Detection of Shiga toxin-producing *Escherichia coli* (STEC) serogroups other than the top-7 STEC, isolated from cattle feces, by multiplex PCR assays

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INTRODUCTION

- Shiga toxin producing *Escherichia coli* (STEC) are major foodborne pathogens.
- Among STEC, seven serogroups, O26, O45, O103, O111, O121, O145, and O157, called top-7 STEC, cause a majority of foodborne STEC infections in humans.
- These STEC reside in the hindgut of cattle and are shed in the feces, which is the major cause of contamination of beef, produce, and water sources.
- Cattle harbor several other serogroups (n=108) of STEC, however, not all have been reported to cause human illness (Table 1).
- Eleven multiplex PCR assays were developed to identify 113 serogroups of STEC, however, only 108 of those serogroups are known to be present in cattle.

OBJECTIVE

Our objective was to identify the serogroups of STEC, isolated from cattle feces, that were not positive for the top-7 STEC, by multiplex PCR assays targeting specific genes for each serogroup.

MATERIALS AND METHODS

- 241 strains isolated from pen-floor fecal samples from 8 feedlots in the central U.S. were used.
- Samples were collected over a 12 week period from June 2014 to August 2014.
- All 241 strains of *E. coli* were positive for the *stx1* (Shiga toxin 1), *stx2* (Shiga toxin 2), or both, and/or the *eae* (intimin) genes, but negative for the top-7 STEC serogroups.
- Eleven sets of multiplex PCR assays developed and validated with known serogroups, which targeted the *wzx* and *wzy* genes in the O-antigen gene cluster of specific serogroups for STEC, were used for the identification of the serogroups.
- Each assay had a different annealing temperature and varied between 25 or 30 PCR cycles (Table 2).
- For each assay, known serogroups of STEC were used as the positive control.

Table 1. Serogroups of Shiga toxin-producing *E. coli* that have been detected in cattle, and the ones known to cause human illness are highlighted.

108 Serogroups identified in cattle									
O1	O2	O3	O4	O5	O6	O7	O8	O10	O11
O15	O16	O17	O18	O19	O20	O21	O22	O23	O25
O29	O35	O37	O38	O39	O40	O42	O43	O46	O49
O51	O53	O55	O62	O63	O69	O70	O74	O75	O76
O79	O80	O81	O82	O84	O85	O86	O87	O88	O90
O91	O92	O93	O96	O98	O101	O102	O105	O107	O108
O109	O110	O112	O113	O114	O115	O116	O118	O119	O120
O124	O125	O126	O128	O130	O131	O132	O136	O139	O140
O141	O146	O147	O148	O149	O150	O152	O153	O154	O156
O159	O160	O161	O163	O165	O168	O169	O170	O171	O172
O174	O175	O176	O177	O178	O179	O181	O182		

RESULTS

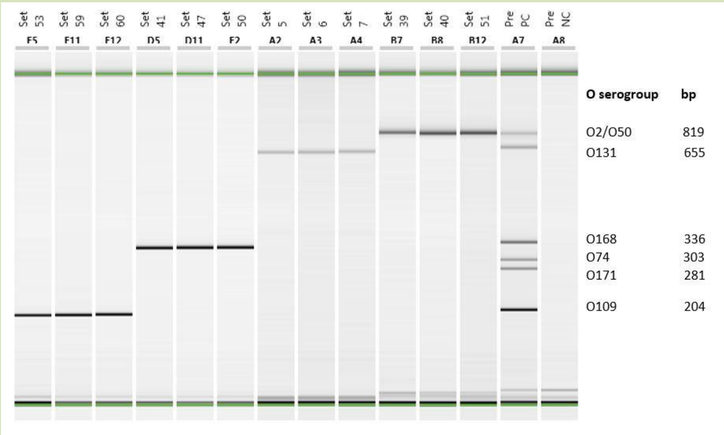
Table 2. Eleven sets of multiplex PCR assays designed to detect 113 serogroups of Shiga toxin-producing *E. coli.*

PCR set	Serogroups	PCR cycles	Annealing Temperature
Set-1	O4, O86, O84, O156, O80, O168, O91, O109	25	65°C
Set-2	O128, O138, O123/O186, O120, O119, O13/O129/O135, O175, O171, O22, O5	30	65°C
Set-3	O6, O98, O181, O75, O33, O116, O150, O79, O25	30	64°C
Set-4	O78, O2/O50, O146, O76, O178, O126, O113, O118/O151, O15, O147	30	63°C
Set-5	O62/O68, O7, O163, O136, O8, O92, O87, O55, O20	30	61°C
Set-6	O141, O153, 132, O130, O108, O96, O88, O107/O117, O74, O38, O39, O115	30	66°C
Set-7	O70, O53, O69, O51, O17/44/73/77/106, O43, O40, O37, O35, O28 (O42/28ac), O18 (18ab/18ac), O1	30	63°C
Set-8	O139, O125 (125ab/125ac), O124/O164, O90/O127, O102, O105, O85, O82, O81, O148, O140	25	68°C
Set-9	O169, O161, O154, O114, O110, O93, O149, O49, O21	25	63°C
Set-10	O165, O160, O182, O179, O46/O134, O177, O176, O174, O172, O170, O159, O152	25	59°C
Set-11	O131, O19, O16, O63, O23, O29, O101/O162, O112ab, O11, O10, O3	30	62°C

Table 3: Multiplex PCR-based identification of serogroups of Shiga toxin-producing *Escherichia coli* (n=241), other than the top-7, isolated from feedlot cattle feces.

Serogroups	Prevalence	Enterohemorrhagic *E. coli*			Shiga toxin-producing *E. coli*		
	(%)	*stx*1	*stx*2	*eae*	*stx*1	*stx*2	*eae*
O20	1(.4)	-	-	-	-	1	-
O35	1(.4)	-	-	-	-	1	-
O76	1(.4)	-	-	-	1	-	-
O96	1(.4)	-	-	-	-	1	-
O113	1(.4)	-	-	-	1	1	-
O118	1(.4)	1	-	1	-	-	-
O175	1(.4)	-	1	1	-	-	-
O98	1(.4)	1	-	1	-	-	-
O102	2(.8)	-	-	-	-	2	-
O178	2(.8)	-	-	-	2	2	-
O136	3(1.3)	-	-	-	-	3	-
O171	4(1.7)	-	-	-	-	4	-
O74	5(2.1)	5	-	5	-	-	-
O8	7(2.9)	-	-	-	4	5	-
O104	12(5.0)	-	-	-	12	-	-
O2	20(8.3)	-	-	-	7	18	-
O131	28(11.6)	-	-	-	-	28	-
O109	40(16.6)	-	35	35	4	1	-
O168	72(29.9)	-	-	-	-	72	-
Unknown	38(15.8)	-	-	-	-	38	-

Image 1. Gel image for the four most predominant serogroups for the eight feedlots.



RESULTS

Of the 241 strains from the eight feedlots, 203 strains (84.2%) belonged to 19 serogroups and 38 (15.8%) were unidentified. The 19 serogroups included O168, O109, O131, O2, O104, O8, O74, O171, O136, O178, O102, O98, O175, O11, O113, O96, O76, O35, and O20.

CONCLUSIONS/SIGNIFICANCE

- Cattle are reservoirs for many STEC serogroups, including those that are not a part of the top-7.
- The four most predominant serogroups for the eight feedlots were: O168 (72/241; 29.9%), O109 (40/241; 16.6%), O131 (28/241; 11.6%), and O2 (20/241; 8.3%) (Fig. 1).
- Serogroup O168 has been isolated from post-weaned pigs with diarrhea (Ho et. al. 2013).
- Serogroup O109 has been isolated from samples of cheese made with raw milk in France and poses a potential health risk in human consumers (Vernozy-Roznad et. al. 2005).
- Serogroup O131 has been isolated from fecal samples of chickens with diarrhea (Wani et. al. 2004).
- Serogroups O2 has been isolated from humans with diarrhea and urinary tract infections (Rizvi et. al. 2003).
- The dominance of a few serogroups of STEC that do not belong to the top-7 STEC in cattle feces suggests potential risk to cause illness in humans.

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