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Comparison of Agar Media for Detection and Quantification of Shiga Toxin–Producing *Escherichia coli* in Cattle Feces

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ABSTRACT

The isolation and quantification of non-O157 Shiga toxin–producing *Escherichia coli* (STEC) from cattle feces are challenging. The primary objective of this study was to evaluate the performance of selected agar media in an attempt to identify an optimal medium for the detection and quantification of non-O157 STEC in cattle feces. Comparison studies were performed using CHROMagar STEC, Possé differential agar (Possé), Possé modified by the reduction or addition of antimicrobials, STEC heart infusion washed blood agar with mitomycin C (SHIBAM), and SHIBAM modified by the addition of antimicrobials. Fourteen STEC strains, two each belonging to serogroups O26, O45, O103, O111, O121, O145, and O157, were used to test detection in inoculated fecal suspensions at concentrations of 10^2 or 10^3 CFU/g. One STEC strain from each of these seven serogroups was used to estimate the concentration of recovered STEC in feces inoculated at 10^3, 10^4, or 10^5 CFU/g. Significantly more suspensions ($P < 0.05$) were positive for STEC when plated on Possé containing reduced concentrations of novobiocin and potassium tellurite compared with SHIBAM, but not SHIBAM modified by containing these same antimicrobials at the same concentrations. Numerically, more suspensions were positive for STEC by using this same form of modified Possé compared with Possé, but this difference was not statistically significant. More suspensions were positive for STEC cultured on CHROMagar STEC compared with those on Possé ($P < 0.05$) and on modified Possé ($P = 0.05$). Most inoculated fecal suspensions below 10^4 CFU/g of feces were underestimated or not quantifiable for the concentration of STEC by using CHROMagar STEC or modified Possé. These results suggest that CHROMagar STEC performs better than Possé or SHIBAM for detection of STEC in bovine feces, but adjustments in the concentrations of novobiocin and potassium tellurite in the latter two media result in significant improvements in their performance.

Key words: Agar; Cattle; *Escherichia coli*; Shiga toxin–producing *Escherichia coli*

Some strains of Shiga toxin–producing *Escherichia coli* (STEC) are enteric pathogens of humans that cause hemorrhagic colitis and hemolytic-uremic syndrome *(36)*. STEC are naturally found in the intestinal tract of ruminants; shed in ruminant feces *(17)*; and commonly transmitted to humans through the ingestion of contaminated food *(10),* particularly beef products *(8)*. Thus, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) declared seven serogroups of intimin-positive STEC that account for the majority of disease due to STEC in the United States as adulterants in raw, nonintact beef *(37)*.

Detection of STEC is critical for food safety, clinical diagnosis, and determination of environmental prevalence. Epidemiological studies *(41)* and clinical laboratories *(20, 23, 32)* have used sorbitol MacConkey agar to detect sorbitol-negative *E. coli* O157:H7, which lacks the ability to ferment sorbitol within 24 h. The addition of antimicrobials such as cefixime and potassium tellurite to agar media has further improved detection of *E. coli* O157:H7 *(29)*. However, most non-O157 STEC ferment sorbitol and have varying levels of resistance to these antimicrobials *(15)*. Therefore, sorbitol MacConkey agar is not a suitable agar medium to detect non-O157 STEC, and its use would result in undetected non-O157 STEC *(19)*.

Collectively, non-O157 STEC organisms are estimated to account for ~64% of foodborne illnesses caused by STEC in the United States *(30),* with 71% of the non-O157 STEC infections due to STEC O26, O45, O103, O111, O121, and O145 *(6)*. Although non-O157 STEC detection in clinical patients has increased *(14),* detection of these organisms has remained challenging in food and environmental sources due to their diversity and resemblance to background flora *(12)*. Agar media designed to detect non-O157 STEC have relied on fermentation of specific carbohydrates, β-galactosidase activity, and resistance to antimicrobials *(16, 27)*. Isolation remains an important step in the identification of an organism and confirmation of results of screening assays. Hyperspectral imaging *(42),* latex agglutination *(24),* PCR *(2),* and other nucleic acid detection methods *(4)* might be used to identify an isolate on the basis of spectral "fingerprint," serotype, virulence gene content, or genes that represent STEC serogroups, respectively.

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Concentration estimates of STEC are important for assessing the risk of STEC in the food chain *(9)*. Concentration has been determined by use of spiral plate methods for STEC O157:H7 *(3, 28)* or real-time PCR assays that quantify total STEC load or specific serogroups of STEC *(1, 7, 22, 31)*. Real-time PCR assays using multiple targets can detect genes contributed by multiple organisms. Presence of background organisms that contain some, but not all, of the gene targets often leads to overestimation of STEC concentration in a sample. To determine the concentration of certain STEC serogroups, organism isolation by spiral plating could be used to determine whether the O-group, Shiga toxin (*stx*), and intimin (*eae*) genes were all contributed from one organism.

The primary objective of this study was to evaluate the performance of selected agar media in an attempt to identify an optimal medium for the detection and quantification in cattle feces of those non-O157 STEC that constitute adulterants in raw, nonintact beef by the USDA-FSIS. In addition, we addressed the same media for detection and quantification of STEC O157:H7 because it, too, is an adulterant. Hence, this study evaluated the performance of selected agar media for the detection and quantification of STECs O26, O45, O103, O111, O121, O145, and O157 (STEC-7) in cattle feces. This study compared CHROMagar STEC, Possé differential agar (Possé) *(27),* modifications of Possé, STEC heart infusion washed blood agar with mitomycin C (SHIBAM), and a modification of SHIBAM for the detection of STEC-7 in inoculated cattle fecal suspensions. In addition, CHROMagar STEC and a modification of Possé were used to establish a quantification method for STEC-7 in inoculated cattle fecal suspensions by spiral plating.

MATERIALS AND METHODS

Bacterial strains and preparation of inoculum. All strains used in this study were STEC strains. They were obtained either from Dr. Shannon Manning (Michigan State University), Dr. David Renter (Kansas State University), Dr. John Luchansky (USDA, Agricultural Research Service, Eastern Regional Research Center), or our laboratory collection. Frozen stock cultures (–80°C) of strains were streaked onto Luria-Bertani agar plates (Luria-Bertani broth, Miller [BD, Sparks, MD] containing 15.0 g/liter of agar) without antibiotics or with 100 mg/liter rifampin (Sigma, St. Louis, MO) and incubated at 37°C for 24 h. Single colonies of wild-type or rifampin-resistant strains were inoculated into Luria-Bertani broth without or with 100 mg/liter rifampin, respectively, and then incubated for 24 h at 37°C in static cultures. Serial 10-fold dilutions of inocula were prepared in buffered peptone water (BPW) and used to inoculate fecal suspensions at concentrations of 10^2 or 10^3 CFU/g of feces for detection studies and 10^3, 10^4, or 10^5 CFU/g of feces for quantification studies. BPW was used as an uninoculated control.

Sample collection. Fecal samples from fresh floor pats voided by steers housed one per pen in an enclosed, environmentally controlled animal containment facility at the University of Nebraska–Lincoln were collected and processed as described previously *(35)*. Samples of feces originating from eight different steers on 11 dates were used in the experiments. In brief, 1 g of cattle feces was suspended in 9 ml of *E. coli* broth (Oxoid Ltd., Hampshire, UK) and vortexed for 1 min. After inoculation, fecal suspensions were vortexed for 20 s and enriched for 6 h at 40°C in a static culture.

Agar media. Agar media and their designations are listed in Table 1. CHROMagar STEC (DRG Int., Springfield, NJ) was prepared according to the manufacturer's instructions, and CHROMagar STEC with rifampin was prepared by adding rifampin at 100 mg/liter and no CHROMagar STEC supplement. Possé differential medium was prepared as described previously *(27)*. The first modified form of Possé differential medium was made by reducing the concentrations of novobiocin (Sigma) from 8.0 to 5.0 mg/liter and potassium tellurite (Sigma) from 2.5 to 0.15 mg/liter, and by adding cefixime trihydrate (Sigma) at 0.05 mg/liter (mPossé1). A second modified form of Possé as described by Stromberg et al. *(33)* was made by reducing novobiocin from 8.0 to 5.0 mg/liter and potassium tellurite from 2.5 to 0.5 mg/liter (mPossé2). Modified Possé with rifampin was prepared by adding rifampin to Possé differential agar at 100 mg/liter, with reduced bile salts (1.5 g/liter), and containing no novobiocin or potassium tellurite. SHIBAM was prepared as described in the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual (11)* and modified by adding novobiocin and potassium tellurite at 5.0 and 0.5 mg/liter, respectively (mSHIBAM).

Detection of STEC-7 on mPossé2 and SHIBAM. Two independent experiments were performed to compare mPossé2 and SHIBAM for the detection of STEC. After fecal suspensions were inoculated and enriched in *E. coli* broth for 6 h, 490-μl aliquots were diluted in 490 μl of phosphate-buffered saline with 0.05% Tween 20 and subjected to three separate immunomagnetic separation (IMS) treatments by using a KingFisher Flex Magnetic Particle Processor (Thermo Scientific, Waltham, MA). The first treatment consisted of 20 μl of anti–*E. coli* O157 IMS Dynabeads (Invitrogen, Carlsbad, CA); the second treatment used a 20-μl pool of O103, O111, and O145 IMS beads (Abraxis LLC, Warminster, PA); and the third treatment used a 20-μl pool of O26, O45, and O121 IMS beads (Abraxis). After IMS, the beads were dropped into 1.0 ml of BPW. The bead suspension was serial diluted in BPW, and 50 μl of each bead suspension diluted 100-fold was spread plated onto mPossé2 and SHIBAM and incubated at 37°C for 18 h. On mPossé2, $\leq$20 red/blue-purple and green colonies were picked and $\leq$20 enterohemolytic colonies were picked from SHIBAM. Colonies were suspended in 50 μl of water and heated for 10 min at 95°C for use as DNA template in PCR reactions. Five colonies were pooled and tested by PCR for *stx* by using the methods of Monday et al. *(25)*. Individual colonies from PCR-positive pools were tested by multiplex PCR to confirm that the isolates were the inoculum strain. The multiplex PCR tested for genes individually representing the seven STEC-7 serogroups and four virulence genes, namely, stx_1, stx_2, enterohemorrhagic *E. coli*–hemolysin (*ehxA*), and *eae (2)*. However, the protocol was modified by replacing primers for *eae* and O111 with primers described by Blanco et al. *(5)* and Noll et al. *(26),* respectively. Each reaction contained 10 μl of iQ multiplex powermix (Bio-Rad, Hercules, CA), 1 μl of all primers mixed, resulting in final primer concentrations of 0.21 μM; 2 μl of water; and 7 μl of DNA template. The following reaction conditions were used for PCR: 94°C for 5 min, followed by 28 cycles of 94°C for 30 s and 67°C for 80 s, with a final extension at 68°C for 7 min. Visualization of PCR amplicons was performed on a QIAxcel Advanced system (QIAGEN, Valencia, CA) by using a QIAxcel DNA screening kit (QIAGEN).

TABLE 1. *List of agar media*

Agar media	Designation	Colony phenotype	Antimicrobial concn (mg/liter)			
			Cefixime	Novobiocin	Potassium tellurite	Rifampin
CHROMagar STEC	CHROMagar STEC	Mauve	Unknown[a]	Unknown[a]	Unknown[a]	Unknown[a]
CHROMagar STEC with rifampin	CHROMagar STEC plus Rif	Mauve	0.0[b]	0.0[b]	0.0[b]	100.0[b]
Possé differential agar	Possé	Blue-purple, red-purple, and green	0.0	8.0	2.5	0.0
Modified Possé differential agar with rifampin	mPossé plus Rif	Blue-purple, red-purple, and green	0.0	0.0	0.0	100.0
Modified Possé differential agar 1	mPossé1	Blue-purple, red-purple, and green	0.05	5.0	0.15	0.0
Modified Possé differential agar 2	mPossé2	Blue-purple, red-purple, and green	0.0	5.0	0.5	0.0
STEC heart infusion agar with mitomycin C	SHIBAM	Enterohemolytic	0.0	0.0	0.0	0.0
Modified STEC heart infusion agar with mitomycin C	mSHIBAM	Enterohemolytic	0.0	5.0	0.5	0.0

[a] CHROMagar STEC base and supplement are proprietary formulations. CHROMagar STEC was prepared by adding the supplement to the base according to the manufacturer's directions.

[b] CHROMagar STEC with rifampin was prepared by adding rifampin and no supplement to the base. The antimicrobial concentration shown is the amount added to the base.

Detection of STEC-7 on mPossé2 and mSHIBAM. Three independent experiments were performed to compare mPossé2 and mSHIBAM for the detection of STEC. Fecal suspensions were processed and colonies were tested as described for the comparison between mPossé2 and SHIBAM, with a minor modification. After IMS, the bead suspension was dropped into 1.0 ml of BPW and serial diluted in BPW. Fifty microliters of nondiluted or 10-fold diluted bead suspension was spread plated onto mPossé2 and 50 μl of 100-fold bead suspension was spread plated onto mSHIBAM and incubated at 37°C for 18 h. On mPossé2, ≤20 red/blue-purple and green colonies were picked and ≤20 enterohemolytic colonies were picked from mSHIBAM. Colonies were tested by multiplex PCR as described above *(2, 5, 26)*.

Detection of STEC-7 on CHROMagar STEC, Possé, mPossé1, and mPossé2. Two independent experiments were performed to compare CHROMagar STEC, Possé, mPossé1, and mPossé2 for the detection of STEC. After enrichment, separate aliquots were subjected to IMS treatments with 20 μl of each IMS bead type: O26, O45, O103, O111, O121, O145, and O157. After IMS, the beads were dropped into 1.0 ml of BPW and diluted 10- and 100-fold in BPW. Fifty microliters of each bead suspension was spread plated onto each agar and incubated at 37°C for 18 h. Colonies were picked from plates containing 25 to 250 colonies. On Possé, mPossé1, and mPossé2 plates inoculated with O26, O45, O103, O111, or O157 IMS beads, six or fewer red/blue-purple colonies were picked and for those inoculated with O121 or O145 IMS beads, six or fewer red/blue-purple and green colonies were picked. On CHROMagar STEC, six or fewer mauve colonies were picked per plate. Colonies were suspended in 50 μl of water and heated for 10 min at 95°C for use as DNA template in PCR reactions. Individual colonies were tested by multiplex PCR as described above *(2, 5, 26)*.

Quantification of STEC-7 strains in inoculated cattle fecal suspensions. Rifampin-resistant STEC strains were used to estimate the concentration of STEC recovered on CHROMagar STEC and mPossé2 from inoculated fecal suspensions. Fecal suspensions were inoculated at a concentration of $\sim 10^3$, 10^4, or 10^5 CFU/g of feces in 9 ml of *E. coli* broth, and 50 μl of the unenriched culture was spiral plated in duplicate onto CHROMagar STEC, CHROMagar STEC with rifampin, mPossé2, and mPossé with rifampin by using an Eddy Jet 2 spiral plater (Neutec, Farmingdale, NY). Red/blue-purple and green colonies were counted on mPossé2 and mPossé with rifampin plates and mauve colonies were counted on CHROMagar STEC and CHROMagar STEC with rifampin. In total, 10 or fewer colonies per plate of the target phenotype were picked, and each colony was suspended in 50 μl of water. The suspended colonies were heated for 10 min at 95°C and tested by multiplex PCR *(2, 5, 26)*. Concentration was determined by the proportion of colonies that tested positive for O-group, *stx*, and *eae* that matched that of the inoculum.

Statistical analysis. Detection of STEC on different agar media was compared using a binary distribution in SAS version 9.3 (PROC GLIMMIX, SAS Institute Inc., Cary, NC). Statistically significant results are represented by values of $P < 0.05$.

RESULTS

Agar comparison studies using inoculated cattle fecal suspensions. Two independent experiments were performed for the comparison between mPossé2 and SHIBAM for the recovery of STEC in inoculated fecal suspensions. Of the strains tested, 6 of 14 were detected on both agars, 7 of 14 were detected only on mPossé2, and 1 of 14 was not detected. STEC strains were detected in 11 of 28 fecal suspensions on mPossé2 and 2 of 28 on SHIBAM

when inoculated with 10^2 CFU/g, and in 17 of 28 fecal suspensions on mPossé2 and 4 of 28 on SHIBAM when inoculated with 10^3 CFU/g. In total, STEC strains were detected in 28 of 56 fecal suspensions when plated on mPossé2 compared with 6 of 56 when plated on SHIBAM (Table 2). mPossé2 detected significantly ($P < 0.05$) more STEC-positive suspensions compared with SHIBAM.

In three independent experiments, mPossé2 and mSHIBAM were compared for the recovery of STEC in inoculated fecal suspensions. Of the strains tested, 4 of 14 were recovered on mPossé2 alone, and 10 of 14 were not recovered on either agar. STEC strains were detected in 2 of 42 fecal suspensions on mPossé2 and 0 of 42 on mSHIBAM when inoculated with 10^2 CFU/g, and 4 of 42 fecal suspensions on mPossé2 and 0 of 42 on SHIBAM when inoculated with 10^3 CFU/g. In total, STEC strains were detected in 6 of 84 fecal suspensions plated on mPossé2; however, no STEC were recovered when plated on mSHIBAM (Table 3). mPossé2 detected fewer STEC-positive suspensions than in the previous comparison, and it did not detect significantly more STEC compared with mSHIBAM.

In two independent experiments, CHROMagar STEC, Possé, mPossé1, and mPossé2 were compared for the recovery of STEC in inoculated cattle feces. No strains were detected on all four media, 3 of 14 were recovered on CHROMagar STEC only, 3 of 14 were recovered on all except mPossé1, 2 of 14 were recovered on CHROMagar STEC and mPossé2 only, 1 of 14 was recovered on CHROMagar STEC and Possé only, 1 of 14 was recovered on all except Possé, and 4 of 14 were not recovered. STEC strains were detected in 9 of 28 fecal suspensions on CHROMagar STEC, 4 of 28 on Possé, 1 of 28 on mPossé1, and 4 of 28 on mPossé2 when inoculated with 10^2 CFU/g. When inoculated with 10^3 CFU/g, STEC were detected in 8 of 28 fecal suspensions on CHROMagar STEC, 2 of 28 on Possé, 0 of 28 on mPossé1, and 5 of 28 on mPossé2. In total, STEC were recovered from 17 of 56 fecal suspensions plated on CHROMagar STEC, 9 of 56 on mPossé2, 6 of 56 on Possé, and 1 of 56 on mPossé1 (Table 4). Significantly ($P < 0.05$) more STEC-positive suspensions were detected by plating on CHROMagar STEC compared with Possé and mPossé1, and approached significance ($P = 0.05$) compared with mPossé2. Possé was not significantly different compared with mPossé1 or mPossé2. However, mPossé2 detected significantly ($P < 0.05$) more STEC-positive suspensions compared with mPossé1.

Quantification of STEC by using inoculated cattle fecal suspensions. Rifampin-resistant STEC strains were spiral plated on CHROMagar STEC and mPossé2 to determine whether STEC-7 strains could be quantified on these media. CHROMagar STEC and mPossé were also supplemented with rifampin to suppress background organisms and allow for optimal recovery of STEC. One of seven strains was quantifiable in suspensions inoculated with STEC at 6.2×10^2 to 1.6×10^3 CFU/g. However, when inoculated at 6.2×10^3 to 1.6×10^4 CFU/g, three of seven strains were quantifiable on CHROMagar STEC and CHROMagar STEC supplemented with rifampin, and one of seven strains was quantifiable on mPossé2 and mPossé with rifampin. When inoculated at 6.2×10^4 to 1.6×10^5 CFU/g, six of seven strains were quantifiable on CHROMagar STEC with rifampin and mPossé with rifampin, five of seven strains were quantifiable on CHROMagar STEC, and four of seven strains were quantifiable on mPossé2 (Table 5).

DISCUSSION

Successful detection of STEC in a complex background requires an agar medium that suppresses the growth of background organisms with minimal suppression of the growth of STEC. In addition to reducing background organisms for detection of STEC, other factors such as cost, time to prepare the media, shelf life, and ease of interpreting phenotypes are important in agar selection *(18)*. In general, commercial agars are more expensive but quicker to prepare compared with published agars such as Possé and SHIBAM. STEC are screened based on one phenotype on CHROMagar STEC and SHIBAM. STEC on CHROMagar STEC can vary in their shade of mauve from purple-pink to brown. For SHIBAM, it can be difficult to determine the enterohemolytic phenotype, and other *E. coli* may present as α-hemolytic, which can obscure the target phenotype. On Possé and modifications of Possé, STEC are screened based on three phenotypes (blue-purple, red-purple, and green). It can be difficult to discriminate between blue-purple and red-purple on Possé and modifications of Possé in the fecal background.

The matrix is also important when evaluating agar media. Cattle feces will likely have a higher concentration of nonpathogenic *E. coli* and other background organisms compared with foods. The FDA recommends using Levine's eosin–methylene blue and SHIBAM for isolating non-O157 STEC *(11)*. Lin et al. *(21)* found that SHIBAM was useful for isolating non-O157 STEC from romaine lettuce and tomatoes. Although SHIBAM may be suitable for food products with low numbers of background organisms, the present study found that SHIBAM is not a suitable agar for detection of STEC in cattle feces. Three of the 14 STEC strains tested lacked *ehxA,* which may account for why lower detection was observed on SHIBAM compared with mPossé2. An *ehxA*-negative STEC strain (1234) was the only strain detected on SHIBAM that lacked *ehxA* and it was detected only once. Colony phenotypes can vary when grown in proximity to other organisms from the microflora that are hemolytic. The detection of an *ehxA*-negative strain on SHIBAM highlights the subjectivity of this medium. A significant difference was not found when mSHIBAM was compared with mPossé2, even though no STEC were detected on mSHIBAM.

Agar media designed to detect *E. coli* O157 use potassium tellurite as a means to select against nonpathogenic *E. coli* and other enteric organisms *(29)*. Verhaegen et al. *(39)* compared the isolation of a variety of non-O157 STEC, including those of the O26, O45, O103, O111, and O145 serogroups, on multiple agar media in pure culture. There was a correlation between growth of the STEC strain

TABLE 2. *Comparison between Possé differential agar with reduced novobiocin and potassium tellurite (mPossé2) and STEC heart infusion washed blood agar with mitomycin C (SHIBAM) for the detection of Shiga toxin–producing* E. coli *in inoculated cattle fecal suspensions*

Strain	Serotype[a]	Source	*stx*	*eae*	*ehxA*	Positive suspensions/no. tested at 10^2 CFU/g		Positive suspensions/no. tested at 10^3 CFU/g		Animal (date)[b]	
						mPossé2[c]	SHIBAM	mPossé2	SHIBAM	4927 (2 Sep. 2014)	2437 (8 Sep. 2014)
DEC10B	O26:H11	Human	stx_1	+	+	1/2	1/2	1/2	0/2	+	+
16272	O26:H11	Bovine	stx_1	+	+	1/2	0/2	1/2	0/2	+	+
B8227-C8	O45:H2	Bovine	stx_1	+	−	1/2	0/2	1/2	0/2	+	+
D88-28058	O45:NM	Bovine	stx_1	+	+	1/2	0/2	2/2	1/2	+	+
MT#80	O103:H2	Human	stx_1	+	+	1/2	0/2	1/2	0/2	+	+
236-5	O103:NT	Bovine	stx_1 and stx_2	+	+	1/2	0/2	1/2	1/2	+	+
8266-1	O111:NT	Bovine	stx_1 and stx_2	+	+	1/2	0/2	1/2	0/2	+	+
10049	O111:H11	Bovine	stx_1 and stx_2	+	+	1/2	1/2	2/2	0/2	+	+
DA-37	O121:H19	Human	stx_2	+	+	0/2	0/2	0/2	0/2	+	+
8-084	O121:H19	Bovine	stx_2	+	−	0/2	0/2	2/2	0/2	+	+
GS G5578620	O145:H28	Human	stx_1	+	+	1/2	0/2	1/2	1/2	+	+
1234	O145:H28	Bovine	stx_1 and stx_2	+	−	1/2	0/2	2/2	1/2	+	+
933	O157:H7	Beef	stx_1 and stx_2	+	+	1/2	0/2	1/2	0/2	+	+
S2006#1	O157:H7	Bovine	stx_1 and stx_2	+	+	0/2	0/2	1/2	0/2	+	+
Total						11/28	2/28	17/28	4/28		

[a] NM, nonmotile; NT, not typed.

[b] Identification number used as source of feces for inoculation and date of experiment.

[c] Possé differential agar with 5.0 mg/liter novobiocin and 0.5 mg/liter potassium tellurite.

TABLE 3. *Comparison between Possé differential agar with reduced novobiocin and potassium tellurite (mPossé2) and STEC heart infusion washed blood agar with mitomycin C, novobiocin, and potassium tellurite (mSHIBAM) for the detection of Shiga toxin–producing* E. coli *in inoculated cattle fecal suspensions*

						Positive suspensions/no. tested at 10^2 CFU/g		Positive suspensions/no. tested at 10^3 CFU/g		Animal (date)[b]		
Strain	Serotype[a]	Source	*stx*	*eae*	*ehxA*	mPossé2[c]	mSHIBAM[d]	mPossé2	mSHIBAM	4917 (14 Oct. 2014)	4832 (20 Oct. 2014)	4927 (27 Oct. 2014)
DEC10B	O26:H11	Human	stx_1	+	+	0/3	0/3	2/3	0/3	+	+	+
16272	O26:H11	Bovine	stx_1	+	+	0/3	0/3	1/3	0/3	+	+	+
B8227-C8	O45:H2	Bovine	stx_1	+	−	0/3	0/3	0/3	0/3	+	+	+
D88-28058	O45:NM	Bovine	stx_1	+	+	0/3	0/3	0/3	0/3	+	+	+
MT#80	O103:H2	Human	stx_1	+	+	0/3	0/3	0/3	0/3	+	+	+
236-5	O103:NT	Bovine	stx_1 and stx_2	+	+	1/3	0/3	0/3	0/3	+	+	+
8266-1	O111:NT	Bovine	stx_1 and stx_2	+	+	0/3	0/3	0/3	0/3	+	+	+
10049	O111:H11	Bovine	stx_1 and stx_2	+	+	0/3	0/3	0/3	0/3	+	+	+
DA-37	O121:H19	Human	stx_2	+	+	0/3	0/3	0/3	0/3	+	+	+
8-084	O121:H19	Bovine	stx_2	+	−	0/3	0/3	0/3	0/3	+	+	+
GS G5578620	O145:H28	Human	stx_1	+	+	1/3	0/3	1/3	0/3	+	+	+
1234	O145:H28	Bovine	stx_1 and stx_2	+	−	0/3	0/3	0/3	0/3	+	+	+
933	O157:H7	Beef	stx_1 and stx_2	+	+	0/3	0/3	0/3	0/3	+	+	+
S2006#1	O157:H7	Bovine	stx_1 and stx_2	+	+	0/3	0/3	0/3	0/3	+	+	+
Total						2/42	0/42	4/42	0/42			

[a] NM, nonmotile; NT, not typed.
[b] Identification number of steer used as source of feces for inoculation and date of experiment.
[c] Possé differential agar with 5.0 mg/liter novobiocin and 0.5 mg/liter potassium tellurite.
[d] STEC heart infusion washed blood agar with mitomycin C, 5.0 mg/liter novobiocin, and 0.5 mg/liter potassium tellurite.

TABLE 4. *Comparison between CHROMagar STEC; Possé differential agar; Possé differential agar with novobiocin, cefixime trihydrate, and potassium tellurite (mPossé1); and Possé differential agar with reduced novobiocin and potassium tellurite (mPossé2) for the detection of Shiga toxin–producing* E. coli *in inoculated cattle fecal suspensions*

					Positive suspensions/no. tested at 10^2 CFU/g				Positive suspensions/no. tested at 10^3 CFU/g				Animal (date)[b]			
Strain	Serotype[a]	Source	*stx*	*eae*	CHROMagar STEC	Possé	mPossé1[c]	mPossé2[d]	CHROMagar STEC	Possé	mPossé1	mPossé2	4866 (23 Apr. 2014)	4832 (30 Apr. 2014)	4917 (6 May 2014)	4896 (13 May 2014)
16272	O26:NT	Bovine	stx_1	+	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	+	−	+	−
DEC10E	O26:H11	Bovine	stx_1	+	1/2	1/2	0/2	0/2	1/2	0/2	0/2	1/2	+	−	+	−
B8227-C8	O45:H2	Bovine	stx_1	+	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	+	−	+	−
D88-28058	O45:NM	Bovine	stx_1	+	1/2	0/2	0/2	0/2	1/2	0/2	0/2	0/2	+	−	+	−
236-5	O103:NT	Bovine	stx_1 and stx_2	+	1/2	1/2	0/2	0/2	1/2	1/2	0/2	1/2	+	−	+	−
RW1372	O103:H2	Bovine	stx_1	+	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	+	−	+	−
8266-1	O111:NT	Bovine	stx_1 and stx_2	+	1/2	1/2	0/2	0/2	1/2	1/2	0/2	0/2	+	−	+	−
10049	O111:H11	Bovine	stx_1 and stx_2	+	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	+	−	+	−
DA-37	O121:H19	Human	stx_2	+	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	−	+	−	+
8-084	O121:H19	Bovine	stx_2	+	1/2	0/2	0/2	1/2	1/2	0/2	0/2	0/2	−	+	−	+
1234	O145:H28	Bovine	stx_1 and stx_2	+	0/2	0/2	0/2	1/2	1/2	0/2	0/2	1/2	−	+	−	+
B6820-C1	O145:NM	Bovine	stx_2	+	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	−	+	−	+
S2006#1	O157:H7	Bovine	stx_1 and stx_2	+	1/2	0/2	1/2	1/2	1/2	0/2	0/2	1/2	−	+	−	+
S2006#2	O157:H7	Bovine	stx_1 and stx_2	+	1/2	1/2	0/2	1/2	1/2	0/2	0/2	1/2	−	+	−	+
Total					9/28	4/28	1/28	4/28	8/28	2/28	0/28	5/28				

[a] NM, nonmotile; NT, not typed.

[b] Identification number of steer used as source of feces for inoculation and date of experiment.

[c] Possé differential agar with 5.0 mg/liter novobiocin, 0.05 mg/liter cefixime trihydrate, and 0.15 mg/liter potassium tellurite.

[d] Possé differential agar with 5.0 mg/liter novobiocin and 0.5 mg/liter potassium tellurite.

TABLE 5. *Quantification of Shiga toxin–producing* E. coli *in inoculated cattle feces by a spiral plate method*

Strain	Serotype[a]	Source	*stx*	*eae*	Resistance	Inoculum (CFU/g)	Estimated CFU/g				Animal (date)[b]	
							CHROMagar STEC	CHROMagar STEC+Rif[c]	mPossé2[d]	mPossé +Rif[e]	6393 (11 Feb. 2015)	4532 (13 Jan. 2016)
H30	O26:H11	Human	stx_1	+	Rifampin	1.0×10^3	NQ[f]	NQ	NQ	NQ	+	−
						1.0×10^4	NQ	NQ	NQ	NQ	+	−
						1.0×10^5	NQ	8.2×10^3	NQ	1.1×10^4	+	−
CDC 96-3285	O45:H2	Human	stx_1	+	Rifampin	8.8×10^2	NQ	NQ	NQ	NQ	+	−
						8.8×10^3	6.2×10^3	9.1×10^3	NQ	NQ	+	−
						8.8×10^4	8.0×10^4	1.1×10^5	NQ	1.2×10^4[g]	+	−
CDC 90-3128	O103:H2	Human	stx_1	+	Rifampin	7.7×10^2	NQ	NQ	NQ	NQ	+	−
						7.7×10^3	5.2×10^3[g]	8.1×10^3	NQ	NQ	+	−
						7.7×10^4	1.1×10^5	1.1×10^5	6.1×10^4	4.7×10^4	+	−
JB1-95	O111:NM	Human	stx_1 and stx_2	+	Rifampin	6.2×10^2	NQ	NQ	NQ	NQ	+	−
						6.2×10^3	NQ	NQ	NQ	NQ	+	−
						6.2×10^4	NQ	NQ	NQ	NQ	+	−
CDC 97-3068	O121:H19	Human	stx_2	+	Rifampin	8.9×10^2	NQ	NQ	NQ	NQ	+	−
						8.9×10^3	NQ	NQ	NQ	NQ	+	−
						8.9×10^4	9.7×10^3	9.6×10^4	3.1×10^4	4.8×10^4	+	−
83-75	O145:NM	Human	stx_2	+	Rifampin	9.0×10^2	NQ	NQ	NQ	NQ	+	−
						9.0×10^3	NQ	NQ	1.3×10^4	NQ	+	−
						9.0×10^4	5.8×10^3[g]	8.1×10^3	6.8×10^3	6.6×10^3	+	−
USDA-FSIS 380-94	O157:H7	Salami	stx_1 and stx_2	+	Rifampin	1.6×10^3	5.0×10^2[g]	NQ	NQ	NQ	−	+
						1.6×10^4	4.1×10^3	1.7×10^4	NQ	1.8×10^4	−	+
						1.6×10^5	4.6×10^4	1.9×10^5	1.1×10^4	1.2×10^5	−	+

[a] NM, nonmotile.
[b] Identification number of steer used as source of feces for inoculation and date of experiment.
[c] CHROMagar STEC without the supplement and with 100 mg/liter rifampin.
[d] Possé differential agar with 5.0 mg/liter novobiocin and 0.5 mg/liter potassium tellurite.
[e] Possé differential agar without novobiocin and potassium tellurite and with 100 mg/liter rifampin.
[f] NQ, not quantifiable.
[g] Quantifiable from one of two plates.

on Possé and CHROMagar STEC with the presence of the tellurite resistance gene *terB (39)*. The agars tested in this study had a range of potassium tellurite concentrations from 0.15 to 2.50 mg/liter or an unknown concentration in CHROMagar STEC. mPossé2, which had higher levels of potassium tellurite, detected significantly more STEC-positive fecal suspensions compared with mPossé1. The addition of cefixime as in mPossé1 was used previously to suppress the growth of *Proteus* spp. without suppressing growth of STEC *(15)*. mPossé1 had concentrations of potassium tellurite, novobiocin, and cefixime as recommended by the USDA-FSIS *Microbiology Laboratory Guidebook* for detection and isolation of non-O157 STEC on modified Rainbow agar *(38)*. A higher level of potassium tellurite may increase the detection rate of STEC through suppression of nontarget organisms.

CHROMagar STEC has been evaluated on human stool samples *(13)* and in pure cultures and beef *(12, 40)*. In stool samples, 82% of samples that were Shiga toxin positive were recoverable on CHROMagar STEC *(13)*. Gill et al. *(12)* found lower recovery concentrations on CHROMagar STEC in pure culture and in inoculated beef compared with brain heart infusion agar. These results suggest that some STEC may be inhibited on CHROMagar STEC. In a separate study *(40),* the use of CHROMagar STEC resulted in a greater recovery rate of cold-stressed STEC (7 days at 4°C) in inoculated ground beef compared with recovery on ChromID EHEC agar. Strains are likely to undergo temperature and acid stress through food processing steps or in the environment, making the recovery of sublethally injured cells important. The present study found that some STEC could not be recovered from enriched fecal suspensions on CHROMagar STEC or other agars tested.

Different animals used to obtain fecal suspensions as well as different strains may account for differences in recovery rates between experiments when the same agar was used. Strain-to-strain variation was observed in this study among STEC strains and within STEC of the same serogroup. Variation in STEC strains has been reported previously when comparing detection methods *(18, 40)*. Variation between strain pairs of the same serogroup could be due to possession or lack of *ehxA*, which would influence detection on SHIBAM. Variable recovery rates could also be due to differences in susceptibility to antimicrobials such as potassium tellurite, which was previously found to influence STEC detection *(18, 39)*. Overall, a high false-negative rate was observed, ranging from 39.3 to 100% depending on the media type and inoculum concentration. For a given inoculum concentration and agar comparison, CHROMagar STEC and mPossé2 had relatively low false-negative rates of 39.3 and 67.9%, respectively, while mPossé1 and mSHIBAM had a false-negative rate of 100% in at least one instance. A false-negative test in epidemiological studies would lead to underestimation of STEC prevalence and more dangerously could lead to contaminated products being sold to consumers by the food industry.

In addition to detection of STEC, concentration estimates are important in assessing the risk of STEC. CHROMagar O157 has been used in spiral plate quantification of STEC O157 *(3, 28),* and mPossé2 has been used to quantify STEC in feces, hide, and carcass samples *(34)*. To our knowledge, this is the first report of the use of CHROMagar STEC for spiral plate quantification of STEC-7. For most fecal suspensions, quantification by spiral plating underestimated the concentration of STEC or did not quantify STEC. Even when rifampin was supplemented in the agar media to reduce background organisms, STEC were infrequently enumerated at their true values. In a few suspensions, STEC strains were enumerated at slightly higher concentrations by using CHROMagar STEC with or without rifampin and mPossé with rifampin compared with the inoculum level. Testing of more colonies may give a more accurate concentration value. Although the spiral plate method used here only tested 10 colonies from an agar plate, the benefit of using this approach is that it can test whether multiple genes originate from one organism, unlike current real-time PCR assays *(1, 7, 31)*. Most suspensions were quantifiable when concentrations were above 10^4 CFU/g. Strain-to-strain variation was observed as the STEC O111 strain was not quantifiable at the levels tested compared with other strains tested that were quantifiable on at least one media using the highest inoculum level. These results suggest that some strains can be quantified by spiral plating on CHROMagar STEC or mPossé2, but their enumeration may be underestimated.

In conclusion, there is no single optimal agar medium for detection of STEC-7 in cattle feces. Although this study suggests that CHROMagar STEC and mPossé2 detect more STEC-positive fecal suspensions compared with other agar media tested, some STEC strains remained undetected when using these media. In addition, this study established that CHROMagar STEC and mPossé2 could be used to quantify STEC-7 in cattle feces. Further studies are needed to determine the optimum antimicrobial concentrations in agar media for detection of STEC-7.

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