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Comparison of Enrichment Broths for Supporting Growth of Shiga Toxin-Producing *Escherichia coli*

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Abstract Detection of Shiga toxin-producing *Escherichia coli* (STEC) in complex sample matrices remains challenging. In an attempt to improve detection, nonselective and selective enrichment broths were compared as follows: (1) trypticase soy broth (TSB) was compared with TSB plus novobiocin, vancomycin, rifampicin, bile salts, and potassium tellurite (TSB-NVRBT) for supporting growth of STEC in pure culture; (2) *E. coli* broth (EC), TSB, and TSB plus bile salts (mTSB) were compared for enrichment of STEC O26, O45, O103, O104, O111, O121, O145, and O157 (STEC-8) in inoculated cattle fecal samples; (3) EC, TSB, and mTSB were compared for the detection of STEC-8 in inoculated cattle fecal samples. Fecal samples were inoculated with wild-type STEC-8 or nalidixic acid- or rifampicin-resistant derivatives of the same strains at 100, 1000, or 10,000 colony-forming units per gram (CFU/g) of feces. In pure culture, the mean STEC CFU/mL following enrichment in TSB was 1.17 $\log_{10}$ greater than that in TSB-NVRBT ($P < 0.05$). In inoculated fecal samples, EC enrichment yielded growth of STEC-8 (6.42 $\log_{10}$ CFU/g) that was significantly greater than in TSB (6.23 $\log_{10}$ CFU/g; $P < 0.05$), and numerically but not significantly greater than in mTSB (6.37 $\log_{10}$ CFU/g; $P = 0.60$). Wild-type STEC strains were detected in 43.8 % (21/48) of the samples enriched in EC and mTSB compared to 27.1 % (13/48) of the samples enriched in TSB ($P = 0.15$). Overall, STEC grew significantly better when enriched in EC compared to TSB. Modification of TSB by the addition of bile salts improved the growth and detection of STEC compared to TSB alone.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are the major cause of hemorrhagic colitis and hemolytic-uremic syndrome in human patients [3, 4, 11] and commonly colonize the large intestines of cattle, sheep, and other ruminants, which serve as reservoirs [3, 16]. These organisms are shed in ruminant feces, often in relatively low numbers, in conjunction with high numbers of commensal flora including nonpathogenic *E. coli* [3, 6]. The infectious dose of STEC O157:H7 in humans is very low, estimated to be <50 bacterial cells [18]. Due to the frequent presence of STEC at low concentrations in different sample matrices and given their low infectious dose, enrichment is usually necessary for detection [1, 6, 18].

The majority of illnesses due to STEC in the U.S. are caused by serogroups O26, O45, O103, O111, O121, O145, and O157 (STEC-7) [4]; further, most of these STEC-7 organisms are intimin (*eae*)-positive and hence classified as enterohemorrhagic *E. coli* (EHEC) [11]. Additionally, the O104:H4 outbreak associated with fenugreek sprouts [5] has raised concern about STEC contaminating other food sources, and therefore, STEC-7 and STEC O104:H4 (collectively referred to herein as STEC-8) were addressed in this study.

Many culture-based and -independent methods for the detection of STEC in food and environmental samples include an enrichment step to enhance detection [22]. However, a medium that gives optimal enrichment,

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collectively, for all STEC-7 or -8 has yet to be determined. Trypticase soy broth (TSB) and *E. coli* broth (EC) have been used most commonly for enrichment in support of detection of STEC O157:H7 and non-O157 in studies spanning across all sample matrix types [20]. Strains enriched in TSB were more likely to yield false-negative results compared to strains enriched in EC [21]. Enrichment media have been modified by the addition of antibiotics and selective components depending on the matrix [8]. The addition of novobiocin [9, 21] or a combination of vancomycin, cefsulodin, and cefixime [2] has been reported to inhibit growth of some STEC. The inclusion of bile salts as a selective measure in media has previously been shown to delay the Jameson effect and was therefore interpreted to potentially promote STEC growth [21]. Enrichment time is another important consideration that increases STEC to a detectable level. An enrichment time of 24 h did not enhance the recovery of STEC compared to 6 h [19].

The objectives of this study were to (i) compare the growth of STEC-7 in pure culture using TSB and TSB plus novobiocin (8.0 mg/L), vancomycin (16.0 mg/L), rifampicin (2.0 mg/L), bile salts No. 3 (1.5 g/L), and potassium (K) tellurite (1.0 mg/L; TSB-NVRBT); (ii) evaluate the growth of STEC-8 in cattle fecal samples enriched in EC, TSB, and TSB with 1.5 g/L of bile salts No. 3 (modified TSB; mTSB) using spontaneous antibiotic-resistant mutant derivative strains; and (iii) compare EC, TSB, and mTSB for the detection of STEC-8 in inoculated cattle fecal samples using wild-type (WT) strains.

Materials and Methods

Bacterial Strains, Preparation of Inoculum, and Inoculation of Fecal Samples

All strains used in this study were STEC. Strains used for Experiment 1 were obtained from Dr. Shannon Manning (Michigan State University STEC Center) and Dr. David G. Renter (Kansas State University), or from our laboratory collection (Table 1). Spontaneous nalidixic acid-resistant mutant (Nal^R), spontaneous rifampicin-resistant (Rif^R) mutant, and WT parent strains used for Experiments 2 and 3 were obtained from Dr. John B. Luchansky (USDA, Agricultural Research Service, Eastern Regional Research Center) or were from our laboratory collection (Table 2).

Frozen (−80 °C) stock cultures of strains were streaked onto Luria–Bertani broth, Miller (LB; BD, Sparks, MD) agar plates without antibiotics or with 40 mg/L nalidixic acid (Fisher) or 100 mg/L rifampicin (Sigma-Aldrich) when appropriate and incubated at 37 °C for 24 h. A single colony was inoculated in LB with corresponding antibiotics for 24 h at 37 °C in a stationary culture.

Fresh feces were collected from beef cattle housed one per pen at the University of Nebraska–Lincoln. One gram (g) of cattle feces was suspended in 9 mL of EC (Oxoid Ltd., Hampshire, UK), TSB (BD, Sparks, MD) or mTSB and vortexed for 2 min. Serial tenfold dilutions of the inoculum were prepared in buffered peptone water (BPW) and used to inoculate fecal samples at a concentration of 100, 1000, or 10,000 colony-forming units per gram (CFU/g) of feces, with BPW used as a mock inoculation. Following 20 s of vortexing, samples were enriched for 6 h at 40 °C in a stationary culture, as described by Paddock et al. [13].

Experiment 1: Comparison of TSB and TSB-NVRBT

Frozen stock cultures were streaked onto TSB agar and used to inoculate 5 mL of TSB. Stationary cultures were incubated 24 h at 37 °C. The medium, TSB-NVRBT, was prepared according to Possé et al. [15]. Duplicate stationary cultures of each strain were prepared in TSB and TSB-NVRBT. Following incubation for 18 h at 42 °C, serial tenfold dilutions in BPW were made from each culture and plated on TSB agar. Plates containing TSB agar were incubated for 18 h at 37 °C, and aerobic plate counts (APC) were performed according to the FDA *Bacteriological Analytical Manual* (BAM) [12].

Experiment 2: Evaluating Growth of STEC-8 using Nal^R and Rif^R Strains

Cattle fecal samples were inoculated with Nal^R or Rif^R STEC-8 strains at concentrations of 100, 1000, or 10,000 CFU/g to evaluate the effect of different enrichment broths on STEC growth in two independent experiments. After enrichment, samples were serially diluted and spread plated onto Possé differential agar [14] modified by supplementation with nalidixic acid (40 mg/L) or rifampicin (100 mg/L) with reduced bile salts No. 3 (1.5 g/L) and containing no novobiocin or K tellurite (mPossé1); these plates were incubated 18 h at 37 °C. Plates were counted by standard methods [12], growth was determined as CFU/g, and these values were $\log_{10}$ transformed prior to statistical analysis. Colonies were confirmed to be the respective inoculum strain by PCR [13].

Experiment 3: Detection of STEC-8 Using WT Strains

Cattle fecal samples inoculated with WT STEC at 100, 1000, and 10,000 CFU/g were subjected to immunomagnetic separation (IMS) for the corresponding O-antigen using a

Table 1 Comparison of growth for Shiga toxin-producing *Escherichia coli* strains under pure culture conditions

Strain	Serotype	Source	stx_1	stx_2	*eae*	Mean $\log_{10}$ CFU/mL	
						TSB	TSB-NVRBT
DEC10B	O26:H11	Human	+	−	+	8.64	7.46
97-3250	O26:H11	Human	+	+	+	8.62	7.79
MT#10	O26:NT	Human	+	−	+	7.30	7.11
TB352A	O26:NM	Human	+	−	+	8.63	7.30
DA-10	O26:NM	Human	+	−	+	9.28	7.41
16272	O26:NT	Cattle	+	−	+	10.30	7.34
1577-88	O26:H11	Cattle	+	−	+	8.85	7.68
M103-19	O45:H2	Human	+	−	+	8.79	7.90
MI01-88	O45:H2	Human	+	−	+	9.04	7.87
MI05-14	O45:H2	Human	+	−	+	9.00	8.30
DA-21	O45:H2	Human	+	−	+	9.00	8.20
B8026-C1	O45:H2	Cattle	+	−	+	9.15	8.18
B8227-C8	O45:H2	Cattle	+	−	+	9.04	8.30
MT#80	O103:H2	Human	+	−	+	9.20	7.72
TB154A	O103:H2	Human	+	−	+	8.82	7.54
8419	O103:H25	Human	+	−	+	9.08	7.62
PT91-24	O103:NM	Human	+	−	+	8.92	7.60
6:38	O103:NM	Human	+	−	+	8.83	7.67
3720	O103:H2	Water	+	−	+	8.90	7.62
15612	O103:H11	Cattle	+	−	+	8.85	7.11
6708-87	O103:H2	Goat	+	−	+	9.11	7.91
RD8	O111:H10	Human	−	+	−	9.15	7.63
3215-99	O111:H8	Human	+	+	+	9.15	8.37
0201 9611	O111:H8	Human	+	−	+	8.93	7.81
3007-85	O111:H8	Human	+	+	+	9.18	8.04
95-3208	O111:NM	Human	+	+	+	8.92	8.08
7726	O111:NT	Cattle	+	+	+	9.36	8.18
8266	O111:NT	Cattle	+	+	+	9.34	8.26
10049	O111:H11	Cattle	+	−	+	8.97	7.68
MT#2	O121:H19	Human	−	+	+	8.81	6.98
MT#18	O121:NT	Human	−	+	+	8.95	7.85
DA-5	O121:H19	Human	−	+	+	8.91	7.85
DA-37	O121:H19	Human	−	+	+	8.59	7.63
3377-85	O121:H19	Human	−	+	+	8.85	8.02
1553	O121:H7	Cattle	+	−	−	8.97	7.02
4190	O121:NT	Cattle	+	−	−	9.15	7.96
DEC10I	O145:H16	Human	+	−	+	8.92	7.38
4865/96	O145:H28	Human	−	+	+	8.93	7.76
GS G5578620	O145:H28	Human	+	−	+	9.08	7.83
IH 16	O145:NM	Human	−	+	+	8.81	7.82
75-83	O145:NM	Human	−	+	+	9.11	7.98
1234	O145:H28	Cattle	+	+	+	9.11	8.15
7744	O145:NT	Cattle	+	−	+	9.08	8.08
933	O157:H7	Beef	+	+	+	8.60	7.68
86-24	O157:H7	Human	−	+	+	8.97	7.89
S2006 #1	O157:H7	Cattle	+	+	+	8.93	7.72
S2006 #2	O157:H7	Cattle	+	+	+	8.95	7.77

Table 1 continued

Strain	Serotype	Source	stx_1	stx_2	*eae*	Mean $\log_{10}$ CFU/mL	
						TSB	TSB-NVRBT
S2006 #3	O157:H7	Cattle	+	+	+	8.93	7.72
S2006 #4	O157:H7	Cattle	+	+	+	8.00	7.82
S2006 #5	O157:H7	Cattle	+	+	+	9.34	7.87
S2006 #6	O157:H7	Cattle	+	+	+	9.00	7.99

Strains were incubated in enrichment broth as stationary cultures for 18 h at 42 °C

NT not typed, *TSB* trypticase soy broth, *TSB-NVRBT* trypticase soy broth plus novobiocin, vancomycin, rifampicin, bile salts, and K tellurite

Table 2 Shiga toxin-producing *Escherichia coli* strains used for inoculation of cattle fecal samples

Strain	Serotype	Source	stx_1	stx_2	*eae*	Resistance
DA-10	O26:NM	Human	+	−	+	N
H30	O26:H11	Human	+	−	+	WT, R
B8227-C8	O45:H2	Cattle	+	−	+	N
CDC 96-3285	O45:H2	Human	+	−	+	WT, R
TB154A	O103:H2	Human	+	−	+	N
CDC 90-3128	O103:H2	Human	+	−	+	WT, R
TY-2482	O104:H4	Human	−	+	−	WT, R
0201 9611	O111:H8	Human	+	−	+	N
JB1-95	O111:NM	Human	+	+	+	WT, R
KDHE 55	O121:NT	Human	−	+	+	N
CDC 97-3068	O121:H19	Human	−	+	+	WT, R
IH 16	O145:NM	Human	−	+	+	N
83-75	O145:NM	Human	−	+	+	WT, R
933	O157:H7	Beef	+	+	+	WT, N
USDA FSIS 380-94	O157:H7	Salami	+	+	+	R

NT not typed, *WT* wild-type, *N* nalidixic acid-resistant, *R* rifampicin-resistant

KingFisher™ Flex Magnetic Particle Processor (Thermo Scientific, Waltham, MA). Anti-O157 Dynabeads® (Invitrogen, Carlsbad, CA), and IMS beads for *E. coli* O26, O45, O103, O104, O111, O121, and O145 (Abraxis LLC, Warminster, PA) were used for IMS. After IMS, samples were diluted and spread plated onto CHROMagar™ STEC (DRG International, Mountainside, NJ), Possé differential agar [14], and Possé differential agar modified by having reduced concentrations of novobiocin (5 mg/L) and K tellurite (0.5 mg/L; mPossé2). Colonies were tested by PCR [13] and recorded as detection or no detection. Two independent experiments were conducted for all STEC-8 WT strains.

Detection by PCR

Five colonies were picked from countable plates according to the methods described in the FDA BAM [12], put into 50 µL of ultrapure water, and heated at 95 °C for 10 min for use as DNA template. From these, a single colony was selected at random and tested by multiplex PCR for inoculum-specific O-type and Shiga toxin (*stx*) type as described by Paddock et al. [13] to confirm that colonies on agar plates were that of the respective inoculum strain.

Statistical Analysis

A paired *t* test was used to compare the growth of STEC in TSB to that in TSB-NVRBT (SAS 9.2, PROC GLIMMIX). The restricted maximum likelihood method was used to compare the growth of antibiotic-resistant STEC in inoculated fecal samples, and a χ^2 analysis was used to compare the detection of WT STEC in inoculated fecal samples (SAS 9.2, PROC MIXED, PROC GLIMMIX).

Results and Discussion

Antimicrobials have the potential to inhibit background organisms and allow STEC to grow to high numbers; however, it has been reported that some antimicrobials, e.g.,

novobiocin and K tellurite, can restrict the growth of some STEC [2, 10, 20, 21]. In order to assess whether antimicrobials in an enrichment medium designed for STEC [15] were actually selective against STEC growth, TSB was compared to TSB-NVRBT on a relatively large number of STEC-7 strains. There was a mean CFU/mL $\log_{10}$ reduction of 1.17 for strains grown in TSB-NVRBT compared to TSB and, in both media, strains within and among serogroups varied in their growth (Table 1). Overall, strains grown in TSB had significantly greater growth than strains grown in TSB-NVRBT ($P < 0.05$); this was true for all non-O157 STEC and STEC O157:H7, and growth of STEC O26, O103, and O121 strains was particularly reduced. The ranking of STEC serogroups in descending order of tolerance of the enrichment conditions were O45, O111, O145, O157, O121, O103, and O26. Two strains (one O26 and one O157:H7) exhibited poor growth in TSB with a minimal further reduction in growth in TSB-NVRBT. Since supplementation of TSB with NVRBT resulted in a significant reduction in growth of STEC, we did not test it further and chose instead to focus on TSB and EC for further experiments in bovine fecal samples. EC and TSB without additional antimicrobials (e.g., novobiocin) had been shown in previous studies to be less inhibitory to growth, and EC reportedly most suitable for supporting detection of non-O157 STEC in bovine feces, but these results were based on very few strains or strains of unidentified serogroups, and did not test the effects of bile salts per se [13, 21]. Since EC and TSB vary, respectively, by the presence of lactose versus glucose, and bile salts versus none, we sought to determine whether the addition of bile salts to TSB might make it comparable to EC in performance.

Strains that were Nal^R or Rif^R were used to accurately assess growth in a complex matrix since these antibiotics effectively eliminated the background microflora in fecal samples. All strains had detectable growth in all enrichment media. The APCs resulting from enrichment of fecal samples inoculated at different CFU/g concentrations were significantly different ($P < 0.05$); samples inoculated with 10,000 CFU had the highest APC, 1000 CFU the next highest, and 100 CFU the lowest. This indicated that the cultures had not become saturated by 6 h of enrichment. A longer enrichment time may increase the concentration of STEC in low abundance. Conversely, when the background microflora reaches its maximum level, STEC growth will stop [21], and an extended time may not increase STEC in low abundance.

Strains in inoculated samples grew to 6.42 $\log_{10}$ CFU/g in EC, 6.23 $\log_{10}$ CFU/g in TSB, and 6.37 $\log_{10}$ CFU/g in mTSB. Although growth of STEC-8 in EC appeared only slightly higher compared to that in TSB, a significant difference was observed ($P < 0.05$), and means were numerically but not significantly greater in mTSB compared to TSB (Table 3). All strains isolated from the feces were confirmed to be that of the inoculum by PCR, and no STEC were isolated in non-inoculated samples. Higher growth of the STEC inoculum strains in EC suggests that lactose provided a selective advantage for these organisms, as expected, in contrast to glucose. Also, as expected, the bile salts in EC and mTSB suppressed growth of background microflora and allowed the STEC to grow to a higher level.

Cattle fecal samples were inoculated with WT STEC strains to compare the detected number of STEC positive samples enriched in EC, TSB, and mTSB. Strains inoculated at 10,000 CFU were detected more frequently than strains at 1000 and 100 CFU ($P < 0.05$). One STEC-8 WT strain was detected in 21 of 48 samples enriched in EC and mTSB and in 13 of 48 samples enriched in TSB (Table 4). Although STEC-8 WT strains were detected more frequently in samples enriched in EC and mTSB compared to TSB, a significant difference for broth type was not observed ($P = 0.15$). Increased growth of STEC in EC and mTSB may have led to more frequent detection compared to TSB. In a study comparing four enrichment broths for detection of non-O157 STEC in inoculated radish sprouts and beef, modified EC with novobiocin demonstrated a lower recovery rate compared to modified TSB with bile salts with or

Table 3 Growth of antibiotic-resistant Shiga toxin-producing *Escherichia coli* in inoculated cattle fecal samples

Enrichment broth	Mean $\log_{10}$ CFU/g
EC	6.42[a]
TSB	6.23[b]
mTSB	6.37[ab]

Means with different letters are significantly different ($P < 0.05$)

EC E. coli broth, *TSB* trypticase soy broth, *mTSB* trypticase soy broth plus bile salts

Table 4 Number of samples (%) detected positive for Shiga toxin-producing *Escherichia coli* in 48 inoculated cattle fecal samples

Enrichment broth	Agar media			Total
	CHROMagar™ STEC	Possé	mPossé2	
EC	8 (16.7)	15 (31.3)	16 (33.3)	21 (43.8)
TSB	5 (10.4)	8 (16.7)	8 (16.7)	13 (27.1)
mTSB	9 (18.8)	13 (27.1)	15 (31.3)	21 (43.8)

EC E. coli broth, *TSB* trypticase soy broth, *mTSB* trypticase soy broth plus bile salts

without novobiocin and universal pre-enrichment broth [10]. These findings suggest that modification of EC by adding novobiocin may be inhibitory to STEC detection in food compared to other enrichment broths.

There have been extensive improvements for enrichment of STEC O157:H7 in cattle feces [1, 6, 17], but methods to improve enrichment of non-O157 STEC have yielded inconsistent results [7, 9, 10, 13, 15, 19–22]. Our work suggests that lactose and bile salts in EC provide a more suitable environment compared to the presence of glucose and absence of bile salts as found in TSB for the enrichment of STEC-8.

Conclusion

The addition of several selective antimicrobials to TSB was found to inhibit growth of STEC in pure culture compared to TSB. EC broth yielded significantly greater STEC growth compared to TSB but not TSB plus bile salts in cattle fecal samples.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Barkocy-Gallagher GA, Edwards KK, Nou X, Bosilevac JM, Arthur TM, Shackelford SD, Koohmaraie M (2005) Methods for recovering *Escherichia coli* O157:H7 from cattle fecal, hide and carcass samples: sensitivity and improvements. J Food Prot 68:2264–2268
2. Baylis CL (2008) Growth of pure cultures of verocytotoxin-producing *Escherichia coli* in a range of enrichment media. J Appl Microbiol 105:1259–1265
3. Bettelheim KA (2007) The non-O157 Shiga-toxigenic (verocytotoxigenic) *Escherichia coli*; under-rated pathogens. Crit Rev Microbiol 33:67–87
4. Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, Strockbine NA (2005) Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. J Infect Dis 92:1422–1429
5. Bucholz U, Bernard H, Werber D, Böhmer MM, Remschmidt C, Wilking H, Deleré Y, an der Heiden M, Adlhoch C, Dreesman J, Ehlers J, Ethelberg S, Faber M, Frank C, Fricke G, Greiner M, Höhle M, Ivarsson S, Jark U, Kirchner M, Koch J, Krause G, Luber P, Rosner B, Stark K, Kühne M (2011) German outbreak of *Escherichia coli* O104:H4 associated with sprouts. N Engl J Med 365:1763–1770
6. Fegan N, Higgs G, Vanderlinde P, Desmarchelier P (2004) Enumeration of *Escherichia coli* O157 in cattle faeces using most probable number technique and automated immunomagnetic separation. Lett Appl Microbiol 38:56–59
7. Fukushima H, Gomyoda M (1999) An effective, rapid and simple method for isolation of Shiga toxin-producing *Escherichia coli* O26, O111 and O157 from faeces and food samples. Zentralbl Bakteriol 289:415–428
8. Hussein HS, Bollinger LM (2008) Influence of selective media on successful detection of Shiga toxin-producing *Escherichia coli* in food, fecal, and environmental samples. Foodborne Pathog Dis 5:227–244
9. Hussein HS, Bollinger LM, Hall MR (2008) Growth and enrichment medium for detection and isolation of Shiga toxin-producing *Escherichia coli* in cattle feces. J Food Prot 71:927–933
10. Kanki M, Seto K, Harada T, Yonogi S, Kumeda Y (2011) Comparison of four enrichment broths for the detection of non-O157 Shiga toxin-producing *Escherichia coli* O91, O103, O111, O119, O121, O145 and O165 from pure culture and food samples. Lett Appl Microbiol 53:167–173
11. Kaper JB, Nataro JP, Mobley HLT (2004) Pathogenic *Escherichia coli*. Nat Rev Microbiol 2:123–140
12. Maturin L, Peeler JT (2001) Aerobic plate count. In: Merker RI (ed) Bacteriological analytical manual, 8th ed. revision A U.S. Food and Drug Administration, College Park, MD. http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm. Accessed 25 Feb 2013
13. Paddock Z, Shi X, Bai J, Nagaraja TG (2012) Applicability of a multiplex PCR to detect O26, O45, O103, O111, O121, O145, and O157 serogroups of *Escherichia coli* in cattle feces. Vet Microbiol 156:381–388
14. Possé B, De Zutter L, Heyndrickx M, Herman L (2008) Novel differential and confirmation plating media for Shiga toxin-producing *Escherichia coli* serotypes O26, O103, O111, O145 and sorbitol-positive and -negative O157. FEMS Microbiol Lett 282:124–131
15. Possé B, De Zutter L, Heyndrickx M, Herman L (2008) Quantitative isolation efficiency of O26, O103, O111, O145 and O157 STEC serotypes from artificially contaminated food and cattle faeces samples using a new isolation protocol. J Appl Microbiol 105:227–235
16. Sánchez S, Garciá-Sánchez A, Martínez R, Blanco J, Blanco JE, Blanco M, Dahbi G, Mora A, Hermoso de Mendoza J, Alonso JM, Rey J (2009) Detection and characterisation of Shiga toxin-producing *Escherichia coli* other than *Escherichia coli* O157:H7 in wild ruminants. Vet J 180:384–388
17. Sanderson MW, Gay JM, Hancock DD, Gay CC, Fox LK, Besser TE (1995) Sensitivity of bacteriologic culture for detection of *Escherichia coli* O157:H7 in bovine feces. J Clin Microbiol 33:2616–2619
18. Tilden J Jr, Young W, McNamara AM, Custer C, Boesel B, Lambert-Fair MA, Majkowski J, Vugia D, Werner SB, Hollingsworth Morris JG Jr (1996) A new route of transmission for *Escherichia coli*: infection from dry fermented salami. Am J Public Health 86:1142–1150
19. Verstraete K, De Zutter L, Messens W, Herman L, Heyndrickx M, De Reu K (2010) Effect of the enrichment time and immunomagnetic separation on the detection of Shiga toxin-producing *Escherichia coli* O26, O103, O111, O145 and sorbitol positive O157 from artificially inoculated cattle faeces. Vet Microbiol 145:106–112
20. Vimont A, Vernozy-Rozand C, Delignette-Muller ML (2006) Isolation of *E. coli* O157:H7 and non-O157 STEC in different matrices: review of the most commonly used enrichment protocols. Lett Appl Microbiol 42:102–108
21. Vimont A, Vernozy-Rozand C, Montet MP, Bavai C, Fremaux B, Delignette-Muller ML (2007) Growth of Shiga-toxin producing *Escherichia coli* (STEC) and bovine feces background microflora in various enrichment protocols. Vet Microbiol 123:274–281
22. Wang F, Yang Q, Kase JA, Meng J, Clotilde LM, Lin A, Ge B (2013) Current trends in detecting non-O157 Shiga toxin-producing *Escherichia coli* in food. Foodborne Pathog Dis 10:665–677