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Virulence Properties and Serotypes of Shiga Toxin-Producing *Escherichia coli* from Healthy Australian Cattle

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The virulence properties and serotypes of complex Shiga toxin-producing *Escherichia coli* (cSTEC) were determined in two studies of healthy cattle in eastern Australia. In the first, a snapshot study, 84 cSTEC isolates were recovered from 37 of 1,692 (2.2%) fecal samples collected from slaughter-age cattle from 72 commercial properties. The second, a longitudinal study of three feedlots and five pasture beef properties, resulted in the recovery of 118 cSTEC isolates from 104 animals. Of the 70 serotypes identified, 38 had not previously been reported.

Shiga toxin-producing *Escherichia coli* (STEC) isolates are an important group of food-borne pathogens that can cause severe gastrointestinal diseases in humans and complications such as hemolytic-uremic syndrome (HUS). The most often reported STEC serotype causing disease in humans worldwide is O157:H7, although in Australia non-O157 serotypes such as O111:H− and O113:H21 are more commonly found to cause diseases such as HUS (1, 11). These are two of the over 160 STEC serotypes which have been isolated from human patients around the world; other important serotypes are O5:H−, O26:H−, O26:H11, O103:H2, O145:H−, and O153:H25 (15, 11).

Ruminants, particularly cattle and sheep, are primary sources of STEC, although most studies have focused on the detection of the O157 serogroup (36). In cases where this has been reported in cattle, the serotypes found to predominate seem to differ from one country to another. Pradel et al. (31) reported the most common serotypes from healthy cattle in France as OX3:H2, O113:H21, O113:H4, OX3:H21, O6:H10, OX178:H19, O171:H2, O46:H38, O172:H21, O22:H16, O91:H10, and O91:H21. Wieler et al. (37) found none of these serotypes in a study of bovine STEC isolated in Germany, although the fact that the isolates were from diarrheic calves rather than healthy cattle may have influenced the range of serotypes isolated. In a study of 358 cattle on 78 farms in Japan, 92 STEC isolates were recovered, of which 74 (80%) could be classified into O serogroups. Of these 74 isolates, 50% belonged to serogroups O8, O26, O84, O113, and O116 and 1 belonged to the O157 serogroup (20). Of the 25 serogroups they identified, only 3 (O15, O22, and O113) were also isolated by Pradel et al. (31) and 4 (O26, O103, O111, and O119) were isolated by Wieler et al. (37). In a longitudinal study of a herd of cattle in Germany, STEC isolates were isolated from 63.2% of cattle, and these were classified into 11 serotypes (5). Only one serotype (O91:H21) was also isolated by Pradel et al. (31), and none were isolated by Wieler et al. (37).

Limited studies have been carried out to determine the STEC population of cattle in Australia. Cobbold and Desmarchelier (8) isolated STEC from 16.7% of fecal samples and 4.1% of environmental samples from three dairy herds. Though they were limited to the identification of five serogroups (O111, O26, O6, O146, and O157), the STEC serotypes identified included O26:H11 (10.2% of the STEC isolates) and O157:H7 (11.2% of the STEC isolates), with prevalences in the cattle fecal samples of 1.7 and 1.9%, respectively. In a study of STEC in 204 feedlot cattle, Midgley et al. (26) cultured eight serotypes in the first 5 days after induction, but after 11 days the serotype O136:H16 predominated. More recently, Hallaran and Sumner (16) reported that the O157 serogroup was rarely detected in dairy cattle presented for slaughter in Victoria, Australia, when they isolated this serogroup from only 1 of 505 fecal samples.

The primary feature of STEC isolates is their ability to produce potent cytotoxins encoded by stx_1 and stx_2, but they also have a number of other virulence factors which enhance their pathogenicity (33). Some STEC have acquired the ability to adhere to the intestinal mucosa in an intimate fashion via the attachment and effacement protein, intimin, encoded by the *eaeA* gene (10, 23, 24, 25), and most produce a plasmid-encoded enterohemolysin, encoded by the *ehxA* gene. STEC isolates that cause disease in humans usually have one or both of these virulence-associated factors (3, 6, 19, 34) and have previously been referred to as complex STEC (cSTEC) (18).

The aim of this study was to isolate cSTEC from healthy cattle in eastern Australia. The study consisted of a "snapshot" examination of fecal samples from healthy preslaughter cattle

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from three production systems: pasture beef cattle, feedlot cattle, and dairy cattle. We also performed longitudinal studies of three feedlot and five pasture beef properties using multiplex PCRs for the detection of *stx*$_1$, *stx*$_2$, *eaeA*, and *ehxA* in fecal samples followed by culture of the samples with cSTEC profiles on vancomycin-cefsulodin-cefixime blood agar (BVCCA) plates or MacConkey agar followed by a further multiplex PCR of suspect cSTEC isolates. All cSTEC isolates were serotyped.

In the snapshot study, 1,692 fecal samples were collected from cattle on 72 commercial properties (27 pasture beef properties, 23 feedlot properties, and 22 dairy cattle properties) selected from across the eastern half of New South Wales and Queensland. Typically, 25 fecal samples were collected from individual healthy animals approximately 1 month prior to planned slaughter. Grazing animals were fresh off pasture and were sampled within 4 h of yarding.

Three feedlots and five pasture beef properties were used in the longitudinal studies. Samples from feedlot cattle (25 per property) were collected at induction, 1 month after induction, and prior to slaughter. Fecal samples from pasture beef properties were collected from 21 to 33 cows (pre- or postcalving) and from calves (17 to 27 samples) less than 6 weeks of age. Where possible, fecal samples were also collected from calves at preweaning or weaning (approximately 6 months old) and at about 2 weeks postweaning. Twenty-six drinking water samples were collected from the three feedlots at each sampling and from three of the five pasture beef properties.

In the snapshot study feces were prepared and subjected to PCR for the specific detection of *stx*$_1$, *stx*$_2$, *eaeA*, and *ehxA* as described by Fagan et al. (12). In the longitudinal study, fecal samples were subjected to PCR for the detection of *stx*$_1$, *stx*$_2$, *eaeA*, and *ehxA* as described by Paton and Paton (28) except that for DNA preparation, Instagene matrix (Bio-Rad, Richmond, Calif.) was used as described previously (12). The multiplex PCR described by Paton and Paton (28) was used in the longitudinal study because it had been demonstrated that the multiplex PCR described by Fagan et al. (12) did not detect *stx*$_{2d\text{-}Ount}$ and *stx*$_{2d\text{-}O111/OX3a}$ subtypes (9). Amplified DNA fragments were resolved by gel electrophoresis by using 2% agarose and stained with ethidium bromide. Glycerol stocks of the overnight EC (modified) broth (CM853; Oxoid, Basingstoke, United Kingdom) were stored at −80°C. Twenty milliliters of water was added to double-strength EC (modified) broth and incubated overnight at 37°C, after which PCR was carried out as described by Paton and Paton (28) with the modification described above.

Isolation of cSTEC was carried out on fecal sample EC (modified) broths, which were positive for at least one Shiga toxin and at least one of the other two virulence factors (*eaeA* and *ehxA*). These broths were cultured as described by Hornitzky et al. (18). Briefly, serial 10-fold dilutions of these stored glycerol stocks were diluted (to ensure single colonies) as described above and plated onto MacConkey agar. The dilution that produced single colonies (ideally about 100 colonies per plate) was cultured on two BVCCA plates. Colonies that produced a narrow zone of hemolysis after overnight incubation at 37°C were considered BVCCA positive. Up to 10 BVCCA-positive colonies from each plate per glycerol stock sample were subjected to multiplex PCR (28).

cSTEC were confirmed as being *E. coli*, O serogrouped (O1 to O173), and H typed (H1 to H56) using the methods described by Bettelheim and Thompson (2) and Chandler and Bettelheim (7). All strains were also tested for verotoxicity by the Vero cell assay described by Konowalchuk et al. (21).

TABLE 1. Multiplex PCR data derived from 1,692 fecal samples from 72 herds in the snapshot study

Herd	No. of samples with a cSTEC PCR positive profile[a]		
	Pasture beef	Feedlot	Dairy
1	3	11	15
2	18	15	12
3	3	20	1
4	1	4	3
5	0	8	2
6	2	3	9
7	4	16	3
8	0	6	5
9	6	7	0
10	4	7	0
11	7	17	2
12	1	18	0
13	11	16	0
14	1	17	7
15	0	16	3
16	8	13	13
17	6	4	4
18	20	0	4
19	13	1	0
20	3	3	1
21	2	2	0
22	8	5	11
23	1	6	
24	1		
25	0		
26	4		
27	4		

[a] 25 samples were collected from each herd.

In the snapshot study, 23 of 27 (85.2%) pasture beef properties, 22 of 23 (95.7%) feedlot cattle properties, and 16 of 22 (72.7%) dairy cattle properties had at least one fecal multiplex PCR with a cSTEC virulence factor profile. The number of cSTEC-positive broths for the 25 fecal samples per property ranged from 0 to 15 for the pasture beef cattle, 0 to 20 feedlot cattle, and 0 to 20 for the dairy cattle (Table 1).

In the feedlot samplings at least one cSTEC virulence factor profile was detected during all three samplings, and the highest number of cSTEC profiles (14 of 25 [56%]) were obtained in feedlot 3 at the time of induction. At least one cSTEC fecal broth profile was also obtained in all samplings of cows and calves from the pasture beef properties, and the highest number of positives was 14 of 26 (53.8%) from pasture beef property 3 from the preweaning-weaning sampling (Tables 1 and 2). Four of the nine feedlot water samples and 2 of the 17 pasture beef cattle water samples produced a cSTEC profile.

In the snapshot study 84 cSTEC were isolated from 37 animals (37 of 1692 [2.2%]). These were recovered from 12 of 25 (48%) pasture beef property, 4 of 23 (17.4%) feedlot cattle property, and 4 of 22 (18%) dairy cattle property fecal samples. The three most common cSTEC virulence factor profiles were *stx*$_2$ and *ehxA* (37 of 84 [44.0%]), *stx*$_1$, *stx*$_2$, and *ehxA* (20 of 84 [23.8%]), and *stx*$_1$ and *ehxA* (14 of 84 [16.7%]) The least common profiles were *stx*$_1$, *eaeA*, and *ehxA* (6 of 84 [7.1%]), and

TABLE 2. Number of cSTEC isolates from cattle feces and water in a longitudinal study of feedlot cattle

Feedlot and sample (*n*)	No. of cSTEC isolates[a] at:		
	Induction	1 mo	Preslaughter
1			
Feces (25)	1 (3)	2 (15)	2[b] (7)
Water (1)	0 (0)	0 (1)	1 (1)
2			
Feces (25)	12 (17)	4 (8)	5 (14)
Water (1)	0 (0)	1 (1)	1 (1)
3			
Feces (25)	14 (15)	0 (1)	0 (3)
Water (1)	0 (0)	0 (0)	0 (0)

[a] Numbers in parentheses are numbers of samples with cSTEC profiles after enrichment in EC (modified) broth.
[b] $n = 24$.

stx$_2$ and *eaeA* (6 of 84 [7.1%]), and *stx*$_1$ and *eaeA* was observed once (1.2%) (Table 3).

In the longitudinal study, cSTEC isolates were recovered from all five pasture beef properties and the three feedlots. One hundred eighteen cSTEC isolates were obtained from 104 animals. In the feedlot studies, feedlot 3 had the most cattle (14 of 25; 56%) excreting cSTEC at the induction sampling. However, cSTEC were not recovered from the cattle at the two subsequent samplings, although 1 of 25 and 3 of 25 fecal broths had cSTEC profiles (Table 1). cSTEC were recovered from each sampling from the other two feedlots (Table 1). cSTEC were recovered from 26 of 122 (21.3%) pasture beef cows and 29 of 113 (25.7%) of their calves at sampling 1. These consisted of 2 to 8 positive cows and 3 to 8 positive calves per property (Table 4). As in the snapshot study, *stx*$_2$ and *ehxA* (63 of 118; 53.4%) and *stx*$_1$, *stx*$_2$, and *ehxA* (38 of 118; 32.2%) were the most common cSTEC virulence factor profiles encountered. The least common profiles were *stx*$_1$, *stx*$_2$, *eaeA*, and *ehxA* (2 of 118; 1.7%) and *stx*$_2$, *eaeA*, and *ehxA* (2 of 118; 1.7%)

We did not recover cSTEC from the same animal in feedlot 1 on more than one occasion. In feedlot 2 there were three cattle from which cSTEC isolates were obtained at two samplings, but each sampling yielded a different serotype (O5:H− and O113:H11). cSTEC were cultured only from cattle in the first sampling (induction) from feedlot 3 (Table 2). Three of nine (33.3%) water samples yielded cSTEC (Table 1).

In the pasture beef properties, two samples from one calf in properties 1 and 2 were positive; three in pasture beef property 3, two in pasture beef property 4, and none in pasture beef property 5 were positive. On one occasion the serotype was the same (O2:H−). In pasture beef property 3, one cow and its calf yielded a cSTEC isolate with the same serotype (O8:H19). Of 17 water samples, 1 (5.9%) yielded cSTEC.

Biochemical analyses confirmed all isolates as *E. coli*, and all isolates produced verocytotoxin (data not shown).

Thirty-three serotypes were recovered from the cattle in the snapshot study. One serotype, O76:H7, was cultured from two dairy cattle. All other serotypes were each recovered from only one animal. A list of serotypes and virulence factor profiles for the STEC isolates is provided in Table 3. Forty-seven serotypes were recovered in the longitudinal studies. The most commonly recovered serotypes were O113:H21 (13 animals), O82:H8 (7 animals), O8:H19, and Ont:H8 (both from 6 animals). The cSTEC isolates from the three trough water samples in the feedlots were serotyped as O130:H11, Ont:H14, and O103:H14. The single water sample yielding cSTEC from the pasture beef properties was serotyped as O154:H− (Table 5). The number of O-untypeable strains is probably reflected by the fact that type strains and antisera for O groups beyond O173 are not yet available.

This work involved the recovery of cSTEC, detection of STEC virulence factors, and serotyping of cSTEC from fecal samples from two separate studies of cattle in eastern Australia. The snapshot study involved the examination of fecal samples for the presence of cSTEC from 1,692 healthy, slaughter-age cattle from three production systems. These samples were collected to determine the range of cSTEC serotypes likely to contaminate carcasses at slaughter and enter the food chain for human consumption. The longitudinal study of eight properties in two production systems, i.e., feedlot cattle and pasture beef cattle, was carried out to estimate the prevalence of cSTEC, to determine their serotypes during various stages of

TABLE 3. cSTEC serotypes isolated in the snapshot study

Serotype[a]	Previously reported[b]	No. of isolates (no. of cattle infected)	Virulence factor profile	Property type[c]
O2:H29	Yes	1 (1)	*stx*$_1$, *ehxA*	FB
O3:H7	No	6 (1)	*stx*$_1$, *stx*$_2$, *ehxA*	DC
O8:H19	Yes	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*	PB
O8(OKA):H5	No	2 (1)	*stx*$_2$, *ehxA*	PB
O26:H11	Yes	7 (1)	*stx*$_1$, *ehxA*	PB
O28:H40	No	1 (1)	*stx*$_2$, *ehxA*	DC
O51:H−	No	1 (1)	*stx*$_1$, *ehxA*	PB
O76:H7	No	2 (2)	*stx*$_1$, *stx*$_2$, *ehxA*	DC
O77:H39	No	1 (1)	*stx*$_1$, *ehxA*	PB
O81:H31	No	1 (1)	*stx*$_2$, *ehxA*	PB
O93(O8):H19	No	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*	PB
O93:H19	No	2 (1)	*stx*$_2$, *ehxA*	PB
O104:H7	No	1 (1)	*stx*$_1$, *eae*	PB
		2 (1)	*stx*$_2$, *eae*	PB
O110:H40	No	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*	PB
O113:H21	Yes	21 (1)	*stx*$_2$, *ehxA*	PB
O116:H21	Yes	5 (1)	*stx*$_2$, *ehxA*	PB
O130:H11	No	2 (1)	*stx*$_1$, *stx*$_2$, *ehxA*	PB
O149:H19	No	1 (1)	*stx*$_2$, *ehxA*	PB
O157:H7	Yes	3 (1)	*stx*$_1$, *eaeA*, *ehxA*	FB
O157:H8	Yes	2 (1)	*stx*$_2$, *ehxA*	PB
		1 (1)	*stx*$_1$, *eaeA*, *ehxA*	FB
O163:H−	Yes	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*	PB
Ont:H−	Yes	1 (1)	*stx*$_1$, *ehxA*	PB
		2 (1)	*stx*$_1$, *eaeA*, *ehxA*	FB
Ont:H2	Yes	1 (1)	*stx*$_2$, *eaeA*	DC
Ont:H5	No	1 (1)	*stx*$_2$, *ehxA*	PB
Ont:H7	Yes	1 (1)	*stx*$_2$, *eaeA*	PB
Ont:H11	No	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*	DC
Ont:H14	No	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*	FB
Ont:H16	Yes	3 (1)	*stx*$_1$, *ehxA*	PB
Ont:H19	Yes	3 (1)	*stx*$_1$, *stx*$_2$, *ehxA*	PB
Ont:H21	Yes	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*	PB
Ont:H40	No	1 (1)	*stx*$_2$, *ehxA*	PB
OR:H32	No	1 (1)	*stx*$_2$, *eaeA*	PB
OR:H34	No	1 (1)	*stx*$_2$, *eaeA*	DC

[a] Ont, O untypeable; OR, O rough.
[b] No, not previously reported (www.microbionet.com.au/vtectable.htm).
[c] FB, feedlot beef; DC, dairy cattle; PB, pasture beef.

TABLE 4. Number of cSTEC isolated from cattle feces and water in the longitudinal study of pasture beef cattle

Pasture beef property and sample	No. of cSTEC isolated/no. of samples[a] from:			
	Cows, pre- or postcalving	Calves		
		<6 wk old	Preweaning or weaning	Post weaning
1				
Feces	7/24 (9/24)	8/23 (13/23)	1/26 (2/26)	NS
Water	NS	0/4 (0/4)	0/2 (0/2)	NS
2				
Feces	3/21 (6/21)	7/17 (11/17)	2/19 (6/19)	0/19 (2/19)
Water	0/2 (0/2)	1/2 (1/2)	0/1 (0/1)	0/1 (0/1)
3				
Feces	6/31 (13/31)	4/27 (9/27)	8/26 (14/26)	3/28 (6/28)
Water	NS	NS	NS	NS
4				
Feces	8/23 (11/23)	7/23 (11/23)	NS	NS
Water	NS	NS	NS	NS
5				
Feces	2/33 (1/33)	3/23 (8/23)	5/28 (9/28)	1/28 (1/28)
Water	0/3 (0/3)	0/1 (0/1)	NS	0/1 (1/1)

[a] Numbers in parentheses are numbers of samples with cSTEC profiles after enrichment in EC (modified) broth. NS, not sampled.

cattle development, and to examine the relationship between cSTEC isolates from cows and their calves.

More than 200 serotypes of STEC have been isolated from healthy domestic animals (4, 14, 27, 35, 36). However, only a subset is commonly recovered from humans with serious gastrointestinal and systemic diseases, although more than 160 different serotypes have, on occasion, been recovered. In this study, 202 cSTEC isolates were isolated from 141 cattle. These consisted of 70 serotypes, of which 38 had not been reported previously (www.microbionet.com.au/vtectable.htm). This significantly increases the diversity of STEC serotypes that have been reported from cattle. Eleven serotypes (O3:H7, O8:H19, O26:H11, O76:H7, O113:H21, O130:H11, Ont:H−, Ont;H2, Ont:H11, Ont:H14, and Ont:H21) were common to both studies (Tables 3 and 5).

The most common serotypes identified in this study were O113:H21, recovered from 14 cattle (1 snapshot and 13 longitudinal studies), O82:H8, recovered from 7 cattle (longitudinal studies), and O8:H19, recovered from 7 cattle (1 snapshot and 6 longitudinal studies). O113:H21 is associated with HUS, but the other two serotypes have not been associated with human illness (www.microbionet.com.au/vtectable.htm). O113:H21 was the second most common serotype after OX3:H2 isolated from cattle in France (31), and the O113 serogroup was the second most common serogroup isolated from cattle in Japan (20). Apart from the O111 serogroup, O5:H− and O113:H21 are the most common serotypes associated with HUS in Australia in recent years (1, 11). Other serotypes recovered in this study that are associated with HUS worldwide are O2:H6, O5:H−, O26:H11, O91:H−, O113:H21, O157:H7, O157:H−, and O163:H19. Given that we have identified 70 serotypes in this study but only 8 have been isolated from seriously ill patients, it seems that the majority of cSTEC serotypes in cattle feces are unlikely to cause severe disease in humans, although O113:H21 is the serotype most commonly found in Australian cattle.

Interestingly, only two animals, one from a feedlot and one from a pasture beef property, excreted O157:H7/H− (Tables 2 and 4), the disease-causing serotypes most commonly reported in the international literature. There were no isolations of the O111:H− serotype, which is the serotype responsible for the largest STEC outbreak in humans in Australia. In this outbreak, caused by the consumption of mettwurst contaminated with the O111:H− serotype, 22 children developed HUS, including one fatality (29). To our knowledge this serotype has been reported only once from cattle in Australia (17). In that study, O111:H− was isolated from a cow with a profuse watery diarrhea and a calf from another herd with a history of ill-thrift and diarrhea, although there was no evidence that this serotype was responsible for the diarrhea. The same method was used to obtain the O111 isolates from sick animals, suggesting that the failure to isolate these serotypes in this study indicates a low prevalence in healthy animals. It is also noteworthy that O111:H−/H2 was isolated from diarrheic calves in Germany (37).

In this study, 70 serotypes were isolated. In parallel snapshot studies carried out with sheep (9), 29 serotypes were reported; however, there were only three serotypes (O5:H−, O84:H−, and O91:H−) which were common to both studies, and all were from properties running both cattle and sheep. These serotypes were isolated on only one occasion, and the O5:H− and O84:H− isolates were isolated from calves. This reinforces the suggestion that different STEC serotypes preferentially inhabit particular ruminant species. However, there are some serogroups, such as O157 and O103, which are common to both sheep and cattle and to other, nonruminant species which cause severe disease in humans. It may be that their capacity to colonize a broad host range contributes to their ability to cause disease in humans more readily than serotypes that appear to be confined to single animal species.

Four serotypes were recovered from water samples collected from the intensive-study properties (O103:H14, O130:H11, O154:H−, and Ont:H14). Interestingly, only one serotype, O130:H11, was cultured from cattle, and the other three serotypes have not previously been reported as STEC. It is possible that such STEC isolates may not grow competitively with other STEC isolates in feces under in vitro and in vivo culture conditions and hence have not previously been recovered from cattle, or it may be that these serotypes persist better in aqueous environments.

A correlation between the presence of Shiga toxin and enterohemolysin in fecal *E. coli* derived from humans and cattle has been previously reported (6, 18, 22). Consequently, BVCCA was used as a means of screening fecal enrichment broths for *E. coli* producing enterohemolysin in the hope that these isolates concomitantly contained *stx*. This was confirmed to be an efficient means of detecting cSTEC, as all 118 (Table 5) isolates in the longitudinal herd studies and 77 of 84 (91.7%) (Table 3) isolates in the snapshot study contained *ehxA*. Although the use of BVCCA was effective in detecting cSTEC containing *ehxA*, we did not recover cSTEC that carry *eaeA* without *ehxA* nearly as often. Whether this represents a true reflection of the prevalence of such isolates in vivo or a method that discriminates against their selection has yet to be determined.

TABLE 5. cSTEC serotypes isolated in longitudinal herd studies

Serotype[a]	Previously reported[b]	No. of isolates (no. of cattle infected)	Virulence factor profile	No. of infected cattle						
				Pasture beef				Feedlot		
				Dams	Calves			Induction	2 mo	Preslaughter
					< 6 wk old	Preweaning	Postweaning			
O2:H−	Yes	1 (1)	*stx*$_2$, *ehxA*			1				
O2:H6	Yes	1 (1)	*stx*$_2$, *ehxA*	1						
O2:H8	No	1 (1)	*stx*$_2$, *ehxA*					1		
O3:H7	No	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*					1		
O5:H−	Yes	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*			1				
O5:H7	No	4 (3)	*stx*$_1$, *stx*$_2$, *ehxA*					3		
O6:H34	Yes	4 (3)	*stx*$_2$, *ehxA*	1		1		1		
O8:H19	Yes	6 (6)	*stx*$_2$, *ehxA*	2	3	1				
O26:H11	Yes	5 (4)	*stx*$_1$, *ehxA*	3		1				
		1 (1)	*stx*$_1$, *eae*, *ehxA*			1				
O28:H8	No	3 (3)	*stx*$_2$, *ehxA*				1	2		
O68:H−	No	1 (1)	*stx*$_1$, *ehxA*					1		
O75:H1	No	1 (1)	*stx*$_1$, *ehxA*				1			
O76:H7	No	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*			1				
O81:H21	No	1 (1)	*stx*$_2$, *ehxA*		1					
O82:H8	Yes	7 (7)	*stx*$_1$, *stx*$_2$, *ehxA*	2	3			2		
O82:H40	No	2 (2)	*stx*$_1$, *stx*$_2$, *ehxA*		1	1				
O84:H−	Yes	1 (1)	*stx*$_1$, *eae*, *ehxA*				1			
O91:H−	Yes	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*	1						
O101:H39	No	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*							1
O103:H14	No	1 (water)	*stx*$_2$, *ehxA*							
O108:H7	No	5 (4)	*stx*$_1$, *stx*$_2$, *ehxA*		1	3				
O113:H−	Yes	1 (1)	*stx*$_2$, *ehxA*			1				
O113:H21	Yes	14 (13)	*stx*$_2$, *ehxA*	3	2	2		6		
		2 (2)	*stx*$_1$, *stx*$_2$, *ehxA*					2		
O130:H11	No	5 (3)	*stx*$_2$, *ehxA*			1		1		
		1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*							1
		1 (water)	*stx*$_1$, *stx*$_2$, *ehxA*							
O130:H38	No	3 (2)	*stx*$_1$, *stx*$_2$, *ehxA*					2		
O153:H8	Yes	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*	1						
O154:H−	No	1 (water)	*stx*$_1$, *stx*$_2$, *eaeA*, *ehxA*							
O157:H−	Yes	1 (1)	*stx*$_1$, *stx*$_2$, *eaeA*, *ehxA*							1
O160:H10	No	1 (1)	*stx*$_2$, *ehxA*							1
O163:H19	Yes	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*			1				
Ont:H−	Yes	2 (1)	*stx*$_2$, *ehxA*					1		
		1 (1)	*stx*$_2$, *eaeA*, *ehxA*						1	
Ont:H2	Yes	1 (1)	*stx*$_2$, *ehxA*					1		
Ont:H8	Yes	2 (1)	*stx*$_1$, *stx*$_2$, *ehxA*					1		
		6 (5)	*stx*$_2$, *ehxA*	2	1	2				
Ont:H11	No	2 (2)	*stx*$_2$, *ehxA*	2						
		2 (1)	*stx*$_1$, *ehxA*	1						
		1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*		1					
Ont:H14	No	1 (water)	*stx*$_2$, *ehxA*							
Ont:H21	Yes	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*		1					
Ont:H28	Yes	1 (1)	*stx*$_2$, *ehxA*					1		
		1 (1)	*stx*$_2$, *eaeA*, *ehxA*					1		
Ont:H32	Yes	1 (1)	*stx*$_2$, *ehxA*							1
Ont:H41	No	2 (1)	*stx*$_1$, *eaeA*, *ehxA*						1	
Ont:H49	No	2 (1)	*stx*$_2$, *ehxA*		1					
		1 (1)	*stx*$_2$, *ehxA*		1					
Ont:HR	Yes	3 (3)	*stx*$_2$, *ehxA*			2		1		
OR:H8	Yes	1 (1)	*stx*$_2$, *ehxA*		1					
OR:H31	No	1 (1)	*stx*$_1$, *stx*$_2$, *eaeA*, *ehxA*				1			
OR:H39	No	1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*							1
OR:H−	Yes	1 (1)	*stx*$_2$, *ehxA*					1		
		1 (1)	*stx*$_1$, *eaeA*, *ehxA*		1					
OX3:H8	Yes	4 (3)	*stx*$_2$, *ehxA*	1?	1?			2		
		1 (1)	*stx*$_1$, *stx*$_2$, *ehxA*					1		
OX3:H40	No	1 (1)	*stx*$_2$, *ehxA*	1						

[a] Ont, O nontypeable; OR, O rough.
[b] No, not previously reported (www.microbionet.com.au/vtectable.htm).

It had previously been reported that the PCR described by Fagan et al. (12) failed to amplify stx_{2d} subtypes that appeared to predominate in sheep (9, 32). This prompted us to replace that PCR for fecal screening in the longitudinal studies with the PCR described by Paton and Paton (28), as it was able to detect these stx_{2d} subtypes (32). In a recent study of bovine STEC containing stx_2, the subtypes $stx_{2d\text{-}Ount}$ and $stx_{2d\text{-}O111/OX3a}$ were rarely identified (K. Brett et al., submitted for publication). Hence, the use of the PCR described by Fagan et al. (12) for fecal screening in the snapshot study is unlikely to have significantly underestimated the number of cattle with fecal cSTEC virulence factor profiles.

In the longitudinal herd studies, there was little correlation between the isolation of cSTEC and the recovery of cSTEC from follow-up samples. In the feedlot studies, there were only three occasions in feedlot 2 where cSTEC was twice isolated from the same animal. On two occasions the cSTEC isolates were of different serotypes, and on the third occasion one isolate was not serotyped. In the only previous longitudinal study of feedlot cattle in Australia, serotypes O157:H−, O168:H8, and O136:H16 were identified. Of these, only O157:H− was recovered in this study. In the pasture beef properties, the same serotype (O2:H−) was isolated from one cow and its calf on one occasion, and only one cow and its calf yielded cSTEC isolates with the same serotype (O8:H19). Collectively, these data suggest that STEC represents a dynamic population of fluctuating serotypes which may also be influenced by diet, stress, hormonal levels, and the anatomical development of the gastrointestinal tract. More intensive studies are required to accurately investigate these fluctuations.

This study indicates that there is a very broad range of cSTEC serotypes in Australian cattle. Some serotypes have been found in other studies of cattle in France, Germany, Japan, and Argentina, but there are some serotypes in these studies which seem to be unique to the population under study. The fact that better and cheaper methods are required for the recovery of non-O157 STEC is, no doubt, a limiting factor in the capacity to recover all the serotypes being excreted by cattle at any particular time. The lack of continuity in isolating cSTEC of the same serotype or even isolating cSTEC from the same animals at subsequent samplings in the longitudinal herd studies suggests that there is a more diverse population of STEC in healthy cattle or that more intensive studies using systems such as hydrophobic grid membrane filtration need to be undertaken.

While the diversity of cSTEC serotypes found in this study in cattle and previously reported for sheep (9) may not be considered of great importance from the human viewpoint, it should be noted that a number of these serotypes have been associated with HUS and other human infections (1, 11). Even when the main causative STEC strain in an outbreak was identified (29) as O111:H−, further detailed studies on the serological response of the patients showed that other STEC strains were likely to have contributed to their condition and clinical outcome (13). In the current situation, where very few clinical laboratories around the world look for STEC strains other than O157 strains, these non-O157 STEC strains will not be recognized. Only when methods looking for all STEC isolates regardless of serotype come into general use will the significance of finding such a diversity of STEC serotypes in the feces of domestic food animals be determined. The recent finding (30) that STEC isolates of serotype O113:H21 carry a hitherto-unrecognized novel autoagglutinating adhesin produced by the *saa* gene while lacking *eaeA* draws attention to the possibility that other bovine and ovine isolates may carry further as-yet-unrecognized virulence factors, enhancing their human pathogenicity.

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