K. Walker, K. A. Bettelheim, B. Corney, P. Gill, G. Bailey, and S. P. Djordjevic, Appl. Environ. Microbiol. 68:6439-6445, 2002). Our data suggest that cSTEC serotypes O5:H$^-$, O75:H8, O91:H$^-$, O123:H$^-$, and O128:H2 are well adapted to colonizing the ovine gastrointestinal tract, since they were the most prevalent serotypes isolated from both pasture-fed and lot-fed sheep. Collectively, our data show that Australian sheep are colonized by diverse cSTEC serotypes that are rarely isolated from healthy Australian cattle.

It is becoming increasingly evident that a variety of both commercially raised and wild animal species represent sources of Shiga toxin-producing *Escherichia coli* (STEC) (1, 2, 4–6, 8, 14, 18, 23, 38, 43, 50). STEC strains are responsible for a number of human gastrointestinal diseases, including diarrhea, bloody diarrhea, and hemorrhagic colitis (HC). In a proportion of individuals, these conditions may be complicated by neurological and renal sequelae, including hemolytic uremic syndrome (HUS). STEC strains causing human gastrointestinal disease are referred to as enterohemorrhagic *E. coli* (EHEC), and serogroups O157, O111, O26, O113, and O103 are responsible for many outbreaks and sporadic cases of HUS and HC.

EHEC strains not only express Shiga toxin, but usually possess other key virulence attributes. One of the most significant virulence factors (other than Shiga toxins) is intimin (encoded by the *eae* gene), a surface protein essential for the formation of attaching-and-effacing lesions on gastrointestinal epithelial cells (19). Enterohemolysin (encoded by the *ehxA* gene) is also commonly associated with EHEC populations (10, 23, 52). *E. coli* strains that possess Shiga toxin genes and those that possess *eae* and/or *ehxA* represent subpopulations of STEC that are more likely to be pathogenic for humans, and we refer to these as complex STEC (cSTEC) (27–29).

Serogroup O157 is the most common EHEC serogroup that has been associated with HC and HUS (35, 52, 54), but the importance of non-O157 STEC strains as causes of HC, HUS, and other gastrointestinal diseases is being increasingly recognized as more laboratories use molecular and immunological methods to screen fecal samples. Historically, many laboratories have screened only for serogroup O157 from clinical and environmental samples because of the ease of screening for its biochemical characteristic of inability to ferment sorbitol and because of the availability of specific antisera. As many laboratories have screened only for this serotype, non-O157 EHEC-associated disease is likely to have been underreported (30, 35). Non-O157 STEC should not be overlooked in human disease investigations because of the following: (i) non-O157 STEC strains are more prevalent than O157 STEC in the feces of meat-producing animals, indicating that humans are more likely to become exposed to these STEC strains as contaminants in foods (1, 14, 28, 31, 44); (ii) O157 STEC strains are not commonly isolated from the feces of healthy cattle and sheep in many parts of the world (9, 14, 28, 50); (iii) >100 STEC serotypes have been recovered from patients with HUS (http://www.microbionet.com.au/frames/feature/vtec/brief01.html); and (iv) 435 STEC serotypes have been recovered from hu-

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man infections (http://www.lugo.usc.es/~ecoli/). Furthermore, many STEC strains recovered from healthy food-producing animals possess virulence attributes that are similar to those found in the common EHEC serogroups. It is not clear what combination of virulence attributes distinguishes common EHEC serogroups from the serologically diverse STEC recovered from ruminant and other animal sources, nor is it known if all animal STEC populations are capable of causing disease in humans. Differences between STEC strains that inhabit farm animals and EHEC strains recovered from human patients may be related to the ability to express and secrete essential virulence factors. HUS-associated and bovine-associated STEC strains have been reported to differ in their basal and inducible Stx production characteristics (47). Similarly, differences in the abilities of O157 STEC strains recovered from both human disease-associated and bovine sources to secrete locus of enterocyte effacement proteins have been reported (34).

Epidemiological studies suggest that different serological STEC populations reside in the gastrointestinal tracts of healthy meat-producing animals (4, 5, 8, 9, 14, 28, 44). Recent studies suggest that STEC strains isolated from ovine and bovine feces contain genetically distinct Shiga toxin subtypes (11, 12, 22, 46). For example, stx_{1c} and $stx_{2d\ (Pierard)}$ subtypes are commonly associated with STEC populations recovered from the feces of healthy sheep but not from the feces of healthy cattle (11, 12, 22, 32, 46, 50). STEC containing stx_{1c} and $stx_{2d\ (Pierard)}$ subtypes are not commonly recovered from the feces of patients experiencing HC or HUS (20, 42, 55), but they are isolated from patients with uncomplicated diarrhea (20, 21, 55). Thus, the allele stx_{1c} may be a marker that identifies STEC strains that cause milder disease (7, 11, 21, 32, 56). The allele stx_{2e} has been identified in STEC strains recovered from the feces of swine but not among STEC strains recovered from ruminant species (our unpublished data; 12, 38, 46). Moreover, STEC strains often possess a large (>90 kb) plasmid (EHEC plasmid) whose genetic content has been shown to vary significantly in different STEC serotypes (13), and several putative virulence genes have been mapped to these plasmids. The enterohemolysin gene, *ehxA*, is typically found on this plasmid (13). Collectively, these observations have important epidemiological implications for tracing sources of STEC suspected of causing outbreaks and sporadic episodes of HUS and other gastrointestinal afflictions.

The expression of intimin is central to the formation of attaching-and-effacing lesions during colonization of the intestinal mucosa of bovines and humans and probably plays an important role in tissue tropism. In addition, intimin may be involved in adherence to eukaryote cell surface receptors, such as β-integrins (24, 40, 41). Fourteen intimin subtypes, which cluster into nine distinct phylogenetic families, have so far been described among *E. coli* strains from human and ruminant sources (45). Although various intimin variants have been detected among *E. coli* strains isolated from cattle and sheep, the intimin subtype may be a good prognostic indicator of the potential of STEC isolates derived from ruminant sources to cause disease in humans (45).

Cattle are recognized reservoirs for serologically diverse STEC strains (8, 23, 28, 29, 31, 44). Recently, it has been demonstrated that Gb3 receptors (recognition sites for the B subunit of Shiga toxins) are located on replicating progenitor epithelial cells in the crypts adjacent to submucosa in the bovine gastrointestinal tract. These receptors are absent on human gastrointestinal epithelia (25, 26). Gb3-positive bovine epithelial cells are resistant to a wide range of concentrations of Shiga toxin 1 (Stx1) (23), in contrast to Shiga toxin-sensitive cells, such as Vero cells. In Gb3-positive bovine epithelial cells, Stx1 is internalized, where it is processed by lysosomes and presumably degraded (26). This contrasts with the Shiga toxin processing pathway in Stx1-sensitive cells, where the toxin is transported to the endoplasmic reticulum, nuclear membrane, and nucleus and the Shiga toxin A subunit catalytically cleaves the glycoside bond within 28S rRNA, leading to disruption of protein synthesis (16, 26). These observations led Hoey et al. (26) to hypothesize that the lack of Shiga toxin-mediated toxicity in cattle may confer some selective advantage in this host and probably has contributed to dissemination of STEC among cattle populations. To date, most animal studies have been done in cattle, but the findings may also apply to other host species.

As part of a previous snapshot study of STEC in slaughter age sheep, selected virulence properties (encoded by stx_1, stx_2, *eae*, and *ehxA*) and serotypes of STEC strains from 1,623 fecal samples recovered from 65 geographically distinct mutton sheep and prime lamb properties across the eastern half of Australia were examined (14). Of the 90 cSTEC isolates examined in that study, 63% (57 isolates) possessed serotypes O91:H$^-$, O5:H$^-$, O128:H2, and O123:H$^-$. In this report, we describe the results of a longitudinal study of cSTEC strains isolated from the feces of ewes and their offspring in five flocks over a period of 6 to 8 months, from lambing to postweaning. Isolates were tested for selected virulence gene properties (encoded by stx_1, stx_2, *eae*, and *ehxA*), and their serotypes were determined. We also determined the virulence gene profiles and serotypes of cSTEC strains recovered from two properties where sheep and cattle grazed simultaneously because they represented an opportunity to examine the hypothesis that sheep and cattle are colonized, in the main, by serologically distinct populations of cSTEC. Finally, to determine if dietary changes can influence the excretion patterns of cSTEC, the virulence gene profiles and serotypes of cSTEC were determined from 288 pasture-fed sheep that were transferred to penned enclosures and fed a range of silage- and grain-based diets comprising between 53 and 100% silage with the balance made up of grain.

MATERIALS AND METHODS

Longitudinal studies of ewes and lambs. Five pasture sheep properties (four from New South Wales and one from Tasmania) were used in these studies. Feces were collected from 25 ewes identified by ear tag and from 25 of their lambs (also identified by ear tag) up to an age of 6 weeks. Feces were also collected from the lambs on two other occasions, at preweaning or weaning (at ~6 months of age) and ~2 weeks postweaning. Fecal samples from the Tasmanian property were collected from ewes, lambs <6 weeks of age, and lambs at preweaning only. For prime lambs born in Tasmania, the practice is to leave the lambs with the ewes until slaughter, so no postweaning samples could be collected.

Studies of sheep grazing with cattle. Three geographically separate properties in the New England region of northern New South Wales with a history of running both sheep and cattle simultaneously were identified. Twenty-five fecal samples were collected on a single occasion from each of 25 sheep and 25 cattle

from each property. The 150 fecal samples were tested for the presence of virulence genes and processed for the isolation of cSTEC as described below.

Study of sheep in a silage-grain diet trial. As part of a study undertaken to determine the impacts of various feedlot diets on production qualities in sheep, fecal samples from 288 sheep aged ~6 months were examined for the presence of cSTEC. The sheep (pasture fed) originated from a property located at Glen Innes in northern New South Wales and were transported to Cowra in central New South Wales for the pen trials. Fifty-eight fecal samples were collected and analyzed by multiplex PCR prior to the transportation of sheep to Cowra to identify cSTEC serotypes that had colonized the gastrointestinal tracts of the sheep prior to the commencement of the pen trials. Upon arrival, the sheep were allowed to acclimatize on pasture for 2 weeks and were segregated into 24 pens, each holding 12 animals. Their diets consisted of 100% silage, 90% silage–10% grain, 77% silage–23% grain, and 53% silage–47% grain, with each diet fed to six pens of 12 animals each (72 animals per diet). The grain component comprised 25% lupin and 75% barley. The sheep were maintained on these diets for 2 months and weighed every 2 weeks. Condition scores of the animals were taken at the beginning and conclusion of the trial. The lambs were healthy, with growth rates responding to the metabolizable energy available in each diet. Fecal samples were collected from all 288 animals on two occasions; the first sampling was performed after the animals had been maintained on the diet regimes for 26 days, and the second was performed 28 days later. At the end of the 2-month period, 166 sheep were determined to have reached market weight and were dispatched for slaughter. The remaining 122 animals were transferred to pasture for 1 month to increase their body weight prior to slaughter. Fecal samples from these 122 animals were collected after 1 month on pasture to determine if the dietary change back to pasture had an impact on the cSTEC serotype profiles.

Collection of feces. Fecal samples were collected per rectum (using individual sterile gloves to avoid animal-to-animal cross-contamination) into sterile 50-ml plastic containers. These samples were stored at 4°C overnight prior to transport to the Elizabeth Macarthur Agricultural Institute, New South Wales, Australia.

Multiplex PCR. For the diet trial, feces (50 mg) were incubated in EC (modified) broth (CM853; Oxoid, Basingstoke, United Kingdom) and subjected to PCR for the specific detection of stx_1, stx_2, *eae*, and *ehxA* as described previously by Fagan et al. (17). For the longitudinal study and sheep-with-cattle study, feces were prepared and subjected to PCR for the specific detection of stx_1, stx_2, *eae*, and *ehxA* as described by Paton and Paton (39), except that DNA templates for PCR were prepared using an InstaGene matrix, as described by the manufacturer (Bio-Rad, Richmond, Calif.). *E. coli* colonies cultured from EC (modified) broths from the diet trial, the longitudinal study, and the sheep-with-cattle study that showed a multilplex PCR profile indicative of the presence of at least one *stx* gene and at least one other virulence gene (*eae* and/or *ehxA*) were individually tested using the Paton multiplex PCR. The Paton multiplex PCR was used because it had been recently shown that the Fagan PCR assay unreliably amplifies $stx_{2d(Pierard)}$ subtypes (14). Although the Fagan multiplex PCR is unreliable in the detection of $stx_{2d\ (Peirard)}$ subtypes, previous data (14, 46) suggest that the vast majority of $stx_{2d\ (Peirard)}$-positive cSTEC strains also possess both stx_1 and *ehxA*. Thus, the Fagan multiplex PCR would have identified most, if not all, of these enrichment broths as potentially containing cSTEC. Amplified fragments were resolved by agarose gel electrophoresis as described previously (17). Glycerol stocks of overnight EC (modified) broth were stored at −80°C.

Isolation and serotyping of cSTEC. Only those EC (modified) broths that were positive for Shiga toxin genes (stx_1 and/or stx_2) and other accessory virulence genes (*eae* and/or *ehxA*) were cultured for STEC. Procedures used for the isolation of cSTEC from EC (modified) broths were described previously (14, 28). Standard biochemical tests were used to confirm that isolates of *E. coli* were cSTEC. The procedures employed to serotype cSTEC were described previously (14). *E. coli* isolates that are nonmotile are described as having an H^- flagellum type.

RESULTS

Longitudinal studies of ewes and lambs. Isolates of cSTEC were recovered from all five pasture sheep properties (Table 1). They were recovered from the feces of 42 of 130 (32.3%) ewes, 69 of 125 (55.2%) of their recently born lambs (<6 weeks old), 54 of 123 (43.9%) lambs sampled preweaning or at weaning, and 25 of 97 (25.8%) lambs sampled postweaning. In total, 248 cSTEC isolates, comprising 49 serotypes, were recovered from 196 sheep (Table 2). Of the 248 cSTEC isolates, 194 (78.2%) possessed stx_1, stx_2, and *ehxA*; 41 (16.5%) possessed stx_1 and *ehxA*; 9 (3.6%) possessed stx_1, *eae*, and *ehxA*; 2 (0.8%) possessed stx_1, stx_2, *eae* and *ehxA*; and 2 (0.8%) possessed stx_2 and *ehxA* (Table 2). The intimin gene (*eae*) was observed in 11 isolates, all from lambs, and included eight serotypes: O26:H^-/H11/H21, O103:H38, O128:H^-, O157:H^-, Ont:H^-, and OR:H11. Of the 49 serotypes identified in this longitudinal study, 18 had not previously been reported, and 14 of these were from lambs (http://www.lugo.usc.es/~ecoli/; http://www.microbionet.com.au/frames/feature/vtec//brief01.html). The most prevalent serotypes isolated were O5:H^- (21 sheep; 10.7%), O75:H8 (20 sheep; 10.2%), O91:H^- (17 sheep; 8.6%), O123:H^- (26 sheep; 13.2%), O128:H2 (34 sheep; 17.3%), Ont:H^- (8 sheep; 4.0%), and Ont:HR (9 sheep; 4.6%). All of these serotypes, with the exception of the Ont serogroups, possessed stx_1, stx_2, and *ehxA*. Of 49 serotypes identified in the longitudinal study, 14 serotypes (O5:H^-, O8:H19, O21:H21, O26:H^-/H11/H21, O75:H8, O128:H^-, O153:H^-/H25, O157:H^-, Ont:H4, Ont:H19, and Ont:H21) had previously been isolated from bovine sources (http://www.microbionet.com.au/frames/feature/vtec//brief01.html; http://www.lugo.usc.es/~ecoli/).

TABLE 1. Proportion of fecal samples positive for cSTEC in longitudinal study of pasture sheep

Pasture sheep property	No. of fecal samples positive for cSTEC/no. of fecal samples tested[a]			
	Periparturient ewes	Lambs		
		<6 wk old	Preweaning/weaning	Postweaning
1	17/25 (21/25)	16/25 (22/25)	12/25 (13/25)	12/25 (15/25)
2	15/25 (19/25)	17/25 (19/25)	19/25 (21/25)	7/25 (7/25)
3	1/25 (4/25)	20/25 (22/25)	10/25 (14/25)	3/24 (3/24)
4	6/30 (6/30)	8/25 (21/25)	12/23 (14/23)	3/23 (3/23)
5	3/25 (17/25)	8/25 (13/25)	1/25 (2/25)	Not determined

[a] Numbers in parentheses are numbers of samples with cSTEC PCR profiles after enrichment in EC (modified) broth.

A greater variety of cSTEC serotypes was isolated from lambs than from ewes—46 in lambs and 20 in ewes—and only 7 serotypes were common to both ewes and lambs. Lambs sampled at <6 weeks of age had the highest prevalence of cSTEC and the greatest variety of serotypes. The prevalence and variety of cSTEC declined with the sampling at preweaning and further declined with the postweaning sampling.

Studies of sheep grazing with cattle. Three properties simultaneously running both sheep and cattle were screened for the presence of cSTEC. Multiplex PCR analyses identified cSTEC profiles in 17 of 25 (68%) enrichment broths derived from the feces of sheep on property 1 and 9 of 25 (36%) broths derived from the feces of sheep collected from property 2. For cattle, only 3 of 25 (12%) enrichment broths from property 1 and 1 of 25 (4%) from property 2 showed multiplex PCR profiles indicative of the presence of cSTEC, and only four cSTEC serotypes (3 were Ont:H7, and 1 was O174:H21) were identified. Fecal enrichment broths of cattle and sheep from a third property failed to detect virulence factor profiles indicative of the presence of cSTEC.

Fifteen cSTEC serotypes were recovered exclusively from the feces of 32 sheep positive for a *stx* gene and/or at least one other virulence gene (*ehxA* and/or *eae*). Of the 15 cSTEC serotypes, 6 (O5:H^-, O75:H8, O91:H^-, O123:H^-, O128:H2,

TABLE 2. Complex STEC serotypes isolated in longitudinal flock studies

Serotype[a]	Previously reported[b]	No. of isolates (no. of sheep infected)[c]	Virulence factor profile[d]				No. of infected sheep			
								Lambs		
			stx_1	stx_2	*eae*	*ehxA*	Ewes	<6 wk old	Preweaning	Postweaning
O5:H$^-$	Yes	29 (21)	+	+		+	9	3	6	3
O8:H19	Yes	1 (1)	+	+		+	1			
O21:H21	Yes	1 (1)	+	+		+		1		
O26:H$^-$	Yes	2 (2)	+		+	+		2		
O26:H11	Yes	3 (3)	+		+	+		3		
O26:H21	Yes	1 (1)	+	+	+			1		
O75:H$^-$	Yes	2 (2)	+	+		+			2	
O75:H8	Yes	26 (20)	+	+		+	3	5	8	4
O75:H40	Yes	3 (2)	+	+		+		1	1	
O77:H$^-$	No	1 (1)	+			+		1		
O77~H4	Yes	3 (2)	+			+			2	
O91:H$^-$	Yes	19 (17)	+	+		+	6	3	6	2
O103:H38	No	2 (2)	+	+		+		1		
			+		+	+		1		
O106:H18	No	1 (1)	+	+		+		1		
O107:H$^-$	No	1 (1)	+	+		+				1
O107/O117:H$^-$	No	1 (1)	+			+			1	
O110:H28	No	1 (1)	+	+		+				1
O117:H21	No	1 (1)	+			+	1			
O121:H2	No	1 (1)	+	+		+			1	
O123:H$^-$	Yes	35 (26)	+	+		+	6	8	6	6
O123:H11	No	1 (1)	+	+		+		1		
O128:H$^-$	Yes	5 (3)	+	+		+	1		1	
			+	+	+	+				1
O128:H2	Yes	40 (34)	+	+		+	3	10	14	7
O128:H2/H8	No	2 (2)	+	+		+	2			
O128:H8/H12	No	1 (1)	+	+		+	1			
O128:HR	No	1 (1)	+			+		1		
O128:Hnt	No	1 (1)	+	+		+	1			
O149:H2	No	2 (2)	+	+		+			1	
O153:H$^-$	Yes	5 (3)	+	+		+		2		
O153:H8	Yes	1 (1)	+	+		+	1			
O153:H25	Yes	3 (3)	+			+		2		
O154:HR	No	1 (1)	+			+		1		
O157:H$^-$	Yes	1 (1)		+	+	+				1
Ont:H$^-$	Yes	12 (8)	+			+	2	5		
			+	+	+	+			1	
Ont:H4	Yes	4 (4)	+			+		2	1	1
Ont:H5/H51	No	1 (1)	+	+		+			1	
Ont:H11	Yes	2 (2)		+		+		1		
			+	+		+	1			
Ont:H14	Yes	1 (1)	+	+		+	1			
Ont:H19	Yes	2 (2)	+	+		+	1		1	
Ont:H21	Yes	1 (1)	+	+		+				1
Ont:H49	Yes	2 (2)	+	+		+	1		1	
Ont:HR	Yes	10 (9)	+			+	2	7		
OR:H$^-$	Yes	2 (2)	+			+		1		
			+	+		+		1		
OR:H2	Yes	7 (5)	+	+		+	2	2	1	
OR:H4	Yes	1 (1)	+	+		+	1			
OR:H11	Yes	1 (1)	+		+	+		1		
OR:HR	No	1 (1)	+			+		1		
O174:H2	Yes	1 (1)	+	+		+			1	
O174:HR	No	1 (1)	+			+		1		

[a] Ont, O nontypeable; OR, O rough.
[b] No, not previously reported (http://www.microbionet.com.au/frames/feature/vtec/brief01.html).
[c] Numbers in brackets are the number of sheep excreting the serotype.
[d] +, gene detected by PCR.

and O174:H2) are prevalent in ovine (13) but not bovine (26) feces (Table 3). Serotypes O6:H$^-$, O141:H49, O152:H21, O153:H25, O157:H21, and O174:H19 were isolated but are considered rarer serotypes (http://www.microbionet.com.au/frames/feature/vtec//brief01.html; http://www.lugo.usc.es/~ecoli/). Serotype O6:H$^-$ was previously isolated from Australian sheep (2). Serotype O75:H8 was also one of the most prevalent cSTEC serotypes isolated from both pasture-fed sheep sampled longitudinally and lot-fed sheep fed a range of diets (see below). Serotype OR:H$^-$ was readily identified among cSTEC strains

TABLE 3. cSTEC serotypes from properties running cattle and sheep simultaneously

Serotype	Previously reported	No. of isolates (no. of sheep infected)	No. of isolates (no. of cattle infected)	Virulence factor profile[a]				Property no.
				stx$_1$	*stx*$_2$	*eae*	*ehxA*	
O5:H$^-$	Yes	9 (5)		+	+		+	1
				+			+	1
					+		+	1
O6:H$^-$	Yes	1 (1)		+	+		+	2
O75:H8	Yes	2 (2)		+	+		+	2
				+			+	2
O91:H$^-$	Yes	3 (1)		+	+		+	1
O123:H$^-$	Yes	10 (5)		+	+		+	1
		5 (3)		+	+		+	2
O128:H2	Yes	1 (1)		+	+		+	1
O141:H49	No	2 (1)			+		+	1
O152:H21	No	1 (1)		+			+	1
O153:H25	Yes	2 (1)		+			+	1
O157:H$^-$	Yes	7 (5)		+	+	+	+	1
				+	+		+	1
O157:H21	Yes	1 (1)		+	+	+	+	1
Ont:H7	Yes		3 (1)		+		+	1
Ont:H8	Yes	1 (1)		+		+	+	1
OR:H$^-$	Yes	3 (2)		+	+	+	+	1
				+	+		+	1
O174:H2	Yes	3 (1)		+			+	1
O174:H19	No	1 (1)		+			+	1
O174:H21	Yes	1 (1)	1 (1)		+	+		1

[a] +, gene detected by PCR.

recovered from ovine feces during longitudinal flock studies (Table 2). Serotype Ont:H8 was described in a previous study of cattle STEC (28). Serotype Ont:H7 was isolated only from cattle (property 1), and serotype O174:H21 was isolated from both sheep and cattle at property 1 (one ovine isolate and one bovine isolate). Serotypes O141:H49, O152:H21, and O174:H19 were not previously reported (http://www.microbionet.com.au/frames/feature/vtec//brief01.html; http://www.lugo.usc.es/~ecoli/).

Study of sheep in a silage-grain diet trial. In the silage-grain diet study, cSTEC profiles were detected in 27 of 58 (46.5%; sampling 1), 110 of 285 (38.6%; sampling 2), 117 of 288

TABLE 4. Number of cSTEC serotypes isolated from sheep fed pasture and a range of silage-grain-based diets

Serotype	Previously reported	Virulence factor profile[c]				Sampling 1 (pasture)	Sampling 2 (silage-grain)				Sampling 3 (silage-grain)				Sampling 4 (pasture)
		stx$_1$	*stx*$_2$	*eae*	*ehxA*		100[a]	90	77	53	100	90	77	53	
O5:H$^-$	Yes	+	+		+		1	1	2		1			1	
		+			+							1			
O5:HR	No	+	+	+		1	1								
		+	+	+	+		1								
O8:H2	Yes	+	+		+		1								
O28:H21	Yes	+	+		+			1							
O37:H10	No	+	+	+						1					
O55:H20	No	+	+	+	+										1
O75:H8	Yes	+	+		+	1	2	4	1		2	1	1	1	1
		+			+			1							
O88:H8	Yes	+	+	+			1								
O91:H$^-$	Yes	+	+		+	2	10	3	9	4	7	4	2	3	2
O112ab:H2	Yes	+	+	+	+			1							
O123:H$^-$	Yes	+	+		+		1	2	1				1		2
O128:H2	Yes	+	+		+		3					1	2		1
O158:HR	No	+		+	+					1					
O172:H$^-$	Yes	+	+		+		1								
OR:H7	Yes	+			+	1									
Ont:H49	Yes	+	+	+						1					
Ont:Hnd[b]		+	+		+			1			2		1	1	
Ont:Hnd		+			+	1	2		1						
Ont:Hnd		+	+	+	+			1							
Ont:Hnd		+		+			1								
Ont:Hnd			+	+	+										1

[a] Percent silage.
[b] Ont:Hnd, isolates with Ont serogroup, H type not determined.
[c] +, gene detected by PCR.

(40.6%; sampling 3), and 19 of 122 (15.6%; sampling 4) isolates. Isolates of cSTEC from sheep typically possess a combination of stx_{1c}/stx_1 and $stx_{2d\ (Pierard)}$ subtypes (11, 12, 32, 46, 50). One hundred and nine isolates comprising 16 cSTEC serotypes were isolated from these 288 sheep (Table 4). The most prevalent serotypes isolated were O91:H$^-$ (46 isolates; 42.2%), O75:H8 (15 isolates; 13.8%), O5:H$^-$ (9 isolates; 8.3%), O123:H$^-$ (7 isolates; 6.4%), and O128:H2 (7 isolates; 6.4%) (Table 4). Although these serotypes are commonly isolated from the feces of sheep (8, 13, 45), a number of cSTEC serotypes, including O5:HR, O37:H10, O55:H20, O112ab:H2, and O158:HR, that were not previously reported were recovered. Serotypes O5:HR and O112ab:H2 were isolated from animals when they were fed a diet composed predominantly (at least 90%) of silage. The greatest variety of serotypes was isolated from sheep when their diet was initially changed from pasture to the silage-grain mixture with between 90 and 100% silage. As the percentage of silage decreased and the percentage of grain increased, the variety of serotypes decreased (Table 4). Of 109 isolates, 11 (10.1%) possessed the *eae* gene and had the serotypes O5:HR, O37:H10, O55:H20, O88:H8, O112ab:H2, and O158:HR, along with several Ont serogroup isolates. STEC serotypes O8:H2, O28:H21, O88:H8, and O172:H$^-$ were previously recovered from cattle (http://www.microbionet.com.au/frames/feature/vtec//brief01.html; http://www.lugo.usc.es/~ecoli/).

DISCUSSION

From these studies—a longitudinal study of ewes and lambs, a study of sheep fed silage-grain diets, and a small study of sheep grazing with cattle—a total of 67 cSTEC serotypes were isolated from sheep. The combined results of all three studies suggest that cSTEC strains with the serotypes O5:H$^-$, O75:H8, O91:H$^-$, O123:H$^-$, and O128:H2 are the predominant serotypes that are shed in the feces of sheep and that these serotypes are well adapted for colonization of the ovine gastrointestinal tract.

Several of these ovine cSTEC serotypes (O5:H$^-$, O91:H$^-$, and O128:H2) have been recovered from humans with HUS. O75:H8 STEC has been isolated from the feces of humans, cattle, and sheep and from beef, but to our knowledge this serotype has not been isolated from a human patient experiencing HUS, HC, or diarrhea (http://www.lugo.usc.es/~ecoli/; http://www.microbionet.com.au/frames/feature/vtec/brief01.html). Of the 90 STEC strains isolated during a previous snapshot study of 1,623 fecal samples from slaughter age sheep from 65 geographically distinct mutton sheep and prime lamb properties across the eastern half of Australia (13), cSTEC serotypes O5:H$^-$, O91:H$^-$, O123:H$^-$, and O128:H2 accounted for 63% (57 isolates) of the isolates. Serotype O75:H8 was not identified in the previous snapshot study but in the present studies was commonly recovered from the feces of pasture-fed sheep (26 isolates) and sheep fed a range of silage-grain diets (13 isolates) (Table 4), as well as 2 isolates from sheep grazing pastures with cattle (Table 3). Despite differences in STEC isolation methods and animal-rearing practices and the fact that cSTEC strains were specifically targeted in our studies, serotypes O5:H$^-$, O91:H$^-$, and O128:H2 are prevalent in the feces of sheep in Australia and overseas (2, 4, 8, 13, 31, 45, 46). In addition to the diverse collection of cSTEC strains described in this report, other STEC serotypes have been isolated from sheep (9, 33, 50, 51). All these studies confirm that sheep are colonized by a highly diverse population of STEC serotypes.

In the present study and previous Australian studies, a total of 81 cSTEC serotypes have been recovered from healthy Australian sheep (2, 14, 18). Of these ovine isolates, cSTEC serotypes O5:H$^-$, O8:H19, O41:H49, O84:H$^-$, O91:H$^-$, O153:H8, O157:H$^-$, and OR:H$^-$ have occasionally also been isolated from healthy Australian cattle (28), and serotypes O26:H$^-$/H11 are prevalent in cattle feces. The most prevalent cSTEC serotypes recovered from Australian cattle comprise serotypes O113:H21, O82:H8, O8:H19, and O26:H11 (28), with a further 62 less prevalent serotypes also having been recovered (28). In two large overseas studies, one of non-O157 STEC strains isolated from carcasses in commercial beef-processing plants in the United States (1) and one of 92 STEC isolates recovered from a study of the feces of healthy cattle in Japan (31), the prevalent ovine serotypes O5:H$^-$, O75:H8, O91:H$^-$, O123:H$^-$, and O128:H2 were not isolated. Collectively, these observations further support the hypothesis that serologically distinct cSTEC populations have a predilection to colonize the gastrointestinal tracts of either sheep or cattle (4–6, 9, 14, 28, 31, 50, 51). Furthermore, recent studies have shown that cSTEC populations from sheep and cattle typically possess different Shiga toxin gene subtypes (11, 12, 22, 32, 46), suggesting that cSTEC populations commonly found in sheep and cattle are lysogenized with genetically distinct Shiga toxin-containing bacteriophage.

The STEC serotype O26:H11 is prevalent in cattle feces (23, 27–29) and is also one of the most important serotypes associated with HUS patients in Germany, Great Britain, Australia, the United States, and Italy (3, 15, 36, 37, 48, 49, 53). This serotype is rarely isolated from the feces of healthy sheep (4, 14, 50; http://www.microbionet.com.au/frames/feature/vtec//brief01.html; http://www.lugo.usc.es/~ecoli/). In our longitudinal studies, three cSTEC serotype O26:H11 isolates, containing *eae*, stx_1, and *ehxA*, were detected in the feces of three lambs <6 weeks of age (Table 2). Only one O26:H11 STEC isolate (stx_1 *eae ehxA*) was recovered among 384 STEC isolates in studies of lambs in Spain (9). Previously, the isolation of several O5:H$^-$ isolates from calves was reported, and it was hypothesized that these animals might be capable of being transiently colonized by cSTEC serotypes not typically associated with slaughter age cattle (reference 11 and references therein). The isolation of O26:H11 from young lambs suggests that preweaned lambs are capable of being transiently colonized by atypical cSTEC serotypes. Further studies are required to confirm this hypothesis.

In the longitudinal study, lambs <6 weeks old had the highest prevalence of cSTEC and the greatest variety of serotypes. The majority of serotypes not previously reported were from lambs, and particularly lambs <6 weeks old. The prevalence and variety of cSTEC declined with the sampling at preweaning and further declined with the postweaning sampling. The lambs also carried the majority of serotypes with the *eae* gene. The differences between lambs and ewes may simply be because the lambs were sampled more often than the ewes (on three separate occasions, whereas the ewes were sampled only

once). On the other hand, lambs may have distinct populations of STEC, as many of the serotypes identified in lambs during the longitudinal study were not found in the ewes and had not been identified in adult sheep in an earlier study (14). At <6 weeks of age, lambs have a monogastric digestive system and a diet of ewe's milk. As the lamb matures, the gastrointestinal tract gradually develops into a rumen-based digestive system that is able to digest pasture through the fermentation of cellulose. The differences in cSTEC serotypes may be related to the effect of age on the physiological differences in the digestive tract, on the diet, or on the immune response to these organisms. Further studies with larger numbers of animals are required to determine if this is a biologically meaningful observation.

Our study of sheep on different dietary regimes showed the greatest variety of cSTEC serotypes in animals whose diet had been changed from pasture to 100% silage or 90% silage–10% grain diets. Five previously unreported serotypes were identified in these animals.

To investigate further whether serologically distinct cSTEC serotypes colonize both healthy sheep and cattle, we sampled feces of sheep and cattle simultaneously grazing the same pasture from three geographically unrelated properties. Of 53 cSTEC strains isolated from sheep in this study, we identified 16 serotypes, the majority belonging to serotypes O5:H$^-$, O75:H8, O123:H$^-$, O157:H$^-$, and Ont:H$^-$. We isolated only one serotype, O174:H21 (not a prevalent sheep isolate), from both sheep and cattle and only one serotype, Ont:H7, from cattle alone. Our observation in cattle and sheep grazing the same properties that cSTEC serotypes commonly isolated from sheep feces were not present in cattle feces suggests that adult cattle may be unable to be, or rarely become, colonized by these cSTEC populations. Alternatively, cSTEC strains of ovine origin may be unable to survive in feces long enough to recolonize other grazing ruminant species. Our findings are consistent with those of others (4–6, 9, 50, 51).

In summary, a total of 67 cSTEC serotypes were isolated from the feces of sheep reared on pasture or various combinations of silage and grain. Serotyping studies showed that the cSTEC serotypes O5:H$^-$, O75:H8, O91:H$^-$, O123:H$^-$, and O128:H2 are prevalent in the feces of Australian sheep. STEC strains displaying these serotypes have also regularly been recovered from the feces of sheep raised in other regions of the world (4, 9, 14, 50, 51). Our observations suggest that young lambs carry a more diverse range of STEC strains than adult sheep. Collectively, these observations reconfirm the hypothesis that sheep are colonized by a population of cSTEC strains that are distinct from serotypes recovered from the feces of cattle.

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REFERENCES

1. **Arthur, T. M., G. A. Barkocy-Gallagher, M. Rivera-Betancourt, and M. Koohmaraie.** 2002. Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. Appl. Environ. Microbiol. **68:**4847–4852.
2. **Bettelheim, K. A., J. C. Bensink, and H. S. Tambunan.** 2000. Serotypes of verotoxin-producing (Shiga toxin-producing) *Escherichia coli* isolated from healthy sheep. Comp. Immunol. Microbiol. Infect. Dis. **23:**1–7.
3. **Beutin, L., S. Aleksic, S. Zimmermann, and K. Gleier.** 1994. Virulence factors and phenotypical traits of verotoxigenic strains of *Escherichia coli* isolated from human patients in Germany. Med. Microbiol. Immunol. (Berlin) **183:**13–21.
4. **Beutin, L., D. Geier, H. Steinruck, S. Zimmermann, and F. Scheutz.** 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in 7 different species of healthy domestic animals. J. Clin. Microbiol. **31:**2483–2488.
5. **Beutin, L., D. Geier, S. Zimmermann, S. Aleksic, H. A. Gillespie, and T. S. Whittam.** 1997. Epidemiological relatedness and clonal types of natural populations of *Escherichia coli* strains producing Shiga toxins in separate populations of cattle and sheep. Appl. Environ. Microbiol. **63:**2175–2180.
6. **Beutin, L., D. Geier, S. Zimmermann, and H. Karch.** 1995. Virulence markers of Shiga-like toxin-producing *Escherichia coli* strains originating from healthy domestic animals of different species. J. Clin. Microbiol. **33:**631–635.
7. **Beutin, L., G. Krause, S. Zimmermann, S. Kaulfuss, and K. Gleier.** 2004. Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. J. Clin. Microbiol. **42:**1099–1108.
8. **Blanco, M., J. E. Blanco, J. Blanco, A. Mora, C. Prado, M. P. Alonso, M. Mourino, C. Madrid, C. Balsalobre, and A. Juarez.** 1997. Distribution and characterization of faecal verotoxin-producing *Escherichia coli* (VTEC) isolated from healthy cattle. Vet. Microbiol. **54:**309–319.
9. **Blanco, M., J. E. Blanco, A. Mora, J. Rey, J. M. Alonso, M. Hermoso, J. Hermoso, M. P. Alonso, G. Dahbi, E. A. Gonzalez, M. I. Bernardez, and J. Blanco.** 2003. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from healthy sheep in Spain. J. Clin. Microbiol. **41:**1351–1356.
10. **Boerlin, P., S. A. McEwen, F. Boerlin-Petzold, J. B. Wilson, R. P. Johnson, and C. L. Gyles.** 1999. Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. J. Clin. Microbiol. **37:**497–503.
11. **Brett, K. M., V. Ramachandran, M. A. Hornitzky, K. A. Bettelheim, M. J. Walker, and S. P. Djordjevic.** 2003. stx_{1c} is the most common Shiga toxin 1 subtype among Shiga toxin-producing *Escherichia coli* isolates from sheep but not among isolates from cattle. J. Clin. Microbiol. **41:**926–936.
12. **Brett, K. N., M. A. Hornitzky, K. A. Bettelheim, M. J. Walker, and S. P. Djordjevic.** 2003. Bovine non-O157 Shiga toxin 2-containing *Escherichia coli* isolates commonly possess $stx_{2\text{-EDL933}}$ and/or stx_{2vhb} subtypes. J. Clin. Microbiol. **41:**2716–2722.
13. **Brunder, W., H. Schmidt, M. Frosch, and H. Karch.** 1999. The large plasmids of Shiga-toxin-producing *Escherichia coli* (STEC) are highly variable genetic elements. Microbiology **145:**1005–1014.
14. **Djordjevic, S. P., M. A. Hornitzky, G. Bailey, P. Gill, B. Vanselow, K. Walker, and K. A. Bettelheim.** 2001. Virulence properties and serotypes of Shiga toxin-producing *Escherichia coli* (STEC) from healthy Australian slaughter age sheep. J. Clin. Microbiol. **39:**2017–2021.
15. **Elliott, E. J., R. M. Robins-Browne, E. V. O'Loughlin, V. Bennett-Wood, J. Bourke, P. Henning, G. G. Hogg, J. Knight, H. Powell, and D. Redmond.** 2001. Nationwide study of haemolytic uraemic syndrome: clinical, microbiological, and epidemiological features. Arch. Dis. Child. **85:**125–131.
16. **Endo, Y., K. Tsurugi, T. Yutsudo, Y. O. T. Takeda, and K. Igarashi.** 1988. Site of action of a vero toxin (VT2) from *Escherichia coli* O157:H7 and of Shiga on eukaryotic ribosomes: RNA N-glycosidase activity of the toxins. Eur. J. Biochem. **171:**45–50.
17. **Fagan, P. K., M. A. Hornitzky, K. A. Bettelheim, and S. P. Djordjevic.** 1999. Detection of Shiga-like toxin (stx_1 and stx_2), intimin (*eaeA*), and enterohemorrhagic *Escherichia coli* (EHEC) hemolysin (EHEC *hlyA*) genes in animal feces by multiplex PCR. Appl. Environ. Microbiol. **65:**868–872.
18. **Fegan, N., and P. Desmarchelier.** 1999. Shiga toxin-producing *Escherichia coli* in sheep and pre-slaughter lambs in eastern Australia. Lett. Appl. Microbiol. **28:**335–339.
19. **Frankel, G., A. D. Phillips, M. Novakova, M. Batchelor, S. Hicks, and G. Dougan.** 1998. Generation of *Escherichia coli* derivatives with differing biological activities using site-directed mutagenesis of the intimin C-terminus domain. Mol. Microbiol. **29:**559–570.
20. **Friedrich, A. W., M. Bielaszewska, W. L. Zhang, M. Pulz, T. Kuczius, A. Ammon, and H. Karch.** 2002. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J. Infect. Dis. **185:**74–84.
21. **Friedrich, A. W., J. Borell, M. Bielaszewska, A. Fruth, H. Tschape, and H. Karch.** 2003. Shiga toxin 1c-producing *Escherichia coli* strains: phenotypic and genetic characterization and association with human disease. J. Clin. Microbiol. **41:**2448–2453.
22. **Gobius, K. S., G. M. Higgs, and P. M. Desmarchelier.** 2003. Presence of activatable Shiga toxin genotype (stx_{2d}) in Shiga toxigenic *Escherichia coli* from livestock sources. J. Clin. Microbiol. **41:**3777–3783.

23. **Gyles, C., R. Johnson, A. L. Gao, K. Ziebell, D. Pierard, S. Aleksic, and P. Boerlin.** 1998. Association of enterohemorrhagic *Escherichia coli* hemolysin with serotypes of Shiga-like-toxin-producing *Escherichia coli* of human and bovine origins. Appl. Environ. Microbiol. **64:**4134–4141.
24. **Hartland, E. L., M. Batchelor, R. M. Delahay, C. Hale, S. Mattews, G. Dougan, S. Knutton, I. Connerton, and G. Frankel.** 1999. Binding of intimin from enteropathogenic *Escherichia coli* to Tir and to host cells. Mol. Microbiol. **32:**151–158.
25. **Hoey, D. E. E., C. Currie, R. W. Else, A. Nutikka, C. A. Lingwood, D. L. Gally, and D. G. E. Smith.** 2002. Expression of receptors for verotoxin 1 from *Escherichia coli* O157 on bovine intestinal epithelium. J. Med. Microbiol. **51:**143–149.
26. **Hoey, D. E. E., L. Sharp, C. Currie, C. A. Lingwood, D. L. Gally, and D. G. E. Smith.** 2003. Verotoxin 1 binding to intestinal crypt epithelial cells results in localization to lysosomes and abrogation of toxicity. Cell. Microbiol. **5:**85–97.
27. **Hornitzky, M. A., K. A. Bettelheim, and S. P. Djordjevic.** 2001. The detection of Shiga toxin-producing *Escherichia coli* in diagnostic bovine faecal samples using vancomycin-cefixime-cefsulodin blood agar and PCR. FEMS Microbiol. Lett. **198:**17–22.
28. **Hornitzky, M. A., B. A. Vanselow, K. Walker, K. A. Bettelheim, B. Corney, P. Gill, G. Bailey, and S. P. Djordjevic.** 2002. Virulence properties and serotypes of Shiga toxin-producing *Escherichia coli* from healthy Australian cattle. Appl. Environ. Microbiol. **68:**6439–6445.
29. **Jenkins, C., M. C. Pearce, H. Chart, T. Cheasty, G. A. Willshaw, G. J. Gunn, G. Dougan, H. R. Smith, B. A. Synge, and G. Frankel.** 2002. An eight-month study of a population of verocytotoxigenic *Escherichia coli* (VTEC) in a Scottish cattle herd. J. Appl. Microbiol. **93:**944–953.
30. **Kehl, S. C.** 2002. Role of the laboratory in the diagnosis of enterohemorrhagic *Escherichia coli* infections. J. Clin. Microbiol. **40:**2711–2715.
31. **Kobayashi, H., J. Shimada, M. Nakazawa, T. Morozumi, T. Pohjanvirta, S. Pelkonen, and K. Yamamoto.** 2001. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* from healthy cattle in Japan. Appl. Environ. Microbiol. **67:**484–489.
32. **Koch, C., S. Hertwig, R. Lurz, B. Appel, and L. Beutin.** 2001. Isolation of a lysogenic bacteriophage carrying the stx_{1ox3} gene, which is closely associated with Shiga toxin-producing *Escherichia coli* strains from sheep and humans. J. Clin. Microbiol. **39:**3992–3998.
33. **Kudva, I. T., P. G. Hatfield, and C. J. Hovde.** 1997. Characterization of *Escherichia coli* O157-H7 and other Shiga toxin-producing *E. coli* serotypes isolated from sheep. J. Clin. Microbiol. **35:**892–899.
34. **McNally, A., A. J. Roe, S. Simpson, F. M. Thomson-Carter, D. E. E. Hoey, C. Currie, T. Chakraborty, D. G. E. Smith, and D. L. Gally.** 2001. Differences in levels of secreted locus of enterocyte effacement proteins between human disease-associated and bovine *Escherichia coli* O157. Infect. Immun. **69:**5107–5114.
35. **Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe.** 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5:**607–625.
36. **Meng, J., S. Zhao, and M. P. Doyle.** 1998. Virulence genes of Shiga toxin-producing *Escherichia coli* isolated from food, animals and humans. Int. J. Food. Microbiol. **45:**229–235.
37. **Misselwitz, J., H. Karch, M. Bielazewska, U. John, F. Ringelmann, G. Ronnefarth, and L. Patzer.** 2003. Cluster of hemolytic-uremic syndrome caused by Shiga toxin-producing *Escherichia coli* O26:H11. Pediatr. Infect. Dis. J. **22:**349–354.
38. **Osek, J.** 1999. Prevalence of Shiga toxin genes among *Escherichia coli* strains isolated from pigs. Vet. Rec. **145:**557–558.
39. **Paton, A. W., and J. C. Paton.** 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, *eaea*, enterohemorrhagic *E. coli hlya*, rfb_{O111}, and rfb_{O157}. J. Clin. Microbiol. **36:**598–602.
40. **Phillips, A. D., and G. Frankel.** 2000. Intimin-mediated tissue specificity in enteropathogenic *Escherichia coli* interaction with human intestinal organ cultures. J. Infect. Dis. **181:**1496–1500.
41. **Philips, A. D., S. Navabpour, S. Hicks, G. Dougan, T. Wallis, and G. Frankel.** 2000. Enterohemorrhagic *Escherichia coli* O157:H7 target Peyer's patches in humans and cause attaching/effacing lesions in both human and bovine intestine. Gut **47:**377–381.
42. **Pierard, D., G. Muyldermans, L. Moriau, D. Stevens, and S. Lauwers.** 1998. Identification of new verocytotoxin type 2 variant b-subunit genes in human and animal *Escherichia coli* isolates. J. Clin. Microbiol. **36:**3317–3322.
43. **Pierard, D., L. Vandamme, L. Moriau, D. Stevens, and S. Lauwers.** 1997. Virulence factors of verocytotoxin-producing *Escherichia coli* isolated from raw meats. Appl. Environ. Microbiol. **63:**4585–4587.
44. **Pradel, N., V. Livrelli, C. De Champs, J. B. Palcoux, A. Reynaud, F. Scheutz, J. Sirot, B. Joly, and C. Forestier.** 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. J. Clin. Microbiol. **38:**1023–1031.
45. **Ramachandran, V., K. Brett, M. A. Hornitzky, M. Dowton, K. A. Bettelheim, M. J. Walker, and S. P. Djordjevic.** 2003. Distribution of intimin subtypes among *Escherichia coli* isolates from ruminant and animal sources. J. Clin. Microbiol. **41:**5022–5032.
46. **Ramachandran, V., M. A. Hornitzky, K. A. Bettelheim, M. J. Walker, and S. P. Djordjevic.** 2001. The common ovine Shiga toxin 2-containing *Escherichia coli* serotypes and human isolates of the same serotypes possess a Stx2d toxin type. J. Clin. Microbiol. **39:**1932–1937.
47. **Ritchie, J. M., P. L. Wagner, D. W. K. Acheson, and M. K. Waldor.** 2003. Comparison of Shiga toxin production by hemolytic-uremic syndrome-associated and bovine-associated Shiga toxin-producing *Escherichia coli* isolates. Appl. Environ. Microbiol. **69:**1059–1066.
48. **Scotland, S. M., G. A. Willshaw, H. R. Smith, and B. Rowe.** 1990. Properties of strains of *Escherichia coli* O26:H11 in relation to their enteropathogenic or enterohemorrhagic classification. J. Infect. Dis. **162:**1069–1074.
49. **Thomas, A. H., H. Chart, T. Cheaty, H. R. Smith, J. A. Frost, and B. Rowe.** 1993. Vero cytotoxin-producing *Escherichia coli*, particularly serogroup O157, associated with human infections in the United Kingdom: 1989–91. Epidemiol. Infect. **110:**591–600.
50. **Urdahl, A. M., L. Beutin, E. Skjerve, and Y. Wasteson.** 2002. Serotypes and virulence factors of Shiga toxin-producing *Escherichia coli* isolated from healthy Norwegian sheep. J. Appl. Microbiol. **93:**1026–1033.
51. **Urdahl, A. M., L. Beutin, E. Skjerve, S. Zimmermann, and Y. Wasteson.** 2003. Animal host associated differences in Shiga toxin-producing *Escherichia coli* isolated from sheep and cattle on the same farm. J. Appl. Microbiol. **95:**92–101.
52. **Welinder-Olsson, C., M. Badenfors, T. Cheasty, E. Kjellin, and B. Kaijser.** 2002. Genetic profiling of enterohemorrhagic *Escherichia coli* strains in relation to clonality and clinical signs of infection. J. Clin. Microbiol. **40:**959–964.
53. **Werber, D., A. Fruth, A. Liesegang, M. Littmann, U. Buchholz, R. Prager, H. Karch, T. Breuer, H. Tschape, and A. Ammon.** 2002. A multistate outbreak of Shiga toxin-producing *Escherichia coli* O26:H11 infections in Germany, detected by molecular subtyping surveillance. J. Infect. Dis. **186:**419–422.
54. **Willshaw, G. A., T. Cheasty, H. R. Smith, S. J. O'Brien, and G. K. Adak.** 2001. Verocytotoxin-producing *Escherichia coli* (VTEC) O157 and other VTEC from human infections in England and Wales: 1995–1998. J. Med. Microbiol. **50:**135–142.
55. **Willshaw, G. A., S. M. Scotland, H. R. Smith, and B. Rowe.** 1992. Properties of vero cytotoxin-producing *Escherichia coli* of human origin of O serogroups other than O157. J. Infect. Dis. **166:**792–802.
56. **Zhang, W. L., M. Bielaszewska, T. Kuczius, and H. Karch.** 2002. Identification, characterization, and distribution of a Shiga toxin 1 gene variant (stx_{1c}) in *Escherichia coli* strains isolated from humans. J. Clin. Microbiol. **40:**1441–1446.