Multiplexed PCR Assays for the Detection of Shiga Toxin-Producing *Escherichia coli*, other than the top-7 Serogroups, Isolated from Feedlot Cattle Feces.

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INTRODUCTION

- Shiga toxin-producing *E. coli* are major food borne pathogens that reside in the hindgut of cattle and are shed in the feces which serve as a source of contamination of beef, produce, and water for human infections.
- A majority of foodborne infections are associated with seven serogroups, often called top-7 STEC.
- The top-7 STEC include serogroups O26, O45, O103, O111, O121, O145, and O157.
- Cattle harbor multiple other STEC serogroups (n=113) and some have been associated with human illnesses.
- Eleven sets of multiplex PCR assays have been developed and validated to detect the 113 serogroups of STEC.

OBJECTIVE

Utilize eleven sets of multiplex PCR assays to identify STEC isolates from feedlot cattle feces that did not belong to the top-7 STEC.

MATERIALS AND METHODS

- One hundred and eighteen isolates from fecal samples, collected from a single feedlot located in the Central U.S. in 2013 over a 12 week period, were used in the study.
- The strains tested positive from *stx1* (Shiga toxin 1) only (n=22), *stx2* (Shiga toxin 2) only (n=30), both *stx1* and *stx2* (n=68), and/or *eae* (intimin; n=25), but negative for the serogroup-specific genes of the top-7 STEC.
- Serogroup-specific primers were designed based on the published sequences of the O-gene cluster.
- Eleven sets of multiplex PCR assays targeting serogroup-specific genes (*wzx*, *wzy*, *gnd*, *wbdA*) of 113 serogroups were used to identify the strains (Table 1).
- The PCR conditions consisted of 94º C for 5 minutes, 25 or 30 cycles of 94º C denaturation for 30 seconds, 59-68º C annealing for 30 seconds, 68º C extension for 75 seconds, and the final step of 68º C extension for 7 minutes.
- Each multiplex PCR assay included known strains of STEC as positive controls.

RESULTS

- Of the 118 strains tested in the study, 25 (21.2%) strains belonged to enterohemorrhagic *E. coli* pathotype, 70 (59.3%) strains belonged to Shiga toxin-producing *E. coli* pathotype.
- A total of 95 strains (80.5%) belonged to 11 serogroups and 23 (19.5%) were unidentifiable (Table 2).
- The 11 identified serogroups included the following: O168 (29.7%), O109 (20.3%), O171 (9.3%) O74 (6.8%), O104 (4.2%), O8 (2.5%), O175 (0.8%), O131 (0.8%), O160 (0.8%), and O169 (0.8%).

Figure 1. Predominant Shiga toxin-producing *E. coli* serogroups (O2, O131, O168, O171, O74, and O109), detected by multiplex PCR assays, in feedlot cattle feces .

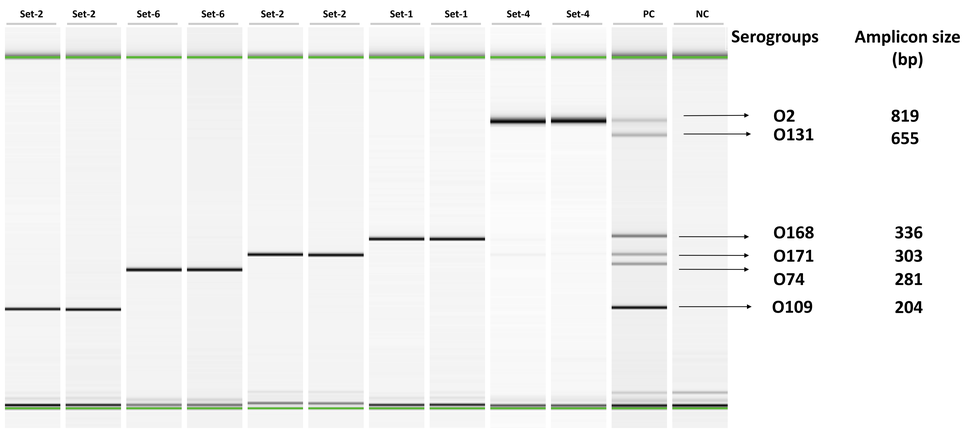

Table 1. Multiplex PCR assays for the detection of Shiga toxin-producing *Escherichia coli* (STEC) serogroups other than the top-7 STEC.

Assays	Genes targeted	Number of O groups	PCR Cycles[a]	Annealing temperature[a]	O groups
Set-1	*wzx/wzy*	8	25	65°C	O4, O80, O84, O86, O91, O109, O156, O168
Set-2	*wzx/wzy*	10	30	65°C	O5, O13/O129/O135, O22, O119, O120, O123/O186, O128, O138, O171, O175
Set-3	*wzx/wzy*	9	30	65°C	O6, O25, O33, O75, O79, O98, O116, O150, O181
Set-4	*wzx/wzy*	10	30	65°C	O2, O15, O76, O78, O113, O118, O126, O146, O147, O178
Set-5	*wzx/wzy/gnd/wbdA*	9	30	65°C	O7, O8, O20, O55, O62/O68, O87, O92, O136, O163
Set-6	*wzx/wzy*	12	30	65°C	O38, O39, O74, O88, O96, O107/O117, O108, O115, O130, O132, O141, O153
Set-7	*wzx/wzy*	12	30	65°C	O1, O17/O44/O73/O77/O106, O18, O28, O35, O37, O40, O43, O51, O53, O69, O70
Set-8	*wzx*	11	25	65°C	O81, O82, O85, O90/O127, O102, O105, O124/O164, O125, O139, O140, O148
Set-9	*wzx*	9	25	65°C	O21, O49, O93, O110, O114, O149, O154, O161, O169
Set-10	*wzx*	12	25	65°C	O46/O134, O152, O159, O160, O165, O170, O172, O174, O176, O177, O179, O182
Set-11	*wzx/wzm*	11	30	65°C	O3, O10, O11, O16, O19 O23, O29, O63, O101/O162, O112, O131
Total		**113**			

[a] All other running conditions were similar for the 11 sets of multiplex PCR assays.

Table 2. Identification of serogroups of Shiga toxin-producing and enterohemorrhagic *Escherichia coli* by multiplex PCR assays

Serogroups	Positive (%)	Enterohemorrhagic *E. coli*			Shiga toxin-producing *E. coli*		
		stx1	*stx2*	*eae*	*stx1*	*stx2*	*eae*
O131	1 (0.8)					1	
O160	1 (0.8)					1	
O169	1 (0.8)					1	
O175	1 (0.8)					1	
O8	3 (2.5)				1	3	
O2	5 (4.2)				1	5	
O104	5 (4.2)				5		
O74	8 (6.8)	8		8			
O171	11 (9.3)	1		1		10	
O109	24 (20.3)		16	16	8		
O168	35 (29.7)				1	34	
Unidentified	23 (19.5)				1	22	

CONCLUSIONS/SIGNIFICANCE

- Cattle harbor multiple STEC serogroups other than the top 7 in the hindgut. The results indicate the diversity of STEC shed in the feces, including the four most predominant groups: O168 (29.7%), O109 (20.3%), O171 (9.3%) and O74 (6.8%).
- The occurrence of STEC serogroups O171, O74, O104, and O8 have been reported in feces of beef (Meichtria et al., 2004) dairy cattle (Gonzales et al., 2016).
- Similar to STEC O157 infections, STEC other than the top 7 associated illnesses can range from cases of sporadic to major outbreaks from mild watery diarrhea to life threatening complications of hemolytic uremic syndrome.
- Food of animal origin, including ground beef and raw milk, are major sources of transmissions of STEC to human consumers.
- Because these strains are either Shiga toxigenic (n=93) or enterohemorrhagic (n=25), the serogroups have the potential to cause human illness.
- The main virulence factor of STEC is production of *stx1* or *stx2* proteins. Human Virulent STEC strains often contain other virulence factors such as intimin (*eae*), a protein essential for the intimate attachment and the formation of attaching and effacing lesions on gastrointestinal epithelial cells thus leading to gastrointestinal illnesses.
- Identifying the different serogroups, other than the top-7, allows for proper assessment of prevalence and potential risk of human illness that are attributed to cattle and subsequent beef products.

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