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Pooling of Immunomagnetic Separation Beads Does Not Affect Detection Sensitivity of Six Major Serogroups of Shiga Toxin–Producing *Escherichia coli* in Cattle Feces†

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ABSTRACT

Shiga toxin–producing *Escherichia coli* (STEC) of the serogroups O26, O45, O103, O111, O121, and O145, often called non-O157 STEC, are foodborne pathogens. Cattle are asymptomatic reservoirs for STEC; the organisms reside in the hindgut and are shed in the feces, which serve as the source of food product contaminations. Culture-based detection of non-O157 STEC involves an immunomagnetic separation (IMS) step to capture the specific serogroups in complex matrices, such as feces. The IMS procedure is time consuming and labor intensive because of the need to subject each fecal sample to six individual beads. Therefore, our objective was to evaluate whether pooling of IMS beads affects sensitivity of non-O157 STEC detection compared with using individual IMS beads. The evaluation was done by comparing detection of serogroups in feces spiked with pure cultures (experiments 1 and 2) and from feces ($n = 384$) of naturally shedding cattle (experiment 3). In spiked fecal samples, detection with pools of three, four, six, or seven beads was similar to, or at times higher than, detection with individual IMS beads. In experiment 3, the proportions of fecal samples that tested positive for the six serogroups as detected by individual or pooled beads were similar. Based on noninferiority tests, detection with pooled beads was not substantially inferior to detection with individual beads ($P < 0.05$). In conclusion, the pooling of IMS beads is a better option for detection of STEC serogroups in fecal samples compared with individual beads because the procedure saves time and labor and has the prospect of a higher throughput.

Shiga toxin–producing *Escherichia coli* (STEC) of the serogroups O26, O45, O103, O111, O121, and O145, often called non-O157 STEC, are foodborne pathogens detected with increasing frequency *(12)*. Similar to the O157:H7 serotype, six serogroups of STEC cause sporadic cases and outbreaks of diarrhea, hemorrhagic colitis, hemolytic uremic syndrome in children, and thrombotic thrombocytopenic purpura in the elderly *(3, 16)*. Cattle are asymptomatic reservoirs for STEC, which are normal inhabitants of the hindgut and are shed in feces. The feces serve as a source of contamination of beef and dairy products and water.

The culture method of non-O157 STEC detection and isolation involves enrichment of feces in a selective broth, which is then subjected to immunomagnetic separation (IMS), followed by plating on a selective medium. The putative colonies are picked based on a phenotypic characteristic of the serogroup, with the final confirmation by immuno- or PCR assay *(6, 20, 22)*. IMS increases sensitivity of detection of STEC, particularly from complex matrices such as feces *(7)*. Detection of six serogroups of non-O157 STEC requires treating each sample individually with six serogroup-specific beads, which makes the culture method a labor-intensive, time-consuming procedure. Pooling IMS beads would allow for fewer IMS cycles to test samples for the six serogroups, resulting in reduced labor, time, and expense. The objective of this study was to determine whether pooling of IMS beads affects detection sensitivity of the six major serogroups of non-O157 STEC in cattle feces compared with use of individual beads.

MATERIALS AND METHODS

Three experiments were performed, two with fecal samples spiked with pure cultures of STEC (experiments 1 and 2) and one with feces from naturally shedding cattle (experiment 3). In each experiment, fecal samples were subjected to individual IMS beads and to combinations of pooled IMS beads to compare detection sensitivity.

Fecal samples spiked with individual or pooled pure cultures of STEC. Strains of seven STEC (Table 1) from our culture collection were used to spike pen-floor fecal samples collected from feedlot cattle in the University Beef Cattle Research Center (Kansas State University, Manhattan). Strains from frozen

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TABLE 1. *Virulence gene profiles of Shiga toxin–producing* Escherichia coli *strains used to spike fecal samples*[a]

E. coli serogroup	Strain	Source	stx_1	stx_2	*eae*	*ehxA*
O26	2013-3-6D	Bovine feces	+	−	+	+
O45[b]	1.2622	Bovine feces	+	−	−	−
O45[b]	CDC-96-3285	Human	+	−	+	+
O103	2013-3-174C	Bovine feces	+	−	+	+
O111	2013-3-390E	Bovine feces	+	−	+	+
O121	KDHE 48	Human	−	+	+	−
O145	2013-3-86C	Bovine feces	+	−	+	+
O157	2013-376D	Bovine feces	+	+	+	+

[a] CDC, Centers for Disease Control and Prevention; KDHE, Kansas Department of Health and Environment.
[b] Strain 1.2622 was used in experiment 1, and strain CDC-96-3285 was used in experiment 2.

protect beads (CryoCare, Key Scientific Products, Round Rock, TX) were grown individually on blood agar (Remel, Lenexa, KS) plates. Single colonies were inoculated into 10 ml of Luria-Bertani broth (BD, Sparks, MD) and incubated at 37°C for 16 h. Then, 100 μl was inoculated into 10 ml of Luria-Bertani broth and was incubated at 37°C until an absorbance of 0.4 (600 nm) was achieved ($\sim 10^8$ CFU/ml). Tenfold serial dilutions (10^{-1} to 10^{-7} dilutions) were prepared in buffered peptone water for each cultured STEC strain. Viable cell counts (CFU per milliliter) were determined by spread plating 100 μl of 10^{-5}, 10^{-6}, and 10^{-7} dilutions on four blood agar plates per dilution. In experiment 1, feces suspended in *E. coli* broth (EC broth; Difco, BD; *(19)*) were inoculated with individual or pooled STEC at 10 CFU/ml of each serogroup and were enriched (6 h at 40°C) before the IMS step. In experiment 2, feces suspended in EC broth were inoculated with individual or pooled STEC at 100 CFU/ml of each serogroup and then were subjected to the IMS step without enrichment.

Experiment 1: feces spiked with pure cultures and subjected to enrichment. Approximately 30 g of each fecal sample ($n = 6$) was suspended in 270 ml of EC broth, and a uniform suspension was achieved with a magnetic stirrer. Fecal suspensions were transferred in 18-ml aliquots to tubes, which were inoculated with individual or a pool of three (O26, O45, and O103; O111, O121, and O145), four (O26, O45, O103, and O157; O111, O121, O145, and O157) or seven (O26, O45, O103, O111, O121, O145, and O157) STEC cultures (Table 2) to obtain a final concentration of 10 CFU/ml of each STEC. The final volume of inoculated fecal suspension was adjusted to 20 ml with buffered peptone water. Fecal suspensions were enriched (40°C for 6 h) and were subjected to IMS in a Kingfisher Flex Magnetic Particle Processor (Thermo Scientific, Waltham, MA) according to the protocol provided by the manufacturer. Enriched fecal suspensions

TABLE 2. *Detection of STEC with individual or pooled IMS beads in cattle feces spiked with individual or pooled STEC*[a]

Fecal samples and IMS beads	Serogroups detected, no. positive						
	O26	O45	O103	O111	O121	O145	O157
Control (not spiked)							
Individual beads	0	1	0	0	0	0	0
Pooled beads: O26+O45+O103 +O111+O121+O145+O157	0	3	2	0	0	0	0
Spiked with individual STEC							
Individual beads	6	0	6	6	2	3	6
Pooled beads:							
O26+O45+O103	6	0	5				
O111+O121+O145				5	4	2	
O26+O45+O103+O157	5	0	5				6
O111+O121+O145+O157				6	1	5	6
O26+O45+O103+O111+O121+ O145+O157	6	1	6	6	3	4	6
Spiked with STEC O26+O45+O103							
Individual beads	6	0	6				
Pooled beads: O26+O45+O103	6	0	6				
Spiked with STEC O111+O121+O145							
Individual beads				6	3	3	
Pooled beads: O111+O121+O145				6	3	2	
Spiked with STEC O26+O45+O103+O157							
Individual beads	6	0	5				6
Pooled beads: O26+O45+O103+O157	5	1	5				5
Spiked with STEC O111+O121+O145 +O157							
Individual beads				5	2	2	5
Pooled beads: O111+O121+O145+O157				6	1	2	5
Spiked with STEC O26+O45+O103+ O111+O121+O145+O157							
Individual beads	6	0	5	5	4	3	6
Pooled beads: O26+O45+O103+ O111+ O121+O145+O157	6	0	5	4	1	0	3

[a] STEC, Shiga toxin–producing *Escherichia coli*; IMS, immunomagnetic separation. $n = 6$.

TABLE 3. *Culture-based detection of six serogroups of non-O157 STEC in cattle feces spiked with six serogroups of STEC with individual or pooled IMS beads*[a]

Fecal samples and IMS beads	Serogroups detected, no. of samples					
	O26	O45	O111	O103	O121	O145
Spiked with individual STEC						
Individual beads	5	1	6	6	5	6
Pooled beads:						
O26+O45+O111	6	0	2			
O103+O121+O145				3	6	6
O26+O45+O103+O111+O121+O145	6	1	6	6	5	6
Spiked with STEC O26+O45+O111						
Individual beads	6	0	6			
Pooled beads:						
O26+O45+O111	6	0	4			
O26+O45+O111+O103+ O121+O145	6	0	6			
Spiked with STEC O103+O121+O145						
Individual beads				6	6	6
Pooled beads:						
O103+O121+O145				6	1	4
O26+O45+O111+O103+ O121+O145				5	5	4

[a] STEC, Shiga toxin–producing *Escherichia coli*; IMS, immunomagnetic separation. $n = 6$.

not spiked with pure cultures and subjected to individual and a pool of seven IMS beads (seven serogroups; Abraxis, Warminster, PA) served as control to detect whether the feces were positive for any of the serogroups before inoculation. Aliquots (860 μl) of enriched sample were mixed with 20 μl of individual IMS beads or 20 μl of each pooled serogroup IMS beads (60 μl of a pool of three IMS beads, 80 μl of a pool of four IMS beads, or 140 μl of a pool of seven IMS beads). Total volume of each sample was adjusted to 1 ml with the addition of sterile EC broth. Fecal suspensions inoculated with individual or a pool of three, four, or seven STEC (10 μl/ml of each serogroup) were subjected to IMS with corresponding individual beads or pools of three, four, or seven IMS beads (Table 2). Following the IMS procedure, 50 μl of each non-O157 bead suspension was spread plated onto chromogenic Possé agar *(21)* modified to include novobiocin at 5 mg/liter and potassium tellurite at 0.5 mg/liter *(20)*. It has been shown that, compared with STEC O157, many non-O157 STEC have a reduced tolerance to novobiocin *(14, 23)* and potassium tellurite *(4, 18)*. As a result, we modified the composition of the Possé medium by decreasing the concentrations of novobiocin (8.0 to 5.0 mg/liter) and potassium tellurite (2.5 to 0.5 mg/liter). The O157 bead suspensions were spread plated onto sorbitol MacConkey agar containing cefixime (0.05 mg/liter) and potassium tellurite (2.5 mg/liter; BD). Plates were incubated for 20 to 24 h at 37°C; and up to six (for individual IMS beads) or up to 10 (for pooled three, four, or seven IMS bead treatments) chromogenic colonies (mauve, green, blue, or purple) from modified Possé medium were randomly picked, inoculated onto blood agar plates, and incubated at 37°C for 24 h. Similarly, from sorbitol MacConkey agar with cefixime and tellurite plates, up to six (for individual beads) or 10 (for pooled three, four, or seven IMS bead treatments) sorbitol-negative colonies were randomly picked, inoculated onto blood agar plates, and incubated at 37°C for 24 h. The colonies (6 or 10) from each non-O157 sample were pooled in distilled water, boiled for 10 min, and centrifuged at 9,300 × *g* for 5 m; and the lysate containing the DNA was tested by a 7-plex PCR *(19)* that targeted the seven serogroup-specific genes (*wzx* gene for O26, O45, O103, O111, and O145; *wbqE* and *wbqF* for O121; and *rfbE* for O157). The amplified DNA was separated on a capillary electrophoresis system in a QIAxcel Advanced System (Qiagen, Valencia, CA) and was analyzed using QIAxcel Screengel software. For the O157 serogroup, the non-sorbitol-fermenting colonies were tested for the O157 antigen by latex agglutination (Oxoid Ltd., Basingstoke, UK); and, if positive, a spot-indole test was performed. Colonies positive for agglutination and indole production were tested by a 6-plex PCR *(2)* that targeted *rfbE, fliC*$_{H7}$, *eae, stx*$_1$, *stx*$_2$, and *ehxA* genes.

Experiment 2: feces spiked with pure cultures and not subjected to enrichment. Twenty pen-floor fecal samples were collected from the University Beef Cattle Research Center, and aliquots of samples were enriched in EC broth and were subjected to a 7-plex PCR assay that targets serogroup specific genes of the seven STEC serogroups *(19)*. Six fecal samples that were negative for all of the seven serogroups were selected to be spiked with pure cultures of STEC. The procedures to prepare the inoculum and determine the concentration of inoculum were as in experiment 1. The final concentration of inoculated fecal suspension in EC broth was adjusted to 100 CFU/ml of each serogroup and was subjected to the IMS procedure without enrichment. Different combinations of pooled IMS beads, O26+O45+O111 and O103+O121+O145, were used in this experiment. Fecal suspensions not spiked with pure cultures and subjected to individual IMS beads served as the control to detect the natural presence of any of the six serogroups. Aliquots (860 μl) of samples were mixed with 20 μl of individual IMS beads or 20 μl of each serogroup IMS beads for pooled bead treatments. The total volume of each sample was adjusted to 1 ml with the addition of sterile EC broth. Fecal suspensions inoculated with individual or pools of three (O26+O45+O111 and O103+O121+O145) or six STEC (O26, O45, O111, O103, O121, and O145) were subjected to IMS with corresponding individual or pools of three or six IMS beads (Table 3). The IMS beads were spread plated onto modified Possé medium, and colonies were picked and tested for serogroups by PCR as previously described.

TABLE 4. *Culture-based detection of six serogroups of non-O157 STEC in cattle feces with individual and pooled IMS beads*[a]

IMS beads	Serogroups, no. (%) of samples positive					
	O26	O45	O103	O111	O121	O145
Individual beads						
O26	95 (24.7)	3	21	0	0	1
O45	40	47 (12.2)	24	3	1	1
O103	25	2	182 (47.4)	1	1	1
O111	36	2	38	27 (7.0)	1	1
O121	31	4	28	1	28 (7.3)	2
O145	36	2	27	1	0	32 (8.3)
Positive with any individual bead[b]	148 (38.5)	52 (13.5)	199 (51.8)	27 (7.0)	30 (7.8)	32 (8.3)
Pooled beads						
O26+O45+O111	155 (40.4)	42 (10.9)	39 (10.2)	29 (7.6)	0	1
O103+O121+O145	51	7	206 (53.6)	2	24 (6.3)	26 (6.8)
Positive with any pooled beads[b]	162 (42.2)	46 (12.0)	206 (53.6)	29 (7.6)	24 (6.3)	26 (6.8)

[a] STEC, Shiga toxin–producing *Escherichia coli*; IMS, immunomagnetic separation. $n = 384$.
[b] Nonredundant samples positive for each serogroup by any individual or pooled IMS beads.

Experiment 3: feces from naturally shedding dairy and feedlot cattle. Pen-floor fecal samples were collected from dairy cows ($n = 48$) at the University Dairy Teaching and Research Center (Kansas State University), from feedlot cattle ($n = 48$) at the Beef Cattle Research Center, and from feedlot cattle ($n = 288$) from six commercial feedlots in Nebraska and Texas. Approximately 2 g of feces was suspended in 18 ml of EC broth, vortexed for 30 s, and incubated at 40°C for 6 h. Enriched fecal suspensions were then subjected to individual beads and to two pools of IMS beads (Table 4). The pooled IMS beads were O26+O45+O111 and O103+O121+O145. For individual IMS beads, 980 μl of enriched sample was mixed with 20 μl of individual IMS beads and was subjected to the IMS procedure. For pooled IMS bead treatments, 940 (pool of three) μl of enriched sample was mixed with 20 μl of each IMS bead in the pool and was subjected to the IMS procedure. Culture procedures following IMS to identify the serogroups were as previously described. For fecal samples ($n = 288$) from the six commercial feedlots, if pooled colonies were positive for any of the six serogroups, then each colony was tested individually by an 11-plex PCR *(1)* to identify the seven serogroups (O157 and six non-O157) and the four major virulence genes (stx_1, stx_2, *eae,* and *ehxA*).

Statistical analysis. We applied a noninferiority test for each serogroup to test the hypothesis that use of pooled IMS beads was not inferior to the use of individual IMS beads for detection of STEC O serogroups in fecal samples subjected to the culture method. A total of 384 (96 and 288 samples from experiment 3) observations corresponding to individual fecal samples were used in the analysis. Noninferiority margins (δ), or the acceptable amount by which the pooling test may differ from the individual IMS testing and still not be considered practically inferior, of 20, 10, and 5% were defined to test the following hypotheses: H_0: $Pp - Pi \geq \delta$ versus H_a: $Pp - Pi < \delta$, where Pp represents the proportion that was positive as determined by pooling IMS beads, Pi the proportion positive based on use of individual IMS beads, and H_0 and H_a the null and alternative hypotheses, respectively. Noninferiority tests were performed using the binomial and noninferiority options in PROC FREQ in SAS 9.3 (SAS Institute Inc., Cary, NC). The identification of samples with pooled IMS beads was based on the use of two pools: pool 1 combined O26, O45, and O111 beads; and pool 2 combined O103, O121, and O145 beads. We have observed a degree of cross-reactivity with IMS beads that suggests binding of nontarget serogroups to a serogroup-specific antibody *(5, 9)*. As a result, a sample was defined as positive for O26 based on pooling if it was positive for O26 based on pool 1 and/or pool 2. For individual IMS, a sample was identified as positive for O26 if the sample was positive for O26 based on any individual IMS bead. The same definitions were used across all serogroups.

RESULTS

Experiment 1. In the control (feces not spiked with STEC), five of the six fecal samples subjected to individual IMS beads were negative and one sample was positive for the O45 serogroup. However, with pooled beads of all six serogroups, three of the six control samples were positive for O45 and two samples were positive for O103 (Table 2). For feces ($n = 6$) spiked with individual STEC or with a pool of three or four STEC and subjected to individual beads or a pool of three or four IMS beads, detection of serogroups of O26, O103, O111, and O157 was six of six, or in a few cases, five of six samples (Table 2). With feces spiked with individual or pooled STEC, the detection of O121 and O145 serogroups was less frequent and ranged from zero to five; however, for the most part, the recovery was similar or even higher with pooled beads compared with individual beads. With O45, the serogroup was detected in only one of the six spiked fecal samples, although three of the six samples not spiked (control) were positive for the O45 serogroup.

Experiment 2. For feces ($n = 6$) spiked with individual STEC and subjected to individual beads or pools of three or six IMS beads, detection of serogroups of O26, O121, and O145 was five of six or six of six. For feces spiked with O103, the detection with a pool of three beads was three of six; but, with a pool of six beads, detection was six of six. With O45, the detection was only one of six spiked samples with individual beads or a pool of six beads and none with a pool of three beads (Table 3). For feces spiked with a pool of

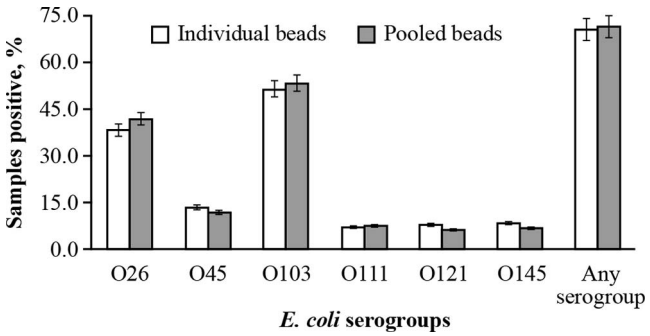

FIGURE 1. *Proportions and 95% confidence intervals (error bars) of fecal samples positive for the six serogroups of non-O157 Shiga toxin–producing* Escherichia coli *in cattle feces (*n = *384) detected with individual or pooled immunomagnetic beads (O26+O45+O111 and O103+O121+145) (experiment 3).*

three STEC subjected to individual beads or a pool of three IMS beads, detection of serogroups O26, O111, O103, and O145 was four, five, or six of six. The recovery of O121 was one of six with a pool of three beads and five of six with a pool of six beads. The O45 was not detected with either individual beads or a pool of three beads.

Experiment 3. A total of 384 fecal samples were subjected to individual beads and two pools of three beads (O26+O45+O111 and O103+O121+O145). All six serogroups were detected in the fecal samples, with O26 and O103 being the two predominant serogroups (Table 4). Because the detection of serogroups was based on the PCR assay of 6 (for individual beads) or 10 (for pooled beads) pooled colonies, serogroups other than the targeted group were also detected. Of the six serogroups, O26 and O103 were detected in a substantial number of fecal samples subjected to other IMS beads. With O26, 95 (24.7%) of 384 fecal samples were positive with O26 beads; but, with the inclusion of nonredundant fecal samples that tested positive with the other five beads, the proportion of samples testing positive increased to 148 (38.5%) of 384. Similarly, with O103, an additional 17 fecal samples were detected as positive with non-O103 IMS beads. With the other four serogroups, only a few (zero to five) additional fecal samples were detected as positive with nonspecific IMS beads. A similar identification of serogroups with nonspecific beads was observed with pooled beads; however, only a few additional fecal samples were identified as positive with nonspecific beads. For example, with O26, only seven additional fecal samples were detected as positive with non-O26 beads. The proportions of fecal samples positive for the six serogroups detected by individual or pooled beads were very similar (within 4%; Fig. 1). The O26 serogroup was detected in 148 (38.5%) of 384 samples tested by individual IMS beads compared with 162 (42.2%) of 384 samples subjected to pooled IMS beads. Likewise, the O103 serogroup was detected in 206 (53.6%) of 384 samples subjected to the pooled IMS beads compared with 199 (51.8%) of 384 detected with individual IMS beads. Individual IMS beads detected a slightly higher number of O45 (52 versus 46), O121 (30 versus 24), and O145 (32 versus 26) serogroups than pooled beads. Application of noninferiority tests indicated that detection of the six serogroups with pooled IMS beads was not substantially

TABLE 5. *Detection of non-O157 STEC and STEC isolates of undetermined serogroups by individual and pooled IMS procedures in fecal samples of feedlot cattle*[a]

Virulence genes (stx_1, stx_2, *eae*)	Non-O157 *E. coli* serogroups							STEC of undetermined serogroups
	O26	O45	O103	O111	O121	O145	Total	
Total serogroups isolated								
Individual IMS	19	6	33	3	3	11	75	36
Pooled IMS	16	5	35	4	4	10	74	40
Shiga toxin 1 (stx_1)								
Individual IMS	1	0	10	3	0	4	18	3
Pooled IMS	0	0	8	4	0	5	17	4
Shiga toxin 2 (stx_2)								
Individual IMS	1	0	0	3	0	3	7	34
Pooled IMS	1	0	0	4	0	1	6	36
Intimin (*eae*)								
Individual IMS	19	0	24	3	0	11	57	5
Pooled IMS	13	0	28	4	0	10	55	1
stx_1+stx_2								
Individual IMS	0	0	0	3	0	0	3	1
Pooled IMS	0	0	0	4	0	0	4	0
stx_1 or stx_2+*eae*								
Individual IMS	2	0	10	3	0	7	22	5
Pooled IMS	1	0	8	4	0	6	19	1

[a] STEC, Shiga toxin–producing *Escherichia coli*; IMS, immunomagnetic separation. $n = 288$.

inferior to individual IMS beads ($P < 0.05$) based on noninferiority margins as low as 5%. The significance of the test was verified by the lower limit of the 90% confidence interval being greater than the noninferiority limit in all tests.

Individual isolates ($n = 288$) from samples from the six commercial feedlots were tested by 11-plex PCR, if DNA from pooled isolates tested positive for one or more of the six serogroups and stx_1 and/or stx_2 genes. A total of 75 and 74 pure cultures of isolates positive for one of the six serogroups were obtained with the individual or the pooled beads procedure, respectively. The three predominant serogroups were O103, O26, and O145 (Table 5). The total numbers of isolates obtained that carried either or both Shiga toxin genes, i.e., STEC, were similar between the individual and pooled IMS procedures (22 versus 19; Table 5). The majority of STEC isolates detected by individual beads and pooled IMS beads were from O103 (10 versus 8) and O145 (7 versus 6) serogroups. All STEC isolates were also positive for the intimin gene. None of the O45 and O121 isolates carried Shiga toxin genes. In addition to the six non-O157 serogroups, a total of 36 and 40 Shiga toxigenic isolates of undetermined serogroups were obtained from individual and pooled IMS procedures, respectively. Only a small number of these STEC isolates carried the intimin gene (Table 5).

DISCUSSION

It is well established that inclusion of an IMS step before plating onto a selective medium in the culture-based detection method improves STEC detection sensitivity in feces or other complex sample matrices *(7, 15, 20, 24)*. In studies requiring detection of the six serogroups of non-O157 STEC, the culture method becomes time consuming and labor intensive because each sample is subjected to six individual IMS procedures, followed by plating onto six plates of selective medium. Therefore, our intent was to determine whether pooling of beads affects the detection sensitivity for the six major serogroups of non-O157 STEC in cattle feces. In two experiments conducted with inoculated feces, the detection of the six serogroups, for the most part, was comparable between the individual and pooled IMS procedures. In the first experiment, the inoculated concentration was low (10 CFU/ml), thus requiring enrichment before the IMS step. Of the six serogroups, the detection of O45 was lower regardless of the IMS procedure. The strain of inoculated O45 may not have survived or grown in the fecal suspension during 6 h of incubation for enrichment. Therefore, in the second experiment, we used a different strain of O45 and increased the concentration of inoculum from 10 to 100 CFU/ml of the fecal suspension and eliminated the enrichment step. Despite the changes, the recovery of the inoculated strain did not improve. It is possible that O45 beads were not specific, although the same beads detected O45 in the uninoculated feces in experiment 1 and in feces of naturally shedding cattle in experiment 3.

Serogroups O26, O103, and O111 showed high recoveries from inoculated feces with both individual beads and pooled IMS beads procedures. The other three serogroups, O45, O121, and O145, were detected less frequently but showed similar recovery between individual and pooled IMS beads. The data generated in the study suggest that pools of three or four beads have similar recovery to that of individual beads. The addition of O157 IMS beads to all pooled treatments showed no change in detection. Different combinations of IMS beads (O26+O45+O103, O26+O45+O111, O111+O145+O121, and O103+O121+O145) showed no change in detection. Because O26 and O103 are the predominant non-O157 serogroups in cattle feces *(6, 8, 9)*, we chose to separate the two serogroups into different pooled IMS combinations, as done in experiments 2 and 3. The separation also resulted in less crowded colonies on inoculated plates, which increased the likelihood of selecting a more complete representation of non-O157 colonies present on the agar plate. Although a pool of six or seven beads had similar recovery to that of a pool of three or four beads, a pool of three beads resulted in less crowded colonies on the medium. Also, based on our previous study *(9)*, a majority of the fecal samples (60 to 70%) from feedlot cattle had three or fewer serogroups per sample. Traditionally, we and others have picked five to six colonies after plating IMS beads on a selective medium *(10, 11, 13, 15, 17)*. With the pooled beads, we chose to increase the number of colonies picked to 10 to enhance the chance for detection of the non-O157 serogroups. Overall, pooling of the beads reduces the number of colonies to be tested by latex agglutination or PCR for serogroup confirmation (18 versus 10 for three serogroups for individual or pooled IMS beads, respectively).

Further evaluation with naturally shedding cattle showed that pools of three IMS beads detected relatively equal numbers of non-O157 serogroups compared with individual IMS beads. We did not include O157 serogroup in the pooled combinations because the O157 serogroup traditionally has been cultured on its own selective medium (for example, sorbitol MacConkey agar with cefixime and tellurite or CHROMagar O157 [BBL, BD, Sparks, MD]) and, in some cases, has been enriched in a different medium (for example, gram-negative broth). Therefore, fecal samples could be tested by individual O157 IMS beads and two pools of three (O26+O45+O111 and O103+O121+O145) IMS beads for the detection of seven STEC. Although a high proportion of fecal samples from feedlot cattle were positive for one or more of the six serogroups, only a small proportion of the isolates obtained in pure culture carried Shiga toxin genes, which were predominantly stx_1. All *stx*-carrying isolates were positive for intimin genes, suggesting that they were the enterohemorrhagic *E. coli* pathotype. A majority of the non-O157 serogroups shed in cattle feces are not Shiga toxigenic, which is in agreement with our previous findings *(6, 9, 20)*.

In experiments using feces inoculated with pure cultures and feces from naturally shedding cattle, the pooling of IMS beads did not affect the sensitivity of detection of six serogroups of non-O157 STEC in cattle feces. The prospect of a test with higher throughput, due to a protocol that saves labor, time, and expense, coupled with the statistical evidence of noninferiority, makes pooling a desirable option for detecting STEC serogroups in bovine fecal samples.

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