JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2003, p. 926–936 Vol. 41, No. 3
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.3.926–936.2003

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

Kim N. Brett,[1,2] Vidiya Ramachandran,[1,2] Michael A. Hornitzky,[1] Karl A. Bettelheim,[3] Mark J. Walker,[2] and Steven P. Djordjevic[1]*

Cooperative Research Centre for Cattle and Beef Quality, Elizabeth Macarthur Agricultural Institute, Camden, New South Wales 2570,[1] Department of Biological Sciences, University of Wollongong, New South Wales 2522,[2] and Microbiological Diagnostic Unit, Public Health Laboratory, Department of Microbiology and Immunology, University of Melbourne, Parkville, Victoria 3052,[3] Australia

Received 5 June 2002/Returned for modification 6 November 2002/Accepted 15 December 2002

Unlike Shiga toxin 2 (stx_2) genes, most nucleotide sequences of Shiga toxin 1 (stx_1) genes from Shiga toxin-producing *Escherichia coli* (STEC), *Shigella dysenteriae*, and several bacteriophages (H19B, 933J, and H30) are highly conserved. Consequently, there has been little incentive to investigate variants of stx_1 among STEC isolates derived from human or animal sources. However stx_{1OX3}, originally identified in an OX3:H8 isolate from a healthy sheep in Germany, differs from other stx_1 subtypes by 43 nucleotides, resulting in changes to 12 amino acid residues, and has been renamed stx_{1c}. In this study we describe the development of a PCR-restriction fragment length polymorphism (RFLP) assay that distinguishes stx_{1c} from other stx_1 subtypes. The PCR-RFLP assay was used to study 378 stx_1-containing STEC isolates. Of these, 207 were isolated from sheep, 104 from cattle, 45 from humans, 11 from meat, 5 from swine, 5 from unknown sources, and 1 from a cattle water trough. Three hundred fifty-five of the 378 isolates (93.9%) also possessed at least one other associated virulence gene (*ehxA*, *eaeA*, and/or stx_2); the combination stx_1, stx_2, and *ehxA* was the most common (175 of 355 [49.3%]), and 90 of 355 (25.4%) isolates possessed *eaeA*. One hundred thirty-six of 207 (65.7%) ovine isolates possessed stx_{1c} alone and belonged to 41 serotypes. Seventy-one of 136 (52.2%) comprised the common ovine serotypes O5:H$^-$, O128:H2, and O123:H$^-$. Fifty-two of 207 isolates (25.1%) possessed an stx_1 subtype; 27 (51.9%) of these belonged to serotype O91:H$^-$. Nineteen of 207 isolates (9.2%) contained both stx_{1c} and stx_1 subtypes, and 14 belonged to serotype O75:H8. In marked contrast, 97 of 104 (93.3%) bovine isolates comprising 44 serotypes possessed an stx_1 subtype, 6 isolates possessed stx_{1c}, and the remaining isolate possessed both stx_{1c} and stx_1 subtypes. Ten of 11 (91%) isolates cultured from meat in New Zealand possessed stx_{1c} (serotypes O5:H$^-$, O75:H8/H40, O81:H26, O88:H25, O104:H$^-$/H7, O123:H$^-$/H10, and O128:H2); most of these serotypes are commonly recovered from the feces of healthy sheep. Serotypes containing stx_1 recovered from cattle rarely were the same as those isolated from sheep. Although an stx_{1c} subtype was never associated with the typical enterohemorrhagic *E. coli* serogroups O26, O103, O111, O113, and O157, 13 human isolates possessed stx_{1c}. Of these, six isolates with serotype O128:H2 (from patients with diarrhea), four O5:H$^-$ isolates (from patients with hemolytic-uremic syndrome), and three isolates with serotypes O123:H$^-$ (diarrhea), OX3:H8 (hemolytic-uremic syndrome), and O81:H6 (unknown health status) represent serotypes that are commonly isolated from sheep.

Ruminants represent one of the largest reservoirs of Shiga toxin-producing *Escherichia coli* (STEC), which is commonly excreted in the feces of meat-producing animals (3, 5, 6, 14, 22, 27, 29, 30, 50). More than 200 different serotypes of STEC have been described so far (13, 22, 28, 61), and more than 150 of these have been reported to have been recovered from humans with hemorrhagic colitis or hemolytic-uremic syndrome (HUS) (http://www.microbionet.com.au). However, a subset of STEC strains (e.g., those of serogroups O26, O103, O111, O113, and O157), often referred to as enterohemorrhagic *E. coli* (EHEC), are more commonly associated with such serious afflictions and often possess associated virulence factors such as plasmid-encoded enterohemolysin and proteins (EaeA, Tir, and Esp) encoded by the locus of enterocyte effacement (LEE) (10, 22, 47). Although genes such as *saa* (45), *iha* (55), *ureC* (23, 36), and *efaI* (39) have been found in association with the genomes of such STEC strains, further studies are needed to determine their role in pathogenesis. Furthermore, differences in levels of secreted LEE proteins among STEC serotypes may also affect virulence (32).

In marked contrast to the expression of stx_2, expression of stx_1 in *E. coli* is regulated by host-derived signals such as the availability of iron and body temperature (11). stx_1 sequences derived from STEC O111:H$^-$ and O48:H21 strains and from three bacteriophages (H19B, 933J, and H30) have been reported to be very similar to the sequence derived from *Shigella dysenteriae* (43) and result in a limited number of amino acid substitutions. Unlike these common stx_1 subtypes, stx_{1OX3} possesses 43 nucleotide mismatches compared to $stx_{1933\text{-}J}$, resulting in 12 amino acid changes (43). Koch et al. (30) have examined the presence of stx_{1OX3} among 148 stx_1-containing *E.*

* Corresponding author. Mailing address: NSW Agriculture, Elizabeth Macarthur Agricultural Institute, Private Mail Bag 8, Camden, NSW, Australia, 2570. Phone: 61-246-406426. Fax: 61-246-406384. E-mail: steve.djordjevic@agric.nsw.gov.au.

coli isolates derived from human and animal sources. The stx_{1OX3} gene was shown to be present in 38 of 48 (79.2%) sheep-derived STEC isolates belonging to serotypes O5:NM, O125:H$^-$, O128:H2, O146:H21, and OX3:H8 but was not present in isolates with serotype O91:NM. These serotypes are commonly recovered from ovine but rarely from bovine sources (5–7, 14, 27, 31, 50). Koch et al., (30) also showed that stx_{1OX3}-positive strains were recovered from humans with diarrhea and that a proportion of these isolates possessed serotypes commonly associated with STEC from ovine sources. These preliminary observations suggest that the association of the stx_{1OX3} gene with ovine STEC strains from other geographical locations requires further investigation.

Zhang et al. (63) investigated the presence of the atypical stx_{1OX3} variant among 212 STEC strains recovered from humans. These authors renamed stx_{OX3}, designating it stx_{1c} (63). Since stx_{1c} has now been identified among serologically diverse STEC strains (30, 58, 63), we retained this nomenclature for our study. Zhang et al. (63) reported stx_{1c} in 36 STEC strains, of which 23 concomitantly possessed stx_{2d}, 12 possessed stx_{1c} alone, and 1 possessed both stx_{1c} and stx_2. Of the 36 stx_{1c}-positive strains, 19 were recovered from asymptomatic patients and 16 were derived from fecal samples of patients with uncomplicated diarrhea. Only a single stx_{1c}-positive isolate was recovered from a patient with HUS. The 36 stx_{1c}-positive isolates belonged to 15 serotypes, none of which included the major STEC serogroups (O26, O103, O111, O113, O145, and O157) (63). Several studies (19, 30, 50, 58, 63) suggest that sheep may be a natural reservoir of stx_{1c}-positive STEC strains that enter the human food chain.

Shiga toxin genes (*stx*) are encoded in the genomes of lambdoid phages (51). Bacteriophage transmission represents the major vehicle in the spread of *stx* among serologically diverse populations of *E. coli* (30, 51) and contributes significantly to the emergence of new STEC clones (51). The location of *stx* downstream of phage lysis genes suggests that phage promoters (57, 59) control the expression of Shiga toxin. Bacteriophages survive better in water than their bacterial hosts and are reported to be more resistant to chlorination and pasteurization (34, 35). Monitoring *stx* subtypes within ruminant and environmental populations of STEC should lead to a better understanding of the movement of bacteriophage within these environments.

Shiga toxins play a major role in inducing vascular injury in the intestinal microcirculation and have been shown to directly affect the intestinal epithelium, although different responses have been reported for different hosts (reviewed in reference 40). More specifically, Shiga toxins perturb cytokine expression patterns as a consequence of their interaction with epithelial cells (1, 56, 62). Rabbit models have been used to demonstrate the ability of stx_1-positive strains to induce more-severe diarrhea and mucosal injury (54), increased inflammatory changes, and elevated mucosal interleukin-1 activity (8) compared with those in rabbits infected with isogenic strains lacking stx_1. The ability of purified stx_1 to induce similar inflammatory responses when inoculated intragastrically into rabbits reinforces these observations (41). However, to our knowledge, the abilities of different Shiga toxin 1 subtypes to induce these physiological changes have not been addressed.

A recent report demonstrating that bovine crypt intestinal epithelium, lining the ileum and jejunum of the small intestine and the cecum and colon of the large intestine, expresses Gb3 receptors for verotoxin 1 (stx_1) from *E. coli* O157:H7 has potentially profound implications for the role of Shiga toxin in the colonization of ruminant species by STEC (25). The hypothesis that Shiga toxin 1 may modulate the bovine immune response is supported by observations that this toxin (i) has been shown to inhibit the activation and proliferation of subpopulations of bovine lymphocytes (33), (ii) suppresses bovine leukemia virus-related spontaneous lymphocyte proliferation (18), and (iii) binds to submucosal lymphoid aggregates (25). Although STEC typically inhabits the gastrointestinal tracts of healthy meat-producing animals, certain subpopulations of STEC may also induce gastrointestinal disease in these animals (9, 60). Wieler et al. (60) showed that the majority of STEC isolates recovered from diarrheic calves concomitantly possessed *eaeA* and stx_1, and they concluded that stx_1-positive strains are more virulent for calves. To our knowledge, the presence of different stx_1 subtypes among STEC isolates derived from cattle has not been intensively investigated.

Previously, we and others have shown that STEC strains that commonly inhabit the gastrointestinal tracts of healthy sheep and cattle represent serologically distinct populations (5–7, 14, 27, 58). Furthermore, it has also been demonstrated that STEC isolates with stx_2 that are commonly recovered from sheep predominantly possess the stx_{2d} subtype (46, 48, 50) whereas bovine stx_2-containing STEC isolates typically possess stx_2 and $stx_{2vha/b}$ subtypes (3; our unpublished data). We considered it important to subtype stx_1 genes in a serologically diverse collection of STEC isolates derived predominantly from ruminants in Australia in order to determine if different stx_1 subtypes also associate with particular hosts. In this study a PCR-restriction fragment length polymorphism (RFLP) assay which differentiates stx_{1c} from the common stx_1-related sequences was developed. The assay was used to characterize 378 stx_1-containing STEC isolates derived predominantly from ovine and bovine sources. We also examined stx_1 subtypes in 45 human STEC strains derived mostly from patients with gastrointestinal and systemic diseases.

MATERIALS AND METHODS

STEC isolates. Three hundred seventy-eight stx_1-containing STEC isolates were used in this study (Table 1); of these, 313 were derived from healthy meat-producing animals, 45 were of human origin, 11 were from a blind study of meat from New Zealand, 5 had unknown histories, 3 were individual isolates from bovines of unknown health status, and 1 was from a water source. Two hundred seven isolates were derived from ovine sources (203 animals) and were obtained from the Elizabeth Macarthur Agricultural Institute (EMAI), Camden, New South Wales, Australia. One hundred four isolates were of bovine origin (83 animals); of these, 99 were obtained from the EMAI and 5 were from the Microbiological Diagnostic Unit (MDU), Melbourne, Australia. The majority of these STEC isolates were collected from healthy sheep and cattle in eastern Australia (14, 27; our unpublished data). Five stx_1-containing isolates from healthy pigs from the EMAI collection were also included. Thirty-seven stx_1-containing human isolates were from the MDU, and eight isolates were from the National Reference Laboratory for Foodborne Diseases, Bern, Switzerland. The 37 human isolates from the MDU included 4 isolates (O81:H6, OR:H$^-$, Ont:H$^-$, and O26:H11) from New Zealand (from individuals of unknown health status), 3 O157:H7 isolates from the 1996 outbreak in Japan (kindly supplied by H. Watanabe), 2 O111:H$^-$ isolates from Italy (kindly provided by A. Caprioli), and a single O157:H7 isolate from the Jack-in-the-Box outbreak in the United States (Table 1). B. Bochner kindly supplied isolates from the United States. These isolates were cultured by previously published procedures (14).

TABLE 1. Virulence factor profiles and stx_1 subtypes among STEC isolates of animal and human origins

Source[a]	Clinical condition[b]	Serotype	No. of isolates	No. of animals or humans	Virulence factor profile				No. of isolates with stx_1 subtype:		
					stx$_1$	*stx*$_2$	*eaeA*	*ehxA*	*stx*$_1$	*stx*$_{1c}$	*stx*$_1$/*stx*$_{1c}$
Ovine	Healthy	O2:H29	1	1	+	−	−	+	1		
Bovine	Healthy	O3:H7	7	2	+	+	−	+	7		
Bovine	Healthy	O5:H$^-$	3	3	+	+	−	+	1	2	
Bovine	Diagnostic	O5:H$^-$	5	1	+	−	+	+	5		
Ovine	Healthy	O5:H$^-$	14	14	+	+	−	+		14	
Ovine	Healthy	O5:H$^-$	7	6	+	−	−	+		7	
Ovine	Healthy	O5:H$^-$	1	1	+	+	−	−		1	
Human/MDU	HUS	O5:H$^-$	1	1	+	−	+	+		1	
Human/MDU	HUS	O5:H$^-$	2	2	+	−	−	+		2	
Human/MDU	HUS	O5:H$^-$	1	1	+	+	−	+		1	
Meat/New Zealand	Unknown	O5:H$^-$	1	1	+	+	−	+		1	
Bovine	Healthy	O5:H7	3	3	+	+	−	+	3		
Ovine	Healthy	O5:HR	1	1	+	+	+	+			1
Ovine	Healthy	O6:H$^-$	1	1	+	+	−	+		1	
Ovine	Healthy	O6:H$^-$	1	1	+	−	−	+		1	
Ovine	Healthy	O8:Hnt	1	1	+	+	−	+		1	
Human/NRLFD	HUS	O8:H8	1	1	+	−	−	−	1		
Bovine	Healthy	O8:H16	1	1	+	+	−	+	1		
Ovine	Healthy	O21:H21	1	1	+	+	−	+		1	
Bovine	Healthy	O26:H$^-$	1	1	+	−	+	+	1		
Ovine	Healthy	O26:H$^-$	2	2	+	−	+	+	2		
Human/MDU	Diarrhea	O26:H$^-$	3	3	+	−	+	+	3		
Unknown/United States	Unknown	O26:H$^-$	1	1	+	−	+	+	1		
Bovine	Healthy	O26:H11	11	7	+	−	+	+	11		
Bovine	Healthy	O26:H11	1	1	+	−	−	+	1		
Bovine	Healthy	O26:H11	1	1	+	−	−	−	1		
Ovine	Healthy	O26:H11	4	4	+	−	+	+	4		
Human/MDU	Diarrhea	O26:H11	3	3	+	−	+	−	3		
Human/MDU	Diarrhea	O26:H11	6	5	+	−	+	+	6		
Human/NRLFD	Diarrhea	O26:H11	2	2	+	−	+	+	2		
Human/New Zealand	Unknown	O26:H11	1	1	+	−	+	+	1		
Bovine	Healthy	O28:H$^-$	1	1	+	−	+	+	1		
Bovine	Healthy	O32/83:H7	1	1	+	−	−	−	1		
Bovine	Healthy	O37:H10	1	1	+	+	+	−			1
Ovine	Healthy	O55:H20	1	1	+	+	+	+			1
Bovine	Healthy	O68:H$^-$	1	1	+	−	−	+	1		
Ovine	Healthy	O69:H8	1	1	+	−	−	−		1	
Ovine	Healthy	O75:H$^-$	1	1	+	+	−	−		1	
Bovine	Healthy	O75:H1	1	1	+	−	−	+	1		
Ovine	Healthy	O75:H8	3	3	+	+	−	−	1	1	1
Ovine	Healthy	O75:H8	1	1	+	−	−	−	1		
Ovine	Healthy	O75:H8	16	16	+	+	−	+	1	2	13
Ovine	Healthy	O75:H8	1	1	+	−	−	+		1	
Meat/New Zealand	Unknown	O75:H8	1	1	+	+	−	+		1	
Ovine	Healthy	O75:H40	1	1	+	−	−	+		1	
Ovine	Healthy	O75:H40	1	1	+	+	−	+		1	
Meat/New Zealand	Unknown	O75:H40	1	1	+	+	−	+		1	
Ovine	Healthy	O77:H4	1	1	+	−	−	+		1	
Ovine	Healthy	O77:H$^-$	1	1	+	−	−	+		1	
Human/New Zealand	Unknown	O81:H6	1	1	+	−	−	+		1	
Ovine	Healthy	O81:H26	1	1	+	−	−	+		1	
Meat/New Zealand	Unknown	O81:H26	1	1	+	−	−	+		1	
Bovine	Healthy	O81:H31	1	1	+	−	−	+	1		
Bovine	Healthy	O82:H40	2	2	+	+	−	+	2		
Bovine	Healthy	O82:H8	6	6	+	+	−	+	6		

Continued

TABLE 1—*Continued*

Source[a]	Clinical condition[b]	Serotype	No. of isolates	No. of animals or humans	Virulence factor profile				No. of isolates with stx_1 subtype:		
					stx_1	stx_2	*eaeA*	*ehxA*	stx_1	stx_{1c}	stx_1/stx_{1c}
Bovine	Healthy	O84:H$^-$	1	1	+	−	+	+	1		
Ovine	Healthy	O84:H$^-$	1	1	+	−	+	+	1		
Bovine	Healthy	O84:H2	1	1	+	−	+	+	1		
Ovine	Healthy	O88:H8	1	1	+	+	+	−			1
Meat/New Zealand	Unknown	O88:H25	1	1	+	−	−	−		1	
Ovine	Healthy	O91:H$^-$	21	20	+	+	−	+	18	3	
Ovine	Healthy	O91:H$^-$	6	6	+	−	−	+	4	2	
Ovine	Healthy	O91:H$^-$	5	5	+	+	−	−	5		
Human/MDU	Healthy	O91:H$^-$	2	2	+	+	−	−	2		
Meat/New Zealand	Unknown	O91:H$^-$	1	1	+	+	−	+	1		
Bovine/Hong Kong	Unknown	O91:H14	1	1	+	+	−	+	1		
Ovine	Diagnostic	O103:H2	1	1	+	−	+	+	1		
Human/MDU	Diarrhea	O103:H2	1	1	+	−	+	+	1		
Ovine	Healthy	O103:H38	1	1	+	+	−	+			1
Meat/New Zealand	Unknown	O104:H7	1	1	+	−	−	−		1	
Meat/New Zealand	Unknown	O104:H$^-$	1	1	+	−	−	−		1	
Ovine	Healthy	O106:H18	1	1	+	+	−	+		1	
Ovine	Healthy	O106:HR	1	1	+	−	+	−		1	
Bovine	Healthy	O108:H7	3	3	+	+	−	+	3		
Bovine	Healthy	O108:H7	1	1	+	−	−	−	1		
Bovine	Healthy	O110:H40	1	1	+	+	−	+	1		
Bovine	Healthy	O111:H$^-$	2	2	+	+	+	+	2		
Human/Italy	Unknown	O111:H$^-$	1	1	+	−	+	−	1		
Human/Italy	Unknown	O111:H$^-$	1	1	+	+	+	−	1		
Unknown/Canada	Unknown	O111:H$^-$	1	1	+	−	+	−	1		
Unknown/United States	Unknown	O111:H$^-$	1	1	+	−	+	−	1		
Unknown/Germany	Unknown	O111:H$^-$	1	1	+	−	+	−	1		
Bovine	Healthy	O111:H8	2	1	+	+	+	+	2		
Ovine	Healthy	O112ab:H2	2	2	+	+	−	−	2		
Ovine	Healthy	O112ab:H2	1	1	+	−	−	−		1	
Ovine	Healthy	O112ab:H2	1	1	+	+	+	+	1		
Bovine	Healthy	O113:H4	1	1	+	+	−	−	1		
Bovine/Canada	Unknown	O113:H4	1	1	+	+	−	−	1		
Bovine	Unknown	O113:H21	1	1	+	−	−	+	1		
Bovine	Unknown	O113:H21	1	1	+	+	−	−	1		
Human/NRLFD	Diarrhea	O117:H7	2	2	+	−	−	−	2		
Bovine	Healthy	O117:H21	1	1	+	−	−	−		1	
Human/NRLFD	Diarrhea	O118:H16	1	1	+	−	+	+	1		
Ovine	Healthy	O121:H2	1	1	+	+	−	+		1	
Ovine	Healthy	O123:H$^-$	1	1	+	−	−	+		1	
Ovine	Healthy	O123:H$^-$	1	1	+	+	+	+		1	
Ovine	Healthy	O123:H$^-$	19	19	+	+	−	+		19	
Human/NRLFD	Diarrhea	O123:H$^-$	1	1	+	+	−	+		1	
Meat/New Zealand	Unknown	O123:H$^-$	1	1	+	+	−	+		1	
Meat/New Zealand	Unknown	O123:H10	1	1	+	+	−	+		1	
Ovine	Healthy	O123:H11	1	1	+	−	−	+		1	
Ovine	Healthy	O128:H2	19	19	+	+	−	+		19	
Ovine	Healthy	O128:H2	5	5	+	−	−	+		5	
Ovine	Healthy	O128:H2	2	2	+	+	−	−		2	
Ovine	Healthy	O128:H2	1	1	+	−	−	−		1	
Human/MDU	Diarrhea	O128:H2	4	4	+	+	−	+		4	
Human/MDU	Diarrhea	O128:H2	2	2	+	+	−	−		2	
Meat/New Zealand	Unknown	O128:H2	1	1	+	+	−	+		1	
Ovine	Healthy	O128:H-/H2	1	1	+	−	−	−		1	
Ovine	Healthy	O128:H8/H2	1	1	+	+	−	+		1	

Continued

TABLE 1—*Continued*

Source[a]	Clinical condition[b]	Serotype	No. of isolates	No. of animals or humans	Virulence factor profile				No. of isolates with stx_1 subtype:		
					stx_1	stx_2	*eaeA*	*ehxA*	stx_1	stx_{1c}	stx_1/stx_{1c}
Ovine	Healthy	O128:Hnt	1	1	+	+	−	+		1	
Bovine	Healthy	O130:H11	2	2	+	+	−	+	2		
Bovine	Healthy	O130:H11	1	1	+	+	−	−	1		
Bovine	Healthy	O130:H38	2	2	+	+	−	+	2		
Bovine	Healthy	O149:H19	1	1	+	+	−	−	1		
Ovine	Healthy	O152:H21	1	1	+	+	−	+		1	
Ovine	Healthy	O153:H$^-$	1	1	+	−	−	−		1	
Bovine	Healthy	O153:H8	1	1	+	+	−	+		1	
Ovine	Healthy	O153:H8	1	1	+	+	−	−		1	
Bovine	Healthy	O153:H21	1	1	+	−	−	−	1		
Ovine	Healthy	O153:H25	3	3	+	−	−	+		3	
Ovine	Healthy	O153:H25	1	1	+	+	−	+		1	
Ovine	Healthy	O153:H25	1	1	+	−	−	−		1	
Bovine	Healthy	O153:HR	1	1	+	+	−	+	1		
Water	N/A	O154:H$^-$	1	1	+	+	+	+	1		
Ovine	Healthy	O154:HR	1	1	+	−	−	+		1	
Bovine	Healthy	O157:H$^-$	1	1	+	+	−	−	1		
Ovine	Healthy	O157:H$^-$	2	2	+	+	+	+	2		
Ovine	Healthy	O157:H$^-$	2	2	+	+	−	+	2		
Porcine	Healthy	O157:H$^-$	5	2	+	−	+	+	5		
Bovine	Healthy	O157:H7	1	1	+	+	+	+	1		
Bovine/Finland	Unknown	O157:H7	1	1	+	+	+	+	1		
Human/Japan	Unknown	O157:H7	1	1	+	+	+	+	1		
Human/Japan	Unknown	O157:H7	1	1	+	+	+	+	1		
Human/United States	Unknown	O157:H7	1	1	+	−	+	+	1		
Unknown/United States	Unknown	O157:H7	1	1	+	+	+	−	1		
Ovine	Healthy	O157:H21	1	1	+	+	+	+	1		
Ovine	Healthy	O158:HR	1	1	+	−	+	+		1	
Bovine	Healthy	O163:H$^-$	1	1	+	+	−	+	1		
Bovine	Healthy	O163:H19	1	1	+	+	−	+	1		
Ovine	Healthy	O163:H19	1	1	+	−	−	−		1	
Ovine	Healthy	O168:H21	1	1	+	−	−	−		1	
Bovine	Healthy	Ont:H1	2	2	+	+	−	+	2		
Bovine	Healthy	Ont:H2	2	1	+	+	−	+	2		
Bovine	Healthy	Ont:H4	1	1	+	−	−	+		1	
Bovine	Healthy	Ont:H8	1	1	+	+	−	+	1		
Ovine	Healthy	Ont:H8	1	1	+	+	+	−			1
Ovine	Healthy	Ont(A):H8	1	1	+	+	−	−		1	
Bovine	Healthy	Ont:H10	1	1	+	−	−	−		1	
Bovine	Healthy	Ont:H11	6	2	+	+	−	+	6		
Bovine	Healthy	Ont:H16	2	1	+	+	−	+	2		
Bovine	Healthy	Ont:H19	1	1	+	+	−	+	1		
Ovine	Healthy	Ont:H19	1	1	+	+	−	−	1		
Bovine	Healthy	Ont:H21	2	2	+	+	−	+	2		
Bovine	Healthy	Ont:H30	1	1	+	+	−	+	1		
Bovine	Healthy	Ont:H41	1	1	+	−	+	+	1		
Ovine	Healthy	Ont:H49	1	1	+	+	+	−	1		
Bovine	Healthy	Ont:H$^-$	2	1	+	−	+	+	2		
Bovine	Healthy	Ont:H$^-$	1	1	+	+	−	+	1		
Ovine	Healthy	Ont:H$^-$	1	1	+	−	−	−		1	
Ovine	Healthy	Ont:H$^-$	2	2	+	+	−	−	2		
Human/MDU	Unknown	Ont:H$^-$	1	1	+	−	+	+	1		
Human/New Zealand	Unknown	Ont:H$^-$	1	1	+	−	+	+	1		
Ovine	Healthy	Ont(A):H$^-$	1	1	+	−	−	+		1	
Ovine	Healthy	Ont:HR	9	7	+	−	−	+		9	

Continued

TABLE 1—*Continued*

Source[a]	Clinical condition[b]	Serotype	No. of isolates	No. of animals or humans	Virulence factor profile				No. of isolates with stx_1 subtype:		
					stx_1	stx_2	*eaeA*	*ehxA*	stx_1	stx_{1c}	stx_1/stx_{1c}
Ovine	Healthy	Ont:Hnt	1	1	+	+	−	−	1		
Human/MDU	Healthy	Ont:Hnt	1	1	+	−	+	+	1		
Ovine	Healthy	OR:H2	2	2	+	+	−	+		2	
Ovine	Healthy	OR:H2	1	1	+	−	−	+		1	
Ovine	Healthy	OR:H4	2	2	+	+	−	+		2	
Human/Japan	Unknown	OR:H7	1	1	+	−	+	+	1		
Bovine	Healthy	OR:H31	1	1	+	+	+	+	1		
Ovine	Healthy	OR:HR	1	1	+	−	−	+		1	
Bovine	Healthy	OR:H$^-$	1	1	+	−	+	+	1		
Ovine	Healthy	OR:H$^-$	2	2	+	+	−	+		2	
Ovine	Healthy	OR:H$^-$	1	1	+	+	−	−		1	
Human/New Zealand	Unknown	OR:H$^-$	1	1	+	−	−	+	1		
Ovine	Healthy	OX3:H2	1	1	+	−	−	−		1	
Bovine	Healthy	OX3:H8	1	1	+	+	−	+	1		
Ovine	Healthy	OX3:H8	1	1	+	+	−	−		1	
Human/NRLFD	HUS	OX3:H8	1	1	+	+	−	−		1	
Ovine	Healthy	OX3:HR	3	3	+	−	−	+		3	

[a] NRLFD, National Reference Laboratory for Foodborne Diseases.
[b] N/A, not applicable.

Multiplex PCR analysis of STEC isolates. All isolates were prepared and subjected to multiplex PCR for detection of STEC virulence factors stx_1, stx_2, *ehxA*, and *eaeA* as described previously (44) with the following modification: for DNA preparation, an Instagene matrix (Bio-Rad, Richmond, Calif.) was used as described previously (17). Amplified PCR products were then resolved by agarose gel electrophoresis (2%, wt/vol) and stained with ethidium bromide (5 μg/ml). Visualization was achieved by UV illumination, and images were captured by using a GelDoc 1000 image analysis station.

***stx*$_1$ subtyping.** The Clustal W program was used to align stx_1 genes deposited in GenBank, and the Mapplot program, accessed via the Australian National Genomic Information Service (ANGIS) (www.angis.org.au), was used to identify restriction enzyme cleavage sites. Since all Shiga toxin 1 gene sequence variants with the exception of stx_{1c} display more than 99% sequence identity with stx_{1933J}, all non-stx_{1c} sequences will be referred to as stx_1 subtypes in this report. To subtype stx_1 sequences, a 603-bp fragment of the gene was amplified by using the Gannon F and R primers (Table 2). Restriction enzymes *Cfo*I and *Rsa*I were used to cut the 603-bp fragment because they were predicted to generate RFLP profiles that readily distinguish stx_{1c} from other stx_1 subtypes. PCR assays were carried out in a 50-μl total volume containing 5 μl of a whole-cell DNA template prepared by using an Instagene matrix (17), 10 mM Tris-HCl (pH 8.3), 10 mM KCl, 2 mM $MgCl_2$, 10 pmol of each primer, 200 μM each deoxynucleoside triphosphate, and 1 U of *Taq* DNA. Thermocycler steps included an initial denaturation step of 94°C for 180 s, followed by 35 cycles of denaturation (94°C for 60 s), annealing (60°C for 60 s), and extension (72°C for 120 s). A final extension step of 72°C for 300 s completed the PCR. The PCR product (3 to 5 μg) was separately digested with 5 U each of *Cfo*I and *Rsa*I in 1× buffer L (Roche Pharmaceuticals) and was incubated at 37°C for a minimum of 4 h. Agarose gel (2%) electrophoresis was used to separate the restricted fragments, and subtypes were identified according to their restriction patterns.

Sequence analysis of *stx*$_1$. The stx_1 genes from two isolates of serotypes O26:H11 (isolate 507) and O5:H$^-$ (isolate 904) were sequenced. These isolates were selected because O26:H11 and O5:H$^-$ are common serotypes of STEC isolates recovered from healthy adult cattle and sheep, respectively. Secondly, serotype O26:H11 possesses an stx_1 RFLP profile that is indistinguishable from those of other stx_1 subtypes, and serotype O5:H$^-$ possesses an stx_1 RFLP profile that is indistinguishable from that of stx_{1c}. A 1,470-bp fragment encoding both A and

TABLE 2. Primers used to amplify and sequence stx_1

Primer name	Primer sequence	Size (bp)	Reference
stx_1 typing			
Gannon F	5′-ACACTGGATGATCTCAGTGG-3′	603	21
Gannon R	5′-CTGAATCCCCCTCCATTATG-3′		
stx_1 sequencing			
Paton 1F	5′-TCGCA-TGAGATCTGACC-3′	1,470	43
Paton 1 R	5′-AACTGACTGAATTGAGATG-3′		
Paton 2 F	5′-ATAAATCGCCATTCGTTGACTAC-3′	180	44
Paton 2 R	5′-AGAACGCCCACTGAGATCATC-3′		
Gannon F	5′-ACACTGGATGATCTCAGTGG-3′	603	21
Gannon R	5′-CTGAATCCCCCTCCATTATG-3′		
Paton 1 F	5′-TCGCATGAGATCTGACC-3′	448	This study
Vidiya 1 R	5′-AATAAGCCGTAGATTATT-3′		

B subunits of all Shiga toxin 1 genes was amplified by using primers Paton 1F and Paton 1R (Table 2) in a reaction volume of 50 μl. PCR was carried out by using 5 μl of a whole-cell DNA template prepared by using an Instagene matrix (17), 10 mM Tris-HCl (pH 8.3), 10 mM KCl, 2 mM $MgCl_2$, 10 pmol of each primer, 200 μM each deoxynucleoside triphosphate, and 1 U of *Taq* DNA polymerase. Thermocycler steps involved an initial denaturation step of 94°C for 180 s, followed by 35 cycles of denaturation (94°C for 30 s), annealing (54°C for 30 s), and extension (72°C for 60 s). A final extension step of 72°C for 300 s completed the PCR. The amplified PCR product was separated by agarose gel electrophoresis (2%) and purified for DNA sequencing by using the QIAquick DNA purification kit (Qiagen, Hilden, Germany). Primers used for sequencing are listed in Table 2. Sequencing reactions were performed by using the Big Dye terminator cycle sequencing ready reaction DNA sequencing kit and electrophoresed on an ABI Prism 377 DNA sequencer (Perkin-Elmer, Santa Clara, Calif.) as described previously (50). Auto Assembler software (Perkin-Elmer) was used to compile and analyze the DNA sequences. Nucleotide and amino acid analyses were performed by using programs accessed via ANGIS. Sequences were compared with those deposited in public databases by using the BlastN and Blast P algorithms (2).

RESULTS

Detection of STEC virulence factors by multiplex PCR. Of 378 stx_1-containing isolates, 207, 104, and 45 were derived from ovine, bovine, and human sources, respectively. Eleven isolates from New Zealand meat, five serotype O157:H$^-$ isolates recovered from porcine feces, five isolates from unknown sources, and a single isolate cultured from a water trough completed the collection. Among the 207 ovine isolates, the predominant virulence factor combinations were represented by 108 isolates (52.2%) with stx_1, stx_2, and *ehxA*; 46 isolates (22.2%) with stx_1 and *ehxA*; 22 isolates (10.6%) with stx_1 and stx_2; 11 isolates (5.3%) with stx_1 alone; 9 isolates (4.3%) with stx_1, *eaeA*, and *ehxA*; and 7 isolates (3.4%) with all four factors. Of 104 bovine isolates, 54 (52.0%) contained stx_1, stx_2, and *ehxA*; 24 (23.1%) contained stx_1, *ehxA*, and *eaeA*; 6 (5.8%) contained stx_1 only; 6 (5.8%) possessed stx_1 and *ehxA*; 7 (6.7%) contained all four factors; 6 (5.8%) possessed stx_1 and stx_2; and 1 (0.96%) possessed stx_1, stx_2, and *eaeA*. Of 45 human isolates, 20 (44.4%) contained stx_1, *ehxA*, and *eaeA*; 6 (13.3%) contained stx_1, stx_2, and *ehxA*; 5 (11.1%) contained stx_1 and stx_2; 4 (8.9%) contained stx_1 and *eaeA*; 3 (6.7%) contained stx_1 alone; 4 (8.9%) contained stx_1 and *ehxA*; 2 (4.4%) contained all four factors; and 1 (2.2%) contained stx_1, stx_2, and *ehxA*. Of the 11 meat isolates, 7 (63.6%) contained stx_1, stx_2, and *ehxA*; 3 (27.3%) contained stx_1 alone; and 1 (9.1%) contained stx_1 and *ehxA*. All five porcine O157:H$^-$ isolates included in this study were positive for stx_1, *eaeA*, and *ehxA*. STEC serotypes and their virulence factor profiles are listed in Table 1.

Development of a PCR-RFLP assay to identify stx_{1c}. RFLP patterns generated by separate digestions of a 603-bp fragment of stx_1 with *Cfo*I and *Rsa*I are shown in Fig. 1A and B, respectively. The 603-bp fragment amplified from STEC isolates possessing stx_1 subtypes (defined as stx_1 subtypes that share more than 99% nucleotide sequence identity with stx_{1933J}) generated fragments of 322, 135, 83, and 63 bp with *Cfo*I and 603 bp with *Rsa*I (Fig. 1, lanes 1 to 5 and 7). However, the 603-bp fragment amplified from STEC isolates possessing stx_{1c} generated fragments of 414 and 189 bp with *Cfo*I and 386 and 217 bp with *Rsa*I, respectively (Fig. 1, lanes 6 and 8 to 11). This assay was used to type stx_1 from 378 stx_1-containing STEC isolates (Table 1).

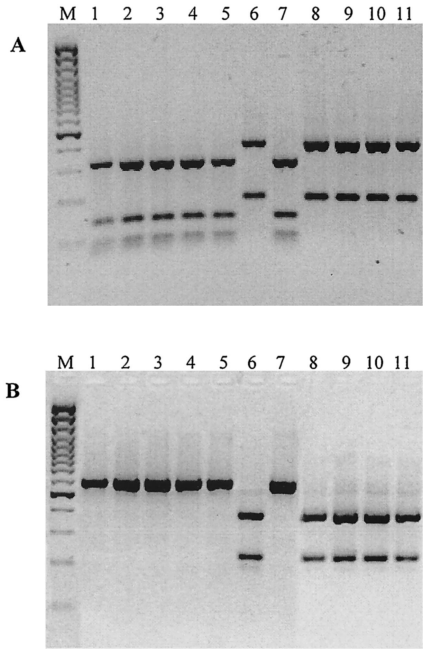

FIG. 1. *Cfo*I (A) and *Rsa*I (B) digests of PCR products obtained with primers Gannon F and Gannon R. Lanes: M, 100-bp Plus marker; 1, O82:H8 (bovine feces); 2, O3:H7 (bovine feces); 3, O26:H11 (bovine feces); 4, OX3:H8 (bovine feces); 5, O130:H11 (bovine feces); 6, O5:H$^-$ (ovine feces); 7, O91:H$^-$ (ovine feces); 8, O123:H$^-$ (ovine feces); 9, O75:H8 (ovine feces); 10, O128:H2 (ovine feces); 11, O123:H$^-$ (ovine feces).

stx_1 subtyping and association with serotype. Of 207 ovine isolates, 136 (65.7%) possessed stx_{1c}, 52 (25.1%) possessed an stx_1 subtype, and 19 (9.2%) concomitantly possessed both stx_{1c} and stx_1 subtypes. Interestingly, STEC isolates that possessed both stx_{1c} and stx_1 were all (except one isolate) of ovine origin and belonged either to serotype O75:H8 (accounting for 14 of the 21 ovine isolates with this serotype) or to one of the following serotypes (each represented by a single isolate): O103:H38, Ont:H8, O88:H8, O55:H20, and O5:HR. A single bovine isolate (serotype O37:H10) possessed both stx_{1c} and a common stx_1 subtype (Table 1).

Ovine STEC isolates positive for stx_{1c} alone comprised 41 serotypes, of which the most common were O128:H2 (with all 28 isolates of this serotype positive for stx_{1c} alone), O5:H$^-$ (all 22 isolates), O123:H$^-$ (all 21 isolates), Ont:HR (all 9 isolates), O153:H25 (all 5 isolates), O91:H$^-$ (5 of 32 isolates), O75:H8 (4 of 21 isolates), and OX3:HR (all 3 isolates). With the exception of isolates with serotypes OR:H$^-$/H2/H4, O6:H$^-$, and O75:H40, the remaining 28 serotypes containing stx_{1c} were represented by a single isolate (Table 1).

Ovine isolates that possessed stx_1 alone belonged to serotypes O26:H$^-$ (with both ovine isolates of this serotype possessing stx_1 alone), O26:H11 (all 4 isolates), O91:H$^-$ (27 of 32 isolates), O157:H$^-$ (4 of 4 isolates), O75:H8 (3 of 21 isolates), O112ab:H2 (3 of 4 isolates), and Ont:H$^-$ (2 of 3 isolates); each of the remaining serotypes was represented by a single isolate (Table 1). Serogroup O26 strains are not commonly isolated from ovine feces (14). Six isolates belonging to serogroup O26 included in this study were recovered from newborn lambs

during intensive sampling on a property that simultaneously grazed sheep and cattle, and their stx_1 subtypes were indistinguishable from stx_1 subtypes found in O26 isolates recovered from cattle.

Of 104 bovine isolates possessing stx_1, 97 (93.3%) contained an stx_1 subtype and belonged to 44 serotypes including O26:H11 (13 of 13 bovine isolates of this serotype), O3:H7 (7 of 7 isolates), O82:H8 (6 of 6 isolates), Ont:H11 (6 of 6 isolates), O5:H$^-$ (6 of 8 isolates), O5:H7 (3 of 3 isolates), O108:H7 (4 of 4 isolates), O82:H40 (2 of 2 isolates), O111:H$^-$ (2 of 2 isolates), O111:H8 (2 of 2 isolates), O113:H4 (2 of 2 isolates), O113:H21 (2 of 2 isolates), O130:H11 (3 of 3 isolates), O130:H38 (2 of 2 isolates), and O157:H7 (2 of 2 isolates); the remaining serotypes were each represented by a single isolate (Table 1). Only 6 of 104 (5.8%) bovine isolates contained stx_{1c}. Those containing stx_{1c} alone belonged to serotypes O5:H$^-$ (accounting for two of eight bovine isolates of this serotype), O153:H8 (one isolate), O117:H21 (one isolate), Ont:H4 (one isolate), and Ont:H10 (one isolate). A single isolate of serotype O37:H10 contained both stx_{1c} and stx_1 subtypes (Table 1).

Of 45 human isolates, 31 were isolated from patients with diarrhea (25 isolates, 7 serotypes) or HUS (6 isolates, 3 serotypes) (Table 1). Two isolates (both serotype O91:H$^-$) were recovered from an asymptomatic patient, and one isolate was from a healthy individual (serotype Ont:Hnt). Of the remaining 11 human isolates, recovered from patients of unknown health status, 4 (serotypes O81:H6, O26:H11, Ont:H$^-$, and OR:H$^-$) were from New Zealand, 3 (2 with serotype O157:H7 and one with OR:H7) were recovered during a HUS outbreak in Japan in 1996, 2 (both serotype O111:H$^-$) were from Italy, 1 (serotype O157:H7, associated with the Jack-in-the-Box-outbreak) was from the United States, and 1 was from Australia (serotype Ont:H$^-$) (Table 1). All six human isolates with serotype O128:H2 (diarrhea), four O5:H$^-$ (HUS) isolates, one isolate with serotype O123:H$^-$ (diarrhea), and one isolate with OX3:H8 (HUS)—all serotypes frequently isolated from sheep—as well as a single isolate of serotype O81:H6 from a patient in New Zealand possessed stx_{1c}. Twelve isolates with serotype O26:H11 and 3 with serotype O26:H$^-$ (14 of these 15 isolates were recovered from patients with diarrhea), both serotypes commonly isolated from bovine feces, possessed an stx_1 subtype. Interestingly, two isolates with serotype O91:H$^-$ (recovered from asymptomatic patients), a common ovine serotype, possessed an stx_1 subtype. Serotype O91:H$^-$ isolates are frequently recovered from ovine feces and are atypical compared with other common ovine STEC serotypes in that they possess an stx_1 subtype. Isolates of serotypes O111:H$^-$ (two isolates from patients of unknown health status), O117:H7 (two, from patients with diarrhea), O157:H7 (three; unknown health status), and Ont:H2 (two; unknown health status), and one isolate each of serotypes O8:H8 (HUS), O103:H2 (diarrhea), O118:H16 (diarrhea), Ont:Hnt (healthy patient), OR:HR (unknown health status), and OR:H$^-$ (unknown health status), all possessed an stx_1 subtype. STEC isolates belonging to the classical EHEC types (serogroups O26, O103, O111, O113, and O157) did not possess stx_{1c} alone, an observation that supports the findings of others (30, 63). Of the 11 isolates from New Zealand meat, those with serotypes O5:H$^-$, O75:H8/H40, O81:H26, O88:H25, O104:H$^-$/H7, O123:H$^-$/H10, and O128:H2 all possessed stx_{1c} while 1 isolate of serotype O91:H$^-$ possessed an stx_1 subtype.

All five O157:H$^-$ isolates recovered from healthy pigs included in this study possessed an stx_1 subtype (Table 1).

stx_1 subtypes in STEC isolates containing the *eaeA* gene. Of 90 STEC isolates that contained *eaeA*, 81 (90%) possessed an stx_1 subtype (Table 1). Of the remaining nine isolates (serotypes O5:HR, O37:H10, O55:H20, O88:H8, and Ont:H8), five contained both common stx_1 and stx_{1c} subtypes and four (serotypes O5:H$^-$, O106:HR, O123:H$^-$, and O158:HR) contained the stx_{1c} subtype (Table 1). These data suggest that STEC isolates containing *eaeA* predominantly possess an stx_1 subtype.

stx_1 sequence analysis. stx_1 from isolates of serotypes O26:H11 (isolate 507) and O5:H$^-$ (isolate 904), which were predicted by RFLP analyses to possess stx_1 and stx_{1c} subtypes, respectively, and which were representative of the two stx_1 RFLP patterns identified in this study, were examined by DNA sequence analysis. The stx_1 sequence derived from serotype O26:H11 showed a single nucleotide difference (A→T) from the stx_{1J933} sequence (GenBank accession no. AF034975.3) (38) in the A subunit (S^{67}→T). The nucleotide sequence derived from serotype O5:H$^-$ showed 100% homology with the reported stx_{1c} gene sequence (accession no. Z36901.1) (43).

DISCUSSION

This study describes the development of a PCR-RFLP assay that differentiates stx_{1c} from other stx_1 subtypes and its application for subtyping stx_1 genes in 378 STEC isolates predominantly from feces of various meat-producing animals and humans. The most striking result of this study was the predominance of stx_{1c} (136 of 207 isolates [65.7%]) in STEC isolated from ovine feces and the infrequent identification of this subtype in STEC from bovine feces (6 of 104 isolates [5.8%]). Of the 207 ovine STEC isolates, 70 (33.8%) belonged to the common ovine serotypes O5:H$^-$, O128:H2, and O123:H$^-$ (5–7, 14, 30, 50, 58; our unpublished data) and were positive for the stx_{1c} gene. Isolates with serotype O91:H$^-$, another commonly reported ovine STEC serotype (14, 30, 58), predominantly possessed an stx_1 subtype (27 of 32 isolates [84.4%]), although 5 isolates possessed stx_{1c}. In similar studies of ovine STEC, all 10 O91:H$^-$ isolates from Germany (30) and 12 O91:H$^-$ isolates from Norway (58) were reported to possess an stx_1 subtype, suggesting that this serotype is rarely infected by lysogenic phage carrying stx_{1c} (see below). Urdahl et al. (58) suggested that STEC isolates belonging to the O91 serogroup might be less host specific than those of other serotypes, since they have been isolated from cattle, sheep, pigs, goats, and humans and are found with different flagellar types including H$^-$, H10, H14, H21, and H49.

In our study, the stx_{1c} gene was identified among a serologically diverse collection of STEC isolates, the vast majority of which have been recovered only from ovine feces (14, 27, 30, 58; our unpublished data). In a study of sheep in Germany, stx_{1c} was detected in 48 stx_1-containing STEC isolates comprising serogroups O5, O125, O128, O146, and OX3 (30). A study in Norway (58) reported stx_{1c} in 73 of 102 (71.6%) stx_1-positive isolates, belonging to 12 different serotypes, from ovine feces; serotypes O5:NM, O6:H10, O91:NM, and O128:NM predom-

inated. A lysogenic bacteriophage carrying the stx_{1c} gene has been isolated from STEC derived from ovine feces, and the ability of the phage to integrate into the genomes of genetically heterogeneous *E. coli* types has been established (30). The authors suggested that the promiscuous nature of this bacteriophage may provide an explanation for the presence of the stx_{1c} gene among serologically diverse populations of STEC belonging to different clonal lineages. Our study confirms and extends these preliminary observations by showing that the stx_{1c} gene is present among 51 STEC serotypes predominantly of ovine origin. The low prevalence of this gene in STEC recovered from bovine sources (6 of 104 isolates [5.8%]) suggests that either phage carrying stx_{1c} are not prevalent in the gastrointestinal tracts of cattle or most serotypes that inhabit cattle are refractory to infection by this phage.

Although STEC strains of serotype O5:H$^-$ are predominantly isolated from sheep and not cattle (5, 14, 58), they are occasionally isolated from the latter. Eight serotype O5:H$^-$ isolates from cattle were included in this study. Three of these STEC isolates were recovered from young calves, and two of these possessed stx_{1c}; the other five O5:H$^-$ isolates were recovered from diarrheic cattle and possessed an stx_1 subtype. Hornitzky et al. have recovered only one O5:H$^-$ STEC isolate from healthy Australian cattle despite intensive fecal sampling (27), and this serotype was not reported among STEC isolates recovered from healthy cattle in studies undertaken in Japan (29), France (49), and Spain (9). However, serotype O5:H$^-$ STEC isolates have previously been recovered from diarrheic calves in Australia (26), the United States (15), Argentina (42), and Germany (60) and from healthy cattle in two studies (22, 28). In one of the studies of healthy cattle (22), all 10 bovine O5:H$^-$ isolates were shown to simultaneously possess stx_1, *eaeA*, and *ehxA*. Furthermore, O5:H$^-$ isolates recovered from diarrheic calves in Germany (60), Argentina (42), and Australia (26) all possessed *eaeA*. O5:H$^-$ isolates recovered from healthy sheep (14, 58; our unpublished data) very rarely possess *eaeA*. These data suggest the existence of different clonal populations of serotype O5:H$^-$, and further genetic studies are in progress to test this hypothesis.

Of 207 ovine stx_1-containing STEC isolates, 20 (9.7%) possessed *eaeA*. Of these, 13 contained an stx_1 subtype, 3 contained stx_{1c}, and the remaining 4 contained both stx_1 and stx_{1c} subtypes. Similarly, 32 of 104 (30.8%) bovine stx_1-containing STEC isolates possessed *eaeA* and none contained stx_{1c}. Of the 165 isolates in this study that possessed the stx_{1c} gene, 137 (83.0%) were found to contain *ehxA*. Isolates with STEC serotypes that are commonly recovered from patients with serious diseases typically carry the *eaeA* gene and also possess the EHEC plasmid, which encodes *ehxA* and other potential virulence-associated factors (10, 22, 61). The majority (82 of 136 [60.3%]) of ovine STEC isolates that possessed stx_{1c} also contained stx_2. Previous studies have shown that STEC isolates recovered from ovine feces typically possess the stx_{2d} subtype (50, 58), a subtype that is not commonly found in STEC isolated from patients with severe disease (19, 46, 47). A study of STEC recovered from sheep in Norway reported that 44 of 57 stx_2-positive isolates possessed stx_{2d} (58). Collectively, these data lend weight to the hypothesis that STEC isolates recovered from sheep feces are not commonly associated with HUS. However, it should be emphasized that we have characterized five STEC isolates from humans with HUS (four O5:H$^-$ isolates and a single OX3:H2 isolate) that did possess stx_{1c}. In addition, 51 of 207 (24.6%) ovine isolates contained an stx_1 subtype, and 41 (80.4%) of these belonged to serogroups (O26, O91, O103, and O157) which have been associated with serious human illnesses in Australia and around the world. Thus, the ability to identify stx_{1c}-positive STEC isolates is of clinical and epidemiological significance, because these isolates are likely to be less pathogenic for humans. Limited studies in Australia (4) indicate that STEC serotypes O123:H$^-$ and O128:H2, serotypes that typically possess stx_{1c} and that are commonly recovered from the feces of sheep, are isolated from humans with diarrhea but without HUS (4). Similarly, investigators reported from a study in Germany (19) that STEC isolates simultaneously possessing stx_{2d} and stx_1 (stx_1 not subtyped) were from asymptomatic patients (11 isolates) or patients with diarrhea but without HUS (26 isolates) and that none of them possessed *eae*. Although the stx_1 subtype was not reported in that study (19), our study shows that stx_{1c} is typically associated with STEC strains that possess stx_{2d}. Furthermore, 16 of these 37 isolates (43.2%) possessed serotypes O75:H8, O91:H$^-$, and O128:H$^-$/H2/Hnt, which are common ovine serotypes. Further studies are required to elucidate the contribution of STEC derived from ovine sources to milder human gastrointestinal conditions such as diarrhea.

Of 104 bovine STEC isolates, 97 (93.3%) contained an stx_1 subtype and comprised 44 serotypes; 22 of these 97 (22.7%) belonged to serogroups O26, O111, O113, O157, and OX3, which are associated with serious disease in humans in Australia (16) and around the world. We and others have recently shown that the majority of bovine STEC isolates that contain stx_2 together with either of the associated virulence factors *ehxA* and *eaeA* possess stx_2 and stx_{2vhb} subtypes (3; our unpublished data). These subtypes are commonly associated with STEC recovered from seriously diseased patients. Collectively, these data suggest that cattle are the reservoir of STEC strains belonging to a diverse collection of serotypes, a subset of which is capable of causing serious human disease.

There is mounting evidence that STEC serotypes that commonly inhabit the gastrointestinal tract of one ruminant species are rarely isolated from other hosts (5–7, 14, 27, 30, 50, 58; our unpublished data). Shiga toxin gene subtypes also appear to associate with particular STEC serotypes and consequently with particular ruminant hosts. For example, stx_2-containing STEC isolates recovered from ovine feces commonly possess stx_{2d} subtypes (46, 50, 58) whereas stx_2-containing STEC isolates commonly recovered from cattle feces typically possess stx_2, stx_{2vha}, and stx_{2vhb} subtypes (3; our published data). Similarly, stx_{2e}-containing STEC is typically isolated from porcine sources and has not to our knowledge been recovered from ovine or bovine sources (14, 26, 27, 30, 50, 58), and stx_{2f}-containing STEC is typically recovered from pigeons (53). These observations are consistent with hypotheses raised by Hoey et al. (25) which suggest that the effects of Shiga toxins on bovine epithelial cells are likely to significantly affect the success of colonization, dissemination, and persistence of STEC in cattle reservoirs. Furthermore, these authors also speculate that genetic heterogeneity among both Shiga toxin subtypes and other associated virulence factors, particularly serotype-dependent variation, may account for differences in

the pathogenicity of different STEC populations for cattle and hence the potential for distribution in humans.

In contrast to these observations, O157 STEC can be isolated from different animal species including humans, cattle, sheep, and swine (12, 14, 24, 37). Irrespective of host source, O157 isolates have never been shown to possess stx_{1c} or stx_{2d} genes and always possess either the stx_1 or the stx_2 and/or stx_{2vh} subtype, or both (3, 19, 63; our unpublished data). In the present study we also show that some serogroups, particularly O75:H8 (14 of 21 isolates), simultaneously possess both stx_1 and stx_{1c} subtypes. Similarly, some bovine STEC isolates have been reported to possess as many as three different stx_2 subtypes (3). Shiga toxin genes are uniformly flanked by bacteriophage-linked sequences in serologically different STEC strains (52, 57). Collectively, these observations support the contention that bacteriophage transmission plays a key role in the spread of Shiga toxin genes among *E. coli* strains and that serotype may influence the outcome of these interactions (30, 51, 52).

ACKNOWLEDGMENTS

K. N. Brett is the recipient of a Commonwealth funded postgraduate scholarship as part of the Beef CRC Mark II. V. Ramachandran is the recipient of a University of Wollongong Postgraduate Research Award and an Overseas Postgraduate Scholarship.

K. N. Brett and V. Ramachandran contributed equally to this work.

We thank A. Burnens for supplying STEC strains from Switzerland.

REFERENCES

1. **Acheson, D. W. K., R. Moore, S. De Breucker, L. Lincicome, M. Jacewicz, E. Skutelsky, and G. T. Keusch.** 1996. Translocation of Shiga toxin across polarized intestinal cells in tissue culture. Infect. Immun. **64:**3294–3300.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **Bertin, Y., K. Boukhors, N. Pradel, V. Livrelli, and C. Martin.** 2001. Stx2 subtyping of Shiga toxin-producing *Escherichia coli* isolated from cattle in France: detection of a new Stx2 subtype and correlation with additional virulence factors. J. Clin. Microbiol. **39:**3060–3065.
4. **Bettelheim, K. A.** 2001. Enterohaemorrhagic *Escherichia coli* O157:H7: a red herring? J. Med. Microbiol. **50:**201–202.
5. **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 seven different species of healthy domestic animals. J. Clin. Microbiol. **31:**2483–2488.
6. **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.
7. **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.
8. **Blake, D. C. I., R. G. Russell, E. Santini, T. Bowen, and E. C. Boedeker.** 1996. Pro-inflammatory mucosal cytokine responses to Shiga-like toxin-1 (SLT-1), p. 75–82. *In* G. T. Keusch and M. Kawakami (ed.), Cytokines, cholera and the gut. IOS Press, Amsterdam, The Netherlands.
9. **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.
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. **Calderwood, S. B., and J. J. Mekalanos.** 1987. Iron regulation of Shiga-like toxin expression in *Escherichia coli* is mediated by the *fur* locus. J. Bacteriol. **169:**4759–4764.
12. **Chapman, P. A., C. A. Siddons, D. J. Wright, P. Norman, J. Fox, and E. Crick.** 1993. Cattle as a possible reservoir of verotoxin-producing *Escherichia coli* O157 infections in man. Epidemiol. Infect. **111:**439–447.
13. **Clarke, R. C., J. B. Wilson, S. C. Read, S. Renwick, K. Rahn, R. P. Johnson, D. Alves, M. A. Karmali, H. Loir, S. A. McEwen, J. Spika, and C. L. Gyles.** 1994. Verocytotoxin-producing *Escherichia coli* (VTEC) in the food chain: preharvest and processing perspectives, p. 17–24. *In* M. A. Karmali and A. G. Goglio (ed.), Recent advances in verocytotoxin-producing *Escherichia coli* infections. Elsevier Science B.V., Amsterdam, The Netherlands.
14. **Djordjevic, S. P., M. A. Hornitzky, G. Bailey, P. Gill, B. Vanselow, K. Walker, and K. Bettelheim.** 2001. Virulence properties and serotypes of Shiga toxin-producing *Escherichia coli* from healthy Australian slaughter-age sheep. J. Clin. Microbiol. **39:**2017–2021.
15. **Dorn, C. R., D. H. Francis, E. J. Angrick, J. A. Willgohs, R. A. Wilson, J. E. Collins, B. H. Jenke, and S. J. Shawd.** 1993. Characteristics of Vero cytotoxin producing *Escherichia coli* associated with intestinal colonisation and diarrhea in calves. Vet. Microbiol. **36:**149–159.
16. **Elliott, E. J., R. M. Robbins-Browne, E. V. O'Loughlin, V. Bennet-Wood, J. Bourke, P. Henning, G. G. Hogg, J. Knight, H. Powell, and D. Redmond.** 2001. Nationwide study of haemolytic uremic syndrome: clinical, microbiological, and epidemiological features. Arch. Dis. Child. **85:**125–131.
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. **Ferens, W. A., and C. J. Hovde.** 2000. Antiviral activity of Shiga toxin 1: suppression of bovine leukemia virus-related spontaneous lymphocyte proliferation. Infect. Immun. **68:**4462–4469.
19. **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.
20. **Gannon, V. P., and C. L. Gyles.** 1990. Characteristics of Shiga-like toxin produced by *Escherichia coli* associated with porcine edema disease. Vet. Microbiol. **24:**89–100.
21. **Gannon, V. P., R. K. King, J. Y. Kim, and E. J. Golsteyn Thomas.** 1992. Rapid and sensitive method for detection of Shiga-like toxin-producing *Escherichia coli* in ground beef using the polymerase chain reaction. Appl. Environ. Microbiol. **58:**3809–3815.
22. **Gyles, C., R. Johnson, A. Gao, K. Ziebell, D. Pierard, S. Aleksic, and P. Boerlin.** 1998. Association of enterohaemorrhagic *Escherichia coli* hemolysin with serotypes of Shiga-like-toxin-producing *Escherichia coli* of human and bovine origins. Appl. Environ. Microbiol. **64:**4134–4141.
23. **Heimer, S. R., R. A. Welch, N. T. Perna, G. Posfai, P. S. Evans, J. B. Kaper, F. R. Blattner, and H. L. T. Mobley.** 2002. Urease on enterohemorrhagic *Escherichia coli*: evidence for regulation by Fur and a *trans*-acting factor. Infect. Immun. **70:**1027–1031.
24. **Heuvelink, A. E., J. T. Zwartkruis-Nahuis, F. L. van den Biggelaar, W. J. Leeuwen, and E. de Boer.** 1999. Isolation and characterisation of verocytotoxin-producing *Escherichia coli* O157 from slaughter pigs and poultry. Int. J. Food Prot. **52:**67–75.
25. **Hoey, D. E. E., C. Currie, R. W. Else, A. Nutikka, C. A. Lingwood, D. 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. **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-cefimine-cefsulodin blood agar and PCR. FEMS Microbiol. Lett. **198:**17–22.
27. **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.
28. **Johnson, R. P., R. C. Clarke, J. B. Wilson, S. C. Read, K. Rahn, S. A. Renwick, K. A. Sandhu, D. Alves, M. A. Karmali, H. Loir, S. A. McEwen, J. S. Spika, and C. L. Gyles.** 1996. Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenic *Escherichia coli*. J. Food Prot. **59:**1112–1122.
29. **Kobayashi, H., J. Shimada, M. Nakazawa, T. Morozumi, T. Pohjanvitra, S. Pelkonnen, and K. Yamamoto.** 2001. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* from healthy cattle in Japan. Appl. Environ. Microbiol. **67:**484–489.
30. **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.
31. **Kudva, I. T., P. G. Hatfield, and C. J. Hovde.** 1999. Characterization of *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* serotypes isolated from sheep. J. Clin. Microbiol. **35:**892–899.
32. **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.
33. **Menge, C., L. H. Wieler, T. Schlapp, and G. Baljer.** 1999. Shiga toxin 1 from *Escherichia coli* blocks activation and proliferation of bovine lymphocyte subpopulations in vitro. Infect. Immun. **67:**2209–2217.
34. **Muniesa, M., and J. Jofre.** 1998. Abundance in sewage of bacteriophages

that infect *Escherichia coli* O157:H7 and that carry the Shiga toxin 2 gene. Appl. Environ. Microbiol. **64:**2443–2448.
35. **Muniesa, M., F. Lucena, and J. Jofre.** 1999. Comparative survival of free Shiga toxin 2-encoding phages and *Escherichia coli* strains outside the gut. Appl. Environ. Microbiol. **65:**5615–5618.
36. **Nakano, M., T. Iida, M. Ohnishi, K. Kurokawa, A. Takahashi, T. Tsukamoto, T. Yasunaga, T. Hayashi, and T. Honda.** 2001. Association of the urease gene with enterohemorrhagic *Escherichia coli* strains irrespective of their serogroups. J. Clin. Microbiol. **39:**4541–4543.
37. **Nakazawa, M., and M. Akiba.** 1999. Swine as a potential reservoir of Shiga toxin-producing *Escherichia coli* O157:H7 in Japan. Emerg. Infect. Dis. **5:**833–834.
38. **Neely, M. N., and D. I. Friedman.** 1998. Functional and genetic analysis of regulatory regions of coliphage H-19B: location of Shiga-like toxin and lysis genes suggest a role for phage functions in toxin release. Mol. Microbiol. **28:**1255–1267.
39. **Nicholls, L., T. H. Grant, and R. M. Robbins-Browne.** 2000. Identification of a novel genetic locus that is required for *in vitro* adhesion of a clinical isolate of enterohaemorrhagic *Escherichia coli* to epithelial cells. Mol. Microbiol. **35:**275–288.
40. **O'Loughlin, E. V., and R. M. Robins-Browne.** 2001. Effect of Shiga toxin and Shiga-like toxins on eukaryotic cells. Microbes Infect. **3:**493–507.
41. **Pai, C. H., J. K. Kelly, and G. L. Meyers.** 1986. Experimental infection of infant rabbits with verotoxin-producing *Escherichia coli*. Infect. Immun. **51:** 16–23.
42. **Parma, A. E., M. E. Sanz, J. E. Blanco, J. Blanco, M. R. Viñas, M. Blanco, N. L. Padola, and A. I. Etcheverria.** 2000. Virulence genotypes and serotypes of verotoxigenic *Escherichia coli* isolated from cattle and foods in Argentina. Eur. J. Epidemiol. **16:**757–762.
43. **Paton, A. W., L. Beutin, and J. C. Paton.** 1995. Heterogeneity of the amino-acid sequences of *Escherichia coli* Shiga-like toxin type-I operons. Gene **153:**71–74.
44. **Paton, A. W., and J. C. Paton.** 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* 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.
45. **Paton, A. W., P. Srimanote, M. C. Woodrow, and J. C. Paton.** 2001. Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-negative Shiga-toxigenic *Escherichia coli* strains that are virulent to humans. Infect. Immun. **69:**6999–7009.
46. **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.
47. **Pierard, D., G. Cornu, W. Proesmans, A. Dediste, F. Jacobs, J. Vande Walle, A. Mertens, J. Ramet, S. Lauwers, and the Belgian Society for Infectology and Clinical Microbiology HUS Study Group.** 1999. Hemolytic-uremic syndrome in Belgium: incidence and association with verocytotoxin-producing *Escherichia coli* infection. Clin. Microbiol. Infect. **5:**16–22.
48. **Pierard, D., L. Van Damme, L. Moriau, D. Stevens, and S. Lauwers.** 1997. Virulence factors of verotoxin-producing *Escherichia coli* isolated from raw meats. Appl. Environ. Microbiol. **63:**4585–4587.
49. **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.
50. **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 stx_{2d} toxin type. J. Clin. Microbiol. **39:**1932–1937.
51. **Schmidt, H., M. Bielaszewska, and H. Karch.** 1999. Transduction of enteric *Escherichia coli* isolates with a derivative of Shiga toxin 2-encoding bacteriophage ϕ3538 isolated from *Escherichia coli* O157:H7. Appl. Environ. Microbiol. **65:**3855–3861.
52. **Schmidt, H.** 2001. Shiga-toxin-converting bacteriophages. Res. Microbiol. **152:**687–695.
53. **Schmidt, H., J. Scheef, S. Morabito, A. Caprioli, L. H. Wieler, and H. Karch.** 2000. A new Shiga toxin 2 variant (Stx2f) from *Escherichia coli* isolated from pigeons. Appl. Environ. Microbiol. **66:**1205–1208.
54. **Sjogren, R., R. Neill, D. Rachmilewitz, D. Fritz, J. Newland, D. Sharpnack, C. Colleton, J. Fondacaro, P. Gemski, and E. Boedeker.** 1994. Role of Shiga toxin 1 in bacterial enteritis: comparison between isogenic *Escherichia coli* strains. Gastroenterology **106:**306–317.
55. **Tarr, P. I., S. S. Bilge, J. C. Vary, Jr., S. Jelacic, R. L. Habeeb, T. R. Ward, M. R. Baylor, and T. E. Besser.** 2000. Iha: a novel *Escherichia coli* O157:H7 adherence-conferring molecule encoded on a recently acquired chromosomal island of conserved structure. Infect. Immun. **68:**1400–1407.
56. **Thorpe, C. M., B. P. Hurley, L. L. Lincicome, M. S. Jacewicz, G. T. Keusch, and D. W. K. Acheson.** 1999. Shiga toxins stimulate secretion of interleukin-8 from intestinal epithelium cells. Infect. Immun. **67:**5985–5993.
57. **Unkmeir, A., and H. Schmidt.** 2000. Structural analysis of phage-borne *stx* genes and their flanking sequences in Shiga toxin-producing *Escherichia coli* and *Shigella dysenteriae* type 1 strains. Infect. Immun. **68:**4856–4864.
58. **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.
59. **Wagner, P. L., M. N. Neely, X. Zhang, and D. W. Acheson.** 2001. Role for a phage promoter in Shiga toxin 2 expression from a pathogenic *Escherichia coli* strain. J. Bacteriol. **183:**2081–2085.
60. **Wieler, L. H., E. Vieler, C. Erpenstein, T. Schlapp, H. Steinruck, R. Bauerfeind, A. Byomi, and G. Baljer.** 1996. Shiga toxin-producing *Escherichia coli* strains from bovines: association of adhesion with carriage of *eaeA* and other genes. J. Clin. Microbiol. **34:**2980–2984.
61. **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:**797–802.
62. **Yamasaki, C., Y. Natori, X. T. Zhang, M. Ohmura, S. Yamasaki, Y. Takeda, and Y. Natori.** 1999. Induction of cytokines in a human colon epithelial cell line by Shiga toxin 1 (Stx1) and Stx2 but not by non-toxic mutant Stx1 which lacks *N*-glycosidase activity. FEBS Lett. **442:**231–234.
63. **Zhang, W., M. Bielaszewska, T. Kuczius, and H. Karch.** 2002. Identification, characterization, and distribution of Shiga toxin 1 gene variant (stx_{1c}) in *Escherichia coli* strains isolated from humans. J. Clin. Microbiol. **40:**1441–1446.