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Brett, Kim; Hornitzky, Michael A; Bettelheim, Karl A; Walker, Mark J.; and Djordjevic, Steven, "Bovine non-O157 Shiga toxin 2-containing Escherichia coli isolates commonly possess stx2-EDL933 and/or stx2vhb subtypes" (2003). *Faculty of Science, Medicine and Health - Papers: part A*. 1971.  
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## Bovine non-O157 Shiga toxin 2-containing *Escherichia coli* isolates commonly possess stx2-EDL933 and/or stx2vhb subtypes

### Abstract

*stx*<sub>2</sub> genes from 138 Shiga toxin-producing *Escherichia coli* (STEC) isolates, of which 127 were of bovine origin (58 serotypes) and 11 of human origin (one serotype; O113:H21), were subtyped. The bovine STEC isolates from Australian cattle carried *ehxA* and/or *eaeA* and predominantly possessed *stx*<sub>2</sub>-EDL933 (103 of 127; 81.1%) either in combination with *stx*<sub>2</sub>vhb (32 of 127; 25.2%) or on its own (52 of 127; 40.4%). Of 22 (90.9%) bovine isolates of serotype O113:H21, a serotype increasingly recovered from patients with hemolytic uremic syndrome (HUS) or hemorrhagic colitis, 20 contained both *stx*<sub>2</sub>-EDL933 and *stx*<sub>2</sub>vhb; 2 isolates contained *stx*<sub>2</sub>vhb only. Although 7 of 11 (63.6%) human O113:H21 isolates associated with diarrhea possessed *stx*<sub>2</sub>-EDL933, the remaining 4 isolates possessed a combination of *stx*<sub>2</sub>-EDL933 and *stx*<sub>2</sub>vhb. Three of the four were from separate sporadic cases of HUS, and one was from an unknown source.

### Keywords

edl933, commonly, subtypes, coli, bovine, containing, 2, shiga, o157, non, stx2vhb, possess, isolates, escherichia, toxin, stx2

### Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

### Publication Details

Brett, K. N., Hornitzky, M. A., Bettelheim, K. A., Walker, M. J. & Djordjevic, S. P. (2003). Bovine non-O157 Shiga toxin 2-containing *Escherichia coli* isolates commonly possess stx2-EDL933 and/or stx2vhb subtypes. *Journal of Clinical Microbiology*, 41 (6), 2716-2722.

## Bovine Non-O157 Shiga Toxin 2-Containing *Escherichia coli* Isolates Commonly Possess *stx*<sub>2-EDL933</sub> and/or *stx*<sub>2vhb</sub> Subtypes

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Received 28 May 2002/Accepted 18 December 2002

***stx*<sub>2</sub> genes from 138 Shiga toxin-producing *Escherichia coli* (STEC) isolates, of which 127 were of bovine origin (58 serotypes) and 11 of human origin (one serotype; O113:H21), were subtyped. The bovine STEC isolates from Australian cattle carried *ehxA* and/or *eaeA* and predominantly possessed *stx*<sub>2-EDL933</sub> (103 of 127; 81.1%) either in combination with *stx*<sub>2vhb</sub> (32 of 127; 25.2%) or on its own (52 of 127; 40.4%). Of 22 (90.9%) bovine isolates of serotype O113:H21, a serotype increasingly recovered from patients with hemolytic uremic syndrome (HUS) or hemorrhagic colitis, 20 contained both *stx*<sub>2-EDL933</sub> and *stx*<sub>2vhb</sub>; 2 isolates contained *stx*<sub>2vhb</sub> only. Although 7 of 11 (63.6%) human O113:H21 isolates associated with diarrhea possessed *stx*<sub>2-EDL933</sub>, the remaining 4 isolates possessed a combination of *stx*<sub>2-EDL933</sub> and *stx*<sub>2vhb</sub>. Three of the four were from separate sporadic cases of HUS, and one was from an unknown source.**

Shiga toxin-producing *Escherichia coli* (STEC) is commonly carried by ruminants (especially cattle) and is an important enteropathogen, causing human diseases ranging from mild diarrhea to more severe conditions such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (7, 15, 22a, 25, 28, 47). Although over 150 serotypes of STEC have been associated with human disease (12, 20), serotype O157:H7 is believed to be responsible for the majority of outbreaks and sporadic cases of HUS in the United States, Japan, and Europe. However, non-O157 STEC is of greater significance in Australia (16) and Argentina (35), and its role in HUS and HC in the United States and Europe is gaining recognition (24).

The most critical factors of STEC associated with severe human disease are the Shiga toxins. STEC can produce one or both of these groups of toxins which comprise two immunologically non-cross-reactive groups (designated Stx1 and Stx2), although other virulence factors such as intimin and enterohemolysin either directly contribute to (or are implicated by association with) the pathogenicity of STEC (11, 21).

*stx*<sub>2</sub> comprises 11 distinct variants (5, 19, 23, 31, 38, 39, 42, 45, 46, 50) and is considered the most important STEC virulence factor associated with human disease (11, 21, 34, 41). More importantly, differences in the degree of pathogenicity of STEC serotypes have been associated with variations in the *stx*<sub>2</sub> subtype (27, 29, 30, 42). The most frequently reported *stx*<sub>2</sub> subtypes are represented by *stx*<sub>2c</sub>, *stx*<sub>2d</sub>, and *stx*<sub>2e</sub> (42, 46, 50).

Although more than 200 different O:H serotypes of STEC have been isolated from cattle (1, 7, 12, 20, 24, 49, 51), there is a paucity of information regarding associations between serotype, *stx*<sub>2</sub> subtype, and virulence factor profiles among STEC

isolates from meat-producing animals (9, 10, 22a, 26, 33, 43). *stx*<sub>2</sub> subtypes *stx*<sub>2-EDL933</sub> and *stx*<sub>2e</sub> (*stx*<sub>2vha</sub> and *stx*<sub>2vhb</sub>) have been described in European studies of bovine STEC; however, their association with serotype has not been reported previously (8, 42, 46). Ramachandran et al. (44) demonstrated that *stx*<sub>2</sub>-containing STEC from ovine feces usually belonged to the *stx*<sub>2d</sub> subtypes (*stx*<sub>2d-Ount/O111/OX3a</sub>).

The purpose of this study was to subtype *stx*<sub>2</sub> genes among a serologically diverse collection of 138 *stx*<sub>2</sub>-containing STEC isolates primarily derived from bovine feces collected from the eastern states of Australia and to compare *stx*<sub>2</sub> subtypes of STEC isolates of serotype O113:H21 (an important serotype increasingly associated with HUS and HC in humans) of bovine and human origin.

A total of 138 STEC isolates, of which 134 were non-O157, were used in this study. These consisted of 127 isolates from cattle and 11 human isolates from individual cases of clinical infections (Table 1). All isolates were prepared and subjected to multiplex PCR for the detection of STEC virulence factors *stx*<sub>1</sub>, *stx*<sub>2</sub>, *ehxA*, and *eaeA* as described previously (14). Amplified PCR products were then resolved by gel electrophoresis through agarose (2% wt/vol) and stained with ethidium bromide (5 μl/ml). Visualization was undertaken using UV illumination, and images were captured using a GelDoc 1000 image analysis station (Bio-Rad).

Bovine STEC isolates with *stx*<sub>2</sub> were subjected to subtyping using typing schemes previously reported (4, 42, 48). For this study, *stx*<sub>2d</sub> subtypes are defined as nucleotide sequence variants of *stx*<sub>2</sub> (*stx*<sub>2d-Ount</sub>, *stx*<sub>2d-O111</sub>, and *stx*<sub>2d-OX3a</sub>), as described previously (42), and are not the potentially mucin-activatable *stx*<sub>2d</sub> variants described by Melton-Celsa et al. (30) (defined as *stx*<sub>2vha</sub> and *stx*<sub>2vhb</sub> in this study). *stx*<sub>2</sub> genes amplified with VT2-e and VT2-f primers and Lin F and Lin R primers were subjected to restriction fragment length polymorphism (RFLP) analysis with the enzymes *Hae*III and *Pvu*II (42) and

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TABLE 1. Virulence factor profiles and *stx*<sub>2</sub> subtypes of STEC of bovine and human origin

Source	Serotype	No. of isolates of animal or human origin	Virulence factor				No. of isolates containing <i>stx</i> <sub>2</sub> variant(s)									
			<i>stx</i> <sub>1</sub>	<i>stx</i> <sub>2</sub>	<i>ehxA</i>	<i>eaeA</i>	<i>stx</i> <sub>2</sub> -EDL <sub>933</sub>	<i>stx</i> <sub>2</sub> vhb	<i>stx</i> <sub>2</sub> d-Ount	<i>stx</i> <sub>2</sub> -EDL <sub>933</sub> / <i>stx</i> <sub>2</sub> vhb	<i>stx</i> <sub>2</sub> -EDL <sub>933</sub> / <i>stx</i> <sub>2</sub> vhb/ <i>stx</i> <sub>1</sub>	<i>stx</i> <sub>2</sub> vhb/ <i>stx</i> <sub>1</sub>	<i>stx</i> <sub>2</sub> -EDL <sub>933</sub> / <i>stx</i> <sub>1</sub>	<i>stx</i> <sub>2</sub> d-OX3a		
B <sup>a</sup>	O2:H8	1	-	+	+	-		1								
B	O3:H7	2	+	+	+	-						1		1		
B	O5:H <sup>-</sup>	2	+	+	+	-										2
B	O5:H7	2	+	+	+	-								1	1	
B	O6:H8	1	-	+	+	-					1					
B	O6:H28	1	-	+	+	-					1					
B	O6:H34	3	-	+	+	-		3								
B	O8(KA):H51	1	-	+	+	-		1								
B	O8:H16	1	+	+	+	-						1				
B	O8:H19	6	-	+	+	-		6								
BD <sup>b</sup>	O21:H21	1	-	+	+	-		1								
B	O28:H8	3	-	+	+	-		3								
B, BD	O28:H40	2	-	+	+	-		2								
B	O49:H <sup>-</sup>	1	-	+	+	+					1					
B	O53:H2	1	-	+	+	-		1								
B	O76:H7	1	-	+	+	+		1								
B	O81:H21	1	-	+	+	-		1								
B	O82:H8	5	+	+	+	-							1		4	
B	O82:H40	2	+	+	+	-									2	
B	O91:H <sup>-</sup>	1	+	+	+	-				1						
B	O91:H21	1	-	+	+	+										1
B	O93:H19	1	-	+	+	-		1								
B	O104:H7	1	-	+	+	-		1								
B	O108:H7	2	+	+	+	-								2		
B	O110:H40	1	+	+	+	-									1	
B	O111:H <sup>-</sup>	1	-	+	+	+									1	
B	O111:H <sup>-</sup>	1	+	+	+	+									1	
BD	O111:H8	1	+	+	+	+									1	
BD	O113:H <sup>-</sup>	1	-	+	+	-					1					
B BD	O113:H21	20	-	+	+	-					20					
B	O113:H21	2	+	+	-	-				2						
H <sup>c</sup> (HUS, HA <sup>d</sup> )	O113:H21	3	-	+	+	-					3					
H (Diarrhea, HUS)	O113:H21	6	-	+	+	-		6								
H (?)	O113:H21	1	-	+	-	-		1								
H (?)	O113:H21	1	-	+	-	-					1					
B	O116:H21	1	-	+	+	-		1								
B	O130:H11	1	-	+	+	-									1	
B	O130:H11	6	-	+	+	-		6								
B	O130:H38	2	+	+	+	-								2		
B	O141:H49	1	-	+	+	-		1								
B	O153:H8	1	+	+	+	-				1						
B	O153:HR	2	+	+	+	-									2	
B, BD	O157:H8	2	-	+	+	-		2								
B	O157:H <sup>-</sup>	1	+	+	+	+								1		
B	O157:H7	1	-	+	+	+				1						
B	O163:H <sup>-</sup>	1	+	+	+	-						1				
B	O163:H19	1	-	+	+	-		1								
B	O163:H19	1	+	+	+	-								1		
B	Ont:H2	2	-	+	+	-		2								
B	Ont:H5	1	-	+	+	-		1								
B	Ont:H7	2	-	+	+	-		1				1				
B	Ont:H8	1	+	+	+	-								1		
B	Ont:H8	3	-	+	+	-		3								
B	Ont:H11	2	+	+	+	-								1	1	
B	Ont:H11	2	-	+	+	-				2						
BD	Ont:H11	1	-	+	+	-				1						
B	Ont:H16	1	+	+	+	-							1			
B	Ont:H21	1	+	+	+	-									1	
B	Ont:H28	1	-	+	+	-		1								
B	Ont:H28	1	+	+	+	-									1	
B	Ont:H30	1	+	+	+	-									1	
B	Ont:H41	1	+	+	+	-									1	
B	Ont:H49	3	-	+	+	-		2				1				
B	Ont:H49	1	+	+	+	-		1								
B	Ont:HR	1	-	+	+	-		1								

Continued on following page

TABLE 1—Continued

Source	Serotype	No. of isolates of animal or human origin	Virulence factor				No. of isolates containing <i>stx</i> <sub>2</sub> variant(s)								
			<i>stx</i> <sub>1</sub>	<i>stx</i> <sub>2</sub>	<i>ehtxA</i>	<i>eaeA</i>	<i>stx</i> <sub>2</sub> -EDL <sub>933</sub>	<i>stx</i> <sub>2</sub> v <sub>hb</sub>	<i>stx</i> <sub>2d</sub> -O <sub>unt</sub>	<i>stx</i> <sub>2</sub> -EDL <sub>933</sub> / <i>stx</i> <sub>2</sub> v <sub>hb</sub>	<i>stx</i> <sub>2</sub> -EDL <sub>933</sub> / <i>stx</i> <sub>2</sub> v <sub>hb</sub> / <i>stx</i> <sub>1</sub>	<i>stx</i> <sub>2</sub> v <sub>hb</sub> / <i>stx</i> <sub>1</sub>	<i>stx</i> <sub>2</sub> -EDL <sub>933</sub> / <i>stx</i> <sub>1</sub>	<i>stx</i> <sub>2d</sub> -OX <sub>3a</sub>	
B	OR:H-	1	-	+	+	-		1							
B	OR:H3	1	-	+	+	-	1								
B	OR:H8	1	-	+	+	-			1						
B	OX3:H8	5	-	+	+	-	5								
B	OX3:H8	1	+	+	+	-						1			
B	OX3:H21	1	-	+	-	+	1								
B	OX3:H40	1	-	+	+	-	1								
Total		138					59	8	2	31	5	11	19	3	

<sup>a</sup> B, feces from healthy cattle unless otherwise specified.

<sup>b</sup> BD, diagnostic fecal samples from cattle.

<sup>c</sup> H, human feces.

<sup>d</sup> HA, hemolytic anemia.

the enzymes *HincII* and *AccI* (28), respectively. In circumstances in which a *stx*<sub>2</sub> gene could not be reliably subtyped using the above-described approach, the gene was amplified with Tyler F and Tyler R primers and subjected to RFLP analysis with the enzymes *MspI*, *NciI*, and *RsaI* (Table 2) (48). PCR products were digested for a minimum of 4 h at 37°C. Fragments were separated by electrophoresis through agarose gels (2% wt/vol), and subtypes were determined according to comparison with profiles reported previously (4, 42, 48).

DNA sequence analysis of both the A and B subunits of two *stx*<sub>2</sub>-containing STEC isolates with serotype O110:H40 and Ont:H11 were undertaken, since RFLP analyses confirmed these isolates possessed *stx*<sub>2</sub> subtypes representative of the majority of the collection. *stx*<sub>2</sub> was amplified using 10 pmol of each of the oligonucleotide primers Stx2F and Stx2R (Table 3). PCRs (50 μl) were carried out containing 2 μl of nucleic acid of a crude whole-cell DNA template prepared using Instagene matrix as described previously (17), 10 mM Tris-HCl (pH 8.3), 10 mM KCl, 2 mM MgCl<sub>2</sub>, 200 μM each deoxynucleoside triphosphate, and 1 U of *Taq* DNA polymerase.

Thermocycler steps involved an initial denaturation step (95°C for 5 min) followed by 35 cycles of denaturation (95°C for 30 s), annealing (60°C for 45 s), and extension (72°C for 90 s). A final extension step of 72°C for 5 min completed the PCR. The amplified PCR product was separated by 2% agarose gel electrophoresis. For DNA sequencing, PCR amplification products were purified using a QIAquick DNA purification kit (Qiagen, Hilden, Germany). Sequencing reactions were performed using the Big Dye terminator cycle sequencing

ready reaction DNA sequencing kit and electrophoresed on an ABI prism 377 DNA sequencer (Perkin Elmer, Foster City, Calif.) as described previously (44). Auto Assembler software (Perkin Elmer) was used to compile and analyze the DNA sequences. Nucleotide and amino acid analysis was performed using programs accessed via the Australian National Genomic Information Service ([www.angis.org.au](http://www.angis.org.au)). Sequences were compared with those deposited in public databases using the BlastN and BlastP algorithms (2).

All 138 isolates possessed *stx*<sub>2</sub> and comprised 58 serotypes. STEC serotypes and their virulence factor profiles are listed in Table 1. A significant proportion of bovine STEC isolates (32 of 127; 25.2%) possessed more than one *stx*<sub>2</sub> variant or a combination of an *stx*<sub>2</sub> variant(s) together with *stx*<sub>1</sub> (35 of 127; 27.6%). Isolates with two copies of *stx*<sub>2</sub> were shown to possess *stx*<sub>2</sub>-EDL<sub>933</sub> and *stx*<sub>2</sub>v<sub>hb</sub> (31 of 127; 24.4%) (Table 1). STEC isolates possessing *stx*<sub>2</sub>-EDL<sub>933</sub> alone were also prevalent (59 of 138; 42.8%). These 59 isolates belonged to 28 serotypes (Table 4). Isolates with *stx*<sub>2</sub>-EDL<sub>933</sub> and *stx*<sub>1</sub> comprised the third most common group (19 of 138; 13.7%), representing 13 serotypes, followed by *stx*<sub>2</sub>v<sub>hb</sub> and *stx*<sub>1</sub> (11 of 138; 7.9%), comprising 9 serotypes. STEC possessing the three Shiga toxin factors *stx*<sub>2</sub>-EDL<sub>933</sub>, *stx*<sub>2</sub>v<sub>hb</sub>, and *stx*<sub>1</sub> was identified with a frequency of 5 of 138 (3.6%) and was represented by 5 serotypes (Table 4). STEC containing *stx*<sub>2</sub>v<sub>hb</sub> alone (8 of 138; 5.8%), *stx*<sub>2d</sub>-O<sub>unt</sub> (2 of 138; 1.4%), or *stx*<sub>2d</sub>-OX<sub>3a</sub> (3 of 138; 2.2%) was isolated infrequently (Table 4). Two bovine isolates of the serotype O5:H<sup>-</sup>, a serotype commonly recovered from ovine feces (14), possessed an *stx*<sub>2d</sub>-OX<sub>3a</sub> subtype; this subtype is commonly associ-

TABLE 2. Restriction fragment sizes used for analysis of *stx*<sub>2</sub>

Primers used to amplify fragment	Restriction enzyme	Expected fragment size(s) for:				Reference
		<i>stx</i> <sub>2</sub> -EDL <sub>933</sub>	<i>stx</i> <sub>2</sub> v <sub>hb</sub>	<i>stx</i> <sub>2d</sub> -O <sub>unt</sub>	<i>stx</i> <sub>2d</sub> -OX <sub>3a</sub>	
VT2-e, VT2-f	<i>HaeIII</i>	348	216, 132	216, 132	167, 132, 49 <sup>a</sup>	42
	<i>PvuII</i>	323, 25 <sup>a</sup>	250, 73, 25 <sup>a</sup>	200, 120, 28 <sup>a</sup>	200, 120, 28 <sup>a</sup>	
Lin F, Lin R	<i>HincII</i>	555, 262, 62	555, 340	880, 15 <sup>a</sup>	880, 15	28
	<i>AccI</i>	544, 351	544, 351	906	544, 351	
Tyler F, Tyler R	<i>MspI</i>	232, 48 <sup>a</sup>	108, 73, 51 <sup>a</sup>		155, 125	48
	<i>NciI</i>	385	159, 126			
	<i>RsaI</i>	216, 69	216, 69			

<sup>a</sup> Fragment too small to visualize under the electrophoresis conditions used.

TABLE 3. Primer pairs used to amplify *stx*<sub>2</sub>

Primer	Sequence (5' to 3')	Size of fragment (bp)	Reference
<i>stx</i> <sub>2</sub> typing			
VT2-e	AATACATTATGGGAAAGTAATA	348	42
VT2-f	TAAACTGCACTTCAGCAAAT		
Lin F	GAACGAAATAATTTATATGT	900 <sup>a</sup>	28
Lin R	TTTGATTGTTACAGTCAT		
Tyler F	AAGAAGATGTTTATGGCGGT	285	48
Tyler R	CACGAATCAGGTTATGCCTC		
<i>stx</i> <sub>2</sub> sequencing			
StxF	TATCTGCGCCGGTCT	1,280	44
StxR	CAAACCKGARCCTGA		
Gannon F	CCATGACAACGGACAGCAGTT	779	18
Gannon R	CCTGTCAACTGAGCAGCACTTTG		
Paton F	GGCAGTGTCTGAACTGCTCC	255	37
Paton R	TCGCCAGTTATCTGACATTCTG		
ST F	AATGCAATGGCGG	200	This study
Stx2R	CAAATCCGGAGCCTGC		
Stx2F2	AATCCAGTACAACGCGCCA	600	This study
ST R	AACGCAGAACTGCTCT		
Tyler F	AAGAAGATGTTTATGGCGGT	285	48
Tyler R	CACGAATCAGGTTATGCCTC		
KBStx2F	AATCCAGTACAACGCGCC	395	This study
KBStx2R	TGCTGAATAATCAGACG		

<sup>a</sup> Amplicons differ by a few nucleotides depending on the variant.

ated with ovine STEC (44). Representative RFLP profiles produced by the digestion of PCR amplification products spanning different regions of *stx*<sub>2</sub> are depicted in Fig. 1 and 2. DNA sequence analysis of *stx*<sub>2</sub> derived from STEC serotype O110:H40 showed 99.8% sequence identity with sequences of *stx*<sub>2-EDL933</sub> (accession number Z37725.1) (36) and the *p* gene derived from phage 933W (13). The two nucleotide polymorphisms that differentiated the *stx*<sub>2</sub> sequence of O110:H40 with *stx*<sub>2-EDL933</sub> did not alter the predicted amino acid sequence. The *stx*<sub>2</sub> gene from an O110:H40 isolate was selected for DNA sequence analysis, because PCR-RFLP analysis indicated that

it was indicative of *stx*<sub>2</sub> genes representative of the majority of bovine isolates used in this study (Fig. 1, lane 6). DNA sequence analysis of the *stx*<sub>2</sub> gene amplified from the Ont:H11 isolate showed 99.5% (5 nucleotide polymorphisms in the A subunit) sequence identity with *stx*<sub>2vhhb</sub>, a sequence variant of *stx*<sub>2c</sub> (accession number X61283.1) (31). These data concur with the results of PCR-RFLP analyses used to type *stx*<sub>2</sub> variants in this study. Of the five nucleotide polymorphisms identified in the A-subunit sequence, only two polymorphisms resulted in a change in the amino acid sequence. The B subunit of *stx*<sub>2vhhb</sub> in Ont:H11 showed nucleotide and predicted amino

TABLE 4. *stx*<sub>2</sub> variant(s) and association with serotype

<i>stx</i> <sub>2</sub> variant(s)	No. (%) of isolates <sup>a</sup>	Serotypes
<i>stx</i> <sub>2-EDL933</sub>	59 (42.8)	O6:H34, O8:(KA):H51, O8:H19, O21:H21, O28:H8, O28:H40, O53:H2, O76:H7, O81:H21, O93:H19, O104:H7, O113:H21, O116:H21, O130:H11, O141:H49, O157:H8, O163:H19, Ont:H2, Ont:H5, Ont:H7, Ont:H8, Ont:H28, Ont:H49, Ont:HR, OR:H3, OX3:H8, OX3:H21, OX3:H40
<i>stx</i> <sub>2-EDL933</sub> / <i>stx</i> <sub>2vhhb</sub>	31 (32.6)	O6:H8, O6:H28, O49:H <sup>-</sup> , O113:H <sup>-</sup> , O113:H21, Ont:H7, Ont:H49, OR:H8
<i>stx</i> <sub>2-EDL933</sub> / <i>stx</i> <sub>1</sub>	19 (10.4)	O5:H7, O82:H8, O82:H40, O110:H40, O111:H <sup>-</sup> , O111:H8, O103:H11, O153:HR, Ont:H11, ONT:H21, Ont:H28, Ont:H30, Ont:H41
<i>stx</i> <sub>2vhhb</sub> / <i>stx</i> <sub>1</sub>	11 (8.0)	O3:H7, O5:H7, O108:H7, O130:H38, O157:H <sup>-</sup> , O163:H19, Ont:H8, Ont:H11, OX3:8
<i>stx</i> <sub>2vhhb</sub>	8 (5.8)	O2:H8, O113:H21, O157:H7, Ont:H11, OR:H <sup>-</sup>
<i>stx</i> <sub>2-EDL933</sub> / <i>stx</i> <sub>2vhhb</sub> / <i>stx</i> <sub>1</sub>	5 (3.6)	O3:H7, O8:H16, O82:H8, O163:H <sup>-</sup> , Ont:H16
<i>stx</i> <sub>2d-OX3a</sub>	3 (2.2)	O5:H <sup>-</sup> , O91:H21
<i>stx</i> <sub>2d-Ount</sub>	2 (1.4)	O91:H <sup>-</sup> , O153:H8

<sup>a</sup> Values are numbers and percentages compared to a total of 138 isolates.

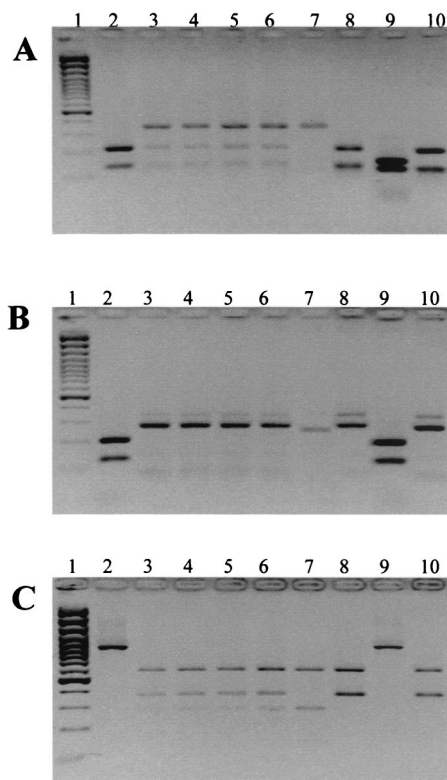


FIG. 1. *Hae*III (A) and *Pvu*II (B) digests of PCR products obtained with VT2-e and VT2-f primers and *Hinc*II (C) digests of PCR products obtained with Lin F and Lin R primers. Lanes: 1, 100-bp Plus marker; 2, *stx*<sub>2</sub>-O<sub>unt</sub> variant of serotype O91:H<sup>-</sup>; 3, *stx*<sub>2</sub>-EDL<sub>933</sub>/*stx*<sub>2</sub>v<sub>hb</sub> variant of serotype O113:H21; 4, *stx*<sub>2</sub>-EDL<sub>933</sub>/*stx*<sub>2</sub>v<sub>hb</sub> variant of serotype O113:H21; 5, *stx*<sub>2</sub>-EDL<sub>933</sub>/*stx*<sub>2</sub>v<sub>hb</sub> variant of serotype O113:H21; 6, *stx*<sub>2</sub>-EDL<sub>933</sub>/*stx*<sub>2</sub>v<sub>hb</sub> variant of serotype O110:H40; 7, *stx*<sub>2</sub>-EDL<sub>933</sub> variant of serotype Ont:H5; 8, *stx*<sub>2</sub>v<sub>hb</sub> variant of serotype Ont:HR; 9, *stx*<sub>2</sub>-OX<sub>3a</sub> variant of serotype O5:H<sup>-</sup>; 10, *stx*<sub>2</sub>v<sub>hb</sub> variant of serotype Ont:H11.

acid sequences identical to those of *stx*<sub>2</sub>v<sub>hb</sub> (accession number X61283.1) (31).

In this study, we determined the *stx*<sub>2</sub> subtypes of 127 bovine STEC isolates belonging to 58 serotypes and showed that Australian bovine STEC which also possesses *ehxA* and/or *eaeA* predominantly possesses either *stx*<sub>2</sub>-EDL<sub>933</sub> or *stx*<sub>2</sub>v<sub>hb</sub> subtypes (alone or in combination with one another). We also demonstrated that several serotypes, particularly O113:H21, simultaneously possess both these subtypes.

In a recent study of 167 *stx*<sub>2</sub>-containing STEC isolates from healthy cattle in France, *stx*<sub>2</sub>v<sub>hb</sub>, *stx*<sub>2</sub>-EDL<sub>933</sub>, and *stx*<sub>2</sub>v<sub>ha</sub> subtypes were commonly identified (5). Our study identified *stx*<sub>2</sub>-EDL<sub>933</sub> and *stx*<sub>2</sub>v<sub>hb</sub> as the predominant subtypes (122 of 127; 96.1%) among Australian bovine STEC isolates; *stx*<sub>2</sub>v<sub>ha</sub>-positive STEC isolates were not observed among any of STEC isolates in our study. Although the serotypes belonging to the *stx*<sub>2</sub>-containing STEC isolates from France were not reported (5), a recent study of 186 STEC isolates from cattle during a 1-year study in France (43) showed that few isolates possessed serotypes in common with those identified in this study. Our data suggest that *stx*<sub>2</sub>v<sub>ha</sub>-positive STEC belongs to serotypes not commonly found in Australian cattle. Alternatively, *stx*<sub>2</sub>v<sub>ha</sub> may only be found in STEC isolates that lack the accessory

virulence factors *ehxA* and/or *eaeA* (termed complex STEC [22]), since all but 4 of the 138 STEC isolates in this study possessed one or both of these factors. In support of the latter hypothesis, the study by Bertin et al. (5) identified enterohemolysin among STEC isolates harboring *stx*<sub>2</sub>-EDL<sub>933</sub> (78%) or a combination of *stx*<sub>2</sub>-EDL<sub>933</sub> and *stx*<sub>2</sub>v<sub>hb</sub> (85%); only 6 of 43 (14%) *stx*<sub>2</sub>v<sub>ha</sub>-containing isolates also possessed *ehxA*, and the presence of *eaeA* was not reported. Furthermore, recent studies in our laboratories have identified *stx*<sub>2</sub>v<sub>ha</sub>-containing STEC in isolates that do not possess *ehxA* and/or *eaeA* (our unpublished data). STEC isolates simultaneously containing three *stx*<sub>2</sub> variants were not observed among the 138 Australian STEC isolates, unlike the results reported by Bertin et al. (5). Furthermore, the untypeable *stx*<sub>2</sub> variant (identified as *stx*<sub>2</sub>-NV<sub>206</sub>) which comprised 24 of 167 (14.4%) of isolates in the study by Bertin et al. (5) was not observed in our Australian collection; 25% of STEC isolates possessing the *stx*<sub>2</sub>-NV<sub>206</sub> possessed *ehxA* (5). Collectively, *stx*<sub>2</sub>-subtyping data suggest that different non-O157 STEC populations predominate in Australia and France. However, the fact that 134 of 138 (97.1%) of the Australian *stx*<sub>2</sub>-containing STEC isolates and 54 of 130 (41.5%) of the *stx*<sub>2</sub>-containing French isolates possessed *ehxA* and/or *eaeA* may bias comparisons between these two STEC populations. Further studies are required to validate these hypotheses.

Serotype O113:H21 is increasingly being isolated from patients with HUS (6, 17, 40) and was the most predominant serotype in *stx*<sub>2</sub>-containing STEC isolates from healthy cattle in Australia (22a). Studies conducted in France (43), Japan (26), and Spain (9, 10, 33) all indicated that O113:H21 is among the most prevalent STEC serotypes recovered from cattle feces. Furthermore, O113:H21 was identified in 10 of 41 (24.4%) STEC isolates recovered from a Canadian study of naturally contaminated beef (3). All of the O113:H21 isolates recovered in our study (22 bovine and 11 human) were *eaeA* negative; 29 of 33 (87.9%) possessed the *ehxA* gene, and only 2 of 33 (6.1%) possessed *stx*<sub>1</sub>. Of 22 (90.9%) bovine isolates of serotype O113:H21, 20 concomitantly possessed both *stx*<sub>2</sub>-EDL<sub>933</sub> and *stx*<sub>2</sub>v<sub>hb</sub> while 2 (9.1%) only possessed *stx*<sub>2</sub>v<sub>hb</sub>. Two O113:H21 isolates

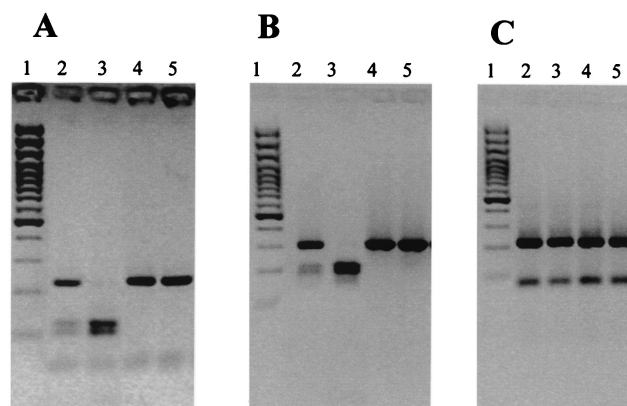


FIG. 2. *Msp*I (A), *Nci*I (B), and *Rsa*I (C) digests of PCR products obtained with Tyler F and Tyler R primers. Lanes: 1, 100-bp Plus marker; 2, *stx*<sub>2</sub>-EDL<sub>933</sub>/*stx*<sub>2</sub>v<sub>hb</sub> variant of serotype O113:H21; 3, *stx*<sub>2</sub>v<sub>hb</sub> variant of serotype Ont:HR; 4, *stx*<sub>2</sub>-EDL<sub>933</sub> variant of serotype Ont:H5; 5, *stx*<sub>2</sub>-EDL<sub>933</sub> variant of serotype Ont:H5.

recovered from HUS patients and a third isolate from a patient with anemia also simultaneously possessed *stx*<sub>2-EDL933</sub> and *stx*<sub>2vhh</sub>. However, four isolates of O113:H21 recovered from human cases of diarrhea, two from patients with HUS, and one isolate from an asymptomatic human individual possessed *stx*<sub>2-EDL933</sub> only. Of the 58 different serotypes identified in our study, 7 serotypes have been reported to cause HUS (O5:H<sup>-</sup>, O91:H<sup>-</sup>, O91:H21, O111:H<sup>-</sup>, O111:H8, O157:H7, and O163:H19).

Ramachandran et al. (44) reported that ovine STEC isolates that also contain *ehxA* and/or *eaeA* predominantly possess *stx*<sub>2d</sub> (*stx*<sub>2d-Ount1</sub>, *stx*<sub>2d-OX3a</sub>, and *stx*<sub>2d-O111</sub>) subtypes. In contrast, the cattle STEC isolates described in this study rarely possessed *stx*<sub>2d</sub> subtypes (5 of 138; 3.6%) and three of these isolates possessed STEC serotypes (O5:H<sup>-</sup> and O91:H<sup>-</sup>) typically found in sheep. Furthermore, none of the ovine isolates examined in the study of Ramachandran et al. (44) contained *stx*<sub>2vhh</sub> and only a single isolate possessed *stx*<sub>2EDL933</sub>, highlighting a dramatic contrast with results reported in the present study of bovine STEC. Bertin et al. (5) showed that 167 bovine *stx*<sub>2</sub>-positive STEC isolates from healthy cattle rarely possessed an *stx*<sub>2d</sub> subtype (8.5% of 167), but their serotype(s) was not reported. Beutin et al. (8) also found that with the exception of a single serotype (O90:H24), cattle and sheep differed with respect to the O:H types of their STEC floras. Together, these and other studies (14, 22, 22a, 32) suggest that genetically and serologically different populations of STEC inhabit the gastrointestinal tracts of cattle and sheep.

This work was supported by funds from the Australian Cooperative Research Centre for Cattle and Beef Quality.

We thank Christine Gillen for technical assistance with sequencing and Kari Gobius for the supply of control DNA preparations containing STEC DNA with the *stx*<sub>2vha</sub> Shiga toxin gene.

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