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Prevalence of *Escherichia coli* O157:H7 from Cull Dairy Cows in New York State and Comparison of Culture Methods Used during Preharvest Food Safety Investigations

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A number of protocols for the cultural detection of *Escherichia coli* O157:H7 in clinical fecal specimens have been proposed. In the present study direct plating of cattle feces was compared to three different broth enrichment protocols, i.e., a protocol with modified *E. coli* broth with novobiocin, a protocol with Trypticase soy broth with cefixime and vancomycin, and a protocol with Gram-Negative Broth with novobiocin, for their relative abilities to detect *E. coli* O157:H7 in feces. In all enrichment protocols, dilutions of the enrichment broths onto 150-mm sorbitol-MacConkey agar plates to which cefixime and tellurite were added were used along with reading of agar plates at both 24 and 48 h. Fecal samples came from a preharvest food safety project in which feces from New York cull dairy cattle from a northeastern packing plant along with experimentally inoculated adult dairy cow feces were tested. The performances of the broth enrichments were comparable to each other, but the broth enrichments were superior to direct plating in their ability to detect *E. coli* O157:H7. Regardless of the culture protocol used, recovery of *E. coli* O157:H7 is more likely from fresh fecal specimens than from frozen samples. An overall prevalence of *E. coli* O157:H7 fecal shedding by New York cull dairy cattle of 1.3% was found in specimens just before processing at the packing plant.

Escherichia coli O157:H7 is an emerging cause of food-borne illness, with over 20,000 cases of infection occurring each year in the United States alone. In humans infections with this serotype may cause bloody diarrhea, and in children infections with this serotype may lead to hemolytic-uremic syndrome (11). Illness is often linked to the consumption of contaminated and undercooked ground beef and unpasteurized fruit juices, but transmission by other means such as person-to-person transmission in child care centers and in families and by swimming in feces-contaminated water is also possible (3, 5). *E. coli* O157:H7 is 1 of over 200 serotypes that are recovered from humans and that produce Shiga-like toxins, i.e., are verocytotoxigenic *E. coli* (VTEC); over 50 of these VTEC serotypes produce bloody diarrhea or hemolytic-uremic syndrome in humans and are thus classified as enterohemorrhagic *E. coli* (15). *E. coli* O157:H7 does not ferment sorbitol, and this fact is used in its isolation on sorbitol-containing bacteriological media. Many non-O157:47 VTEC strains of bovine and other origins have been isolated from humans and have been associated with disease but are not sought in human clinical microbiology laboratories, which generally screen only for O157:H7; these non-O157:47 VTEC strains ferment sorbitol, and there are no convenient culture means to screen for them. The significance of non-O157:47 VTEC strains in human disease is of research interest in a number of laboratories (14, 19, 20).

There are over 36,000 farms in New York State, and half of them have cattle and calves. New York ranks third in the nation in numbers of dairy cows. Cull dairy cows in New York are the source of 1.05×10^8 lb of hamburger produced in New York State each year. Because healthy cattle have been shown to be transient reservoirs of food-borne pathogens such as *E. coli* O157:H7 (6, 13), data on the prevalence of O157:H7 in animals presented to slaughter are critical in the design of risk-based hazard analysis critical control point programs to control pathogens in packing plants.

A number of culture methods for the screening of fecal specimens for *E. coli* O157:H7 are available. Feces may be directly plated onto selective and/or differential agars (8, 17), or feces may be selectively enriched in broth followed by plating onto selective, differential agars (see references 15 and 16 for reviews of agar improvements over the years); this enrichment step may be followed by immunomagnetic separation with beads coated with O157-specific antibody before plating onto agar (8). Various researchers have also used PCR techniques to screen broth enrichment cultures for the presence of O157:H7; often, the primers are specific for the Shiga-like toxin genes and not specifically for O157:H7, although use of primers specific for the H7 flagellum shows promise for improved analytical specificity (9, 10, 18).

In New York, during 1990 and 1991 the U.S. Department of Agriculture's (USDA's) Centers for Epidemiology and Animal Health first studied the prevalence of O157:H7 infections on U.S. dairy farms, including New York, as part of a National Animal Health Monitoring Survey (NAHMS) called the National Dairy Heifer Evaluation Project (1). Beginning in 1995 we started a series of field studies on *E. coli* O157:H7 including a study of its prevalence in New York State's cull dairy cattle. We had an excellent opportunity to evaluate the various bacterial culture methods for the recovery of *E. coli* O157:H7 available at that time. Some laboratories had success in detecting *E. coli* O157:H7 by direct plating of feces onto sorbitol-MacConkey (SMAC) agar plates (8, 17). The National Veterinary Services Laboratories (NVSL), USDA, in various surveys including the National Animal Health Monitoring Surveys

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(NAHMS), had successfully used selective enrichment in modified *E. coli* broth supplemented with novobiocin (mECnov) followed by plating at least 2 dilutions of mECnov onto SMAC agar containing cefixime and potassium tellurite supplements (SMACct) (L. A. Thomas, R. A. Reymann, H. W. Moon, R. A. Schneider, D. R. Cummins, M. G. Beckman, L. Schroeder-Tucker, and K. Ferris, Abstr. 35th Annu. Meet. Am. Assoc. Vet. Lab. Diagnosticians [AAVLD], p. 83, 1992). The Washington State University laboratory used selective broth enrichment in Trypticase soy broth (TSB) supplemented with cefixime and vancomycin (TSBcv), followed by plating of dilutions onto SMACct agar plates (16). In this study we have compared the direct plating, mECnov, and TSBcv methods and another new method, one with Gram-Negative Broth (HAJNA formulation) with novobiocin (GNHAJNAnov) for their relative abilities to detect *E. coli* O157:H7 in the feces of cull dairy cows soon after their arrival at a major packing plant. We also determined, in our evaluation of culture procedures, the point prevalence of *E. coli* O157:H7 shedding in New York cull dairy cattle at the point of slaughter. Other aspects of this group of preharvest food safety studies will be reported elsewhere, such as the effects of transit time to packing plant, weather, and body condition scores, on the shedding status of *E. coli* O157:H7.

MATERIALS AND METHODS

Bacterial strains. *E. coli* O157:H7 ATCC 43894 (American Type Culture Collection, Manassas, Va.) was used as a control strain for experimental inoculation of bovine feces in some experiments. Overnight cultures of *E. coli* O157:H7 grown on Trypticase soy agar with 5% sheep blood (BBL, Becton Dickinson, Cockeysville, Md.) at 37°C were used to produce a standard inoculum in sterile water equivalent to a 0.5 McFarland standard, i.e., approximately 1.5×10^8 CFU/ml on the day of testing; this 0.5 McFarland standard was serially diluted in sterile normal saline solution to produce the inoculum for the tests.

Fecal specimens and target population. Bovine fecal specimens of unknown *E. coli* O157:H7 infection status were received in small metal containers from cattle that were part of a cross-sectional survey of 1,668 culled dairy cattle tested at a major northeastern packing plant. The cows were representative of the population of cull cows purchased by this packing plant and were composed primarily of Holstein cattle. These cattle originated from all major livestock markets and regions of New York State. The bulk of the fecal samples were obtained during five, 4-day sampling periods per month from August to September in 1995. Cows were sampled in pens immediately prior to and up to a few hours prior to slaughter. Other fecal specimens from *E. coli* O157:H7-negative cows were obtained from research dairy cattle at the College of Veterinary Medicine at Cornell University and were contaminated with known amounts of *E. coli* O157:H7 for use as positive controls in experiments.

Identification of *E. coli* O157:H7 colonies. For each test sample, up to 10 bacterial colonies that were cultured on various agar media with or without broth enrichment (see experimental details below) and that did not ferment sorbitol were first screened by a rapid latex test (RIM *E. coli* O157:H7 Latex Test Kit; Remel, Lenexa, Kans.) to ascertain their O157 infection status. If the RIM O157 latex test was positive, the suspect bacterial colony was subcultured onto a Trypticase soy agar with 5% sheep blood agar plate and the plate was incubated overnight at 37°C before screening the next day by the H7 latex test (same RIM *E. coli* O157:H7 Latex Test Kit). If at least the O157 latex test was positive, the bacterial isolate was biochemically identified as *E. coli* by using the Sensititre Automated Microbiology System (SAMS) AP80 panel (Sensititre Microbiology System Division, AccuMed International, Inc., Westlake, Ohio).

If the O157 latex was positive and the H7 latex was negative, the strain was grown overnight in TSB (BBL) at 37°C and was then serially passaged in TSB at least five times to ensure that the isolate was highly motile. Next, a 3-ml aliquot of an overnight broth culture was mixed 1:1 with 3 ml of 1% (vol/vol) formalin in physiological saline solution, and the suspension was used as an antigen in the following test. Two drops of H7 antiserum (Seiken, Denka Seiken Co., Ltd., Tokyo, Japan, obtained from Oxoid-Division of UniPath Co., Ogdensburg, N.Y.) was mixed with 0.5 ml of the formalinized broth culture, thoroughly mixed, covered, and then incubated in a 50°C water bath for 1 h and observed for agglutination. H7-positive isolates were also identified biochemically by using SAMS.

All sorbitol-negative isolates that were biochemically confirmed to be *E. coli* and that were O157 latex positive, whether or not they were H7 latex positive, were tested by PCR as a confirmatory test, according to the manufacturer's directions, by using the BAX O157:H7 Pathogen Detecting System (Qualicon Inc., a subsidiary of E. I. du Pont de Nemours & Company, Wilmington, Del.).

Experiments. Experiments 1, 2A, 2B, 4, and 6 used fecal samples from cull dairy cows of unknown *E. coli* O157:H7 infection status; experiment 6 also used some experimentally contaminated feces as controls. Experiments 3 and 5 used experimentally contaminated feces from adult dairy milking cows.

(i) Experiment 1. Experiment 1 was used to determine the preslaughter prevalence of *E. coli* O157:H7 in 1,668 culled dairy cows at a major northeastern packing plant from August to September 1995.

Fresh fecal samples from these cows were directly plated onto 100-mm SMAC agar plates (SMAC agar; Difco Laboratories, Detroit, Mich.) with no selective additive, and the plates were incubated overnight at 37°C, at which time up to 10 sorbitol-negative colonies were picked for identification (see the protocol described above under Identification of *E. coli* O157:H7 colonies); the plates were reincubated for an additional 24 h, at which time additional colonies were picked if present for screening.

(ii) Experiment 2A. The recovery of O157:H7 from direct plating of feces from experiment 1 was thought to be low. As a result experiment 2 was performed to incorporate a broth enrichment step and to use a more selective and differential agar plating medium than experiment 1; this project was also performed to assess the effects of freezing and thawing on the ability to recover viable O157:H7 bacteria in frozen bovine feces.

A subset ($n = 116$) of the original 1,668 fecal specimens from experiment 1 was randomly chosen for retesting; contained within this subset were 9 of the original 16 O157:H7-positive fecal specimens. These specimens were taken from the −70°C freezer where they had been stored for 7 months and then retested in March 1996 by using the protocol of Washington State University (16). This method used an enrichment step, dilution, and a more selective or differential plating medium to enhance the detection of O157:H7. One gram of feces was added to 9 ml of TSBcv (TSB [BBL] with cefixime [50 ng/ml; Wyeth Ayerst Lederle Laboratories, Division of American Cyanamide Company, Sanford, N.C.] and vancomycin [40 μg/ml; Sigma Chemical Co., St. Louis, Mo.]), and the components were mixed on a vortex mixer and incubated at 37°C for 18 to 24 h. After incubation, serial 10-fold dilutions were made in plain TSB (BBL) in a microtiter plate, and then 0.1 ml of the 10^{-2} and 10^{-4} dilutions was plated onto 150-mm SMACct plates (SMAC agar [Difco Laboratories] to which cefixime [50 ng/ml] and potassium tellurite [2.5 μg/ml] were added) and was evenly spread. The SMACct plates were incubated for 18 to 24 h at 37°C, at which time sorbitol-negative colonies were picked for identification (see protocol described above under Identification of *E. coli* O157:H7 colonies); the SMACct plates were reincubated for an additional 24 h, at which time additional colonies were picked, if present, for screening as detailed above under Identification of *E. coli* O157:H7 colonies.

(iii) Experiment 2B. Experiment 2B was a continuation of experiment 2A. It was done 1 month later (April 1996) and looked at 176 additional frozen fecal specimens from experiment 1. The specimens had been stored at −70°C for 8 months. The experimental procedures were the same as those for experiment 2A.

(iv) Experiment 3. Experiment 3 was done to evaluate a second, different broth enrichment called mECnov (Difco Laboratories) by using the protocol from NVSL at USDA (Thomas et al., Abstr. 35th Annu. Meet. AAVLD). Experiment 3 used experimentally contaminated fresh fecal samples only; i.e., the experiment consisted of making a 0.5 McFarland standard suspension of a fresh culture of *E. coli* O157:H7 ATCC 43894 in sterile water, making serial 10-fold dilutions of this suspension in mECnov from 10^{-1} to 10^{-7}, and then adding 1 g of fresh bovine feces to each tube from an adult dairy cow whose feces were shown to be negative for *E. coli* O157:H7. Testing of the feces of this negative control cow over a 2-week period by selective broth enrichment and selective plating showed that she was not shedding *E. coli* O157:H7. After thorough mixing, the mECnov tubes with feces were incubated at 37°C for 18 to 24 h, and then 0.1 ml from the tube with the 1×10^{-5} dilution (containing 1.5×10^3 CFU/ml) and 0.1 ml from the tube with the 1×10^{-6} dilution (containing 1.5×10^2 CFU/ml) were spread onto 150-mm SMACct plates which were incubated and read for sorbitol-negative colonies as described above for experiments 1, 2A, and 2B. All tubes were tested blindly by the investigators.

(v) Experiment 4. Experiment 4 was done to field test the Washington State protocol used in experiments 2A and 2B with fresh fecal specimens (rather than frozen feces) from cull dairy cattle. Fresh feces from 210 new cull cows were collected and were processed as described above for experiments 2A and 2B.

(vi) Experiment 5. Experiment 5 was performed to briefly evaluate a new third enrichment broth GNHAJNAnov as another potential protocol for use in O157:H7 testing. GNHAJNAnov broth enrichment was compared to the protocols with TSBcv and mECnov to screen experimentally contaminated feces from adult dairy cattle.

The following enrichment broths were used: the new broth, broth for gram-negative organisms (HAJNA formulation, Difco) with novobiocin (20 μg/ml; Difco) (GNHAJNAnov), TSBcv (as described above for experiments 2a, 2b, and 4), and mECnov (as described above for experiment 3). A 0.5 McFarland standard suspension of a fresh culture of *E. coli* O157:H7 ATCC 43894 in sterile water was first made, followed by the preparation of serial 10-fold dilution of this suspension in each different enrichment broth (from 10^{-1} to 10^{-7}) for use as innocula for the experiment. For each of the three types of enrichment broths, one tube of *E. coli* O157:H7 at a concentration of 1.5×10^4 CFU/ml, two tubes of *E. coli* at a concentration of 1.5×10^3 CFU/ml, and three tubes of *E. coli* at a concentration of 1.5×10^2 CFU/ml were produced and used as positive

TABLE 1. Summary of various treatment protocols for of bovine feces used in experiments 1 to 6 in the New York State cull dairy cow preharvest food safety study

Expt no.	No. of cull dairy cows in field study	Experimentally contaminated dairy cow feces used (no. positive/no. negative)	Type of treatment used			
			DIR[a]	TSBcv	mECnov	GNHAJNAnov
1	1,668 (fresh)	No (—[b]/—)	X			
2A	116 (frozen)	No (—/—)		X		
2B	176 (frozen)	No (—/—)		X		
3		Yes (5/—)			X	
4	210 (fresh)	No (—/—)		X		
5		Yes (6/14)		X	X	X
6	88 (fresh)	Yes (24/—)	X	X	X	X

[a] DIR, direct plating without enrichment broth onto plain SMAC agar.
[b] —, not tested.

controls; an additional 14 tubes of each enrichment broth were also used as experimental negative controls; thus, a total of 20 tubes of each enrichment broth (6 positive control tubes and 14 negative control tubes) were included in the experiment. Then, 1 g of fresh bovine feces from an adult dairy cow (previously screened and shown to be negative for O157:H7) was added to each of the 20 tubes and the contents were thoroughly mixed. All tubes were incubated at 37°C for 18 to 24 h. Serial 10-fold dilutions of the contents of each tube were then made by using the respective enrichment broths, after which 0.1 ml each from the tubes with the 10^{-5} and the 10^{-6} dilutions was spread onto 150-mm SMACct plates. The SMACct plates were incubated and screened for sorbitol-negative colonies as described above for experiments 1, 2A, 2B, and 4. All tubes were tested blindly by the investigators.

(vii) Experiment 6. Experiment 6 expanded experiment 5, but this time it was a field study to evaluate the abilities of the same three enrichment broths to detect *E. coli* O157:H7 in fresh fecal samples from cull dairy cattle collected in June 1996; it also differed from experiment 5 by the addition of a comparison of these enrichment broths versus direct culturing of fresh cull cow feces onto SMACct plates. Experimentally contaminated positive control feces were included in the study.

Fresh feces from 88 new cull cows of unknown O157:H7 infection status, in addition to positive controls (consisting of 24 experimentally contaminated fresh fecal samples from dairy cattle shown to be negative for O157:H7 infection), were tested. A protocol similar to that used for experiment 5 was followed; in brief, feces were directly plated onto SMACct plates, which were then incubated for 18 to 24 h and then for an additional 18 to 24 h; sorbitol-negative colonies were picked for identification after each incubation period. Also, at the same time 1 g of each of the 88 cull cow feces was added to 9 ml each of TSBcv, mECnov, and GNHAJNAnov tubes; and the contents were mixed on a vortex mixer and incubated at 37°C for 18 to 24 h. After incubation, serial 10-fold dilutions were made in plain TSB (BBL) in a microtiter plate, and then 0.1 ml each of the 10^{-2} and 10^{-4} dilutions was plated onto 150-mm SMACct plates and evenly spread. The SMACct plates were incubated for 18 to 24 h at 37°C, at which time sorbitol-negative colonies were picked for identification (see the protocol described above under Identification of *E. coli* O157:H7 colonies); the SMACct plates were reincubated for an additional 24 h, at which time additional colonies, if present, were picked for screening. The positive control broths were made up as described above for experiment 5, except that in experiment 6 there were, for each type of enrichment broth, eight tubes with a concentration of 1.5×10^2 CFU/ml, eight tubes with a concentration of 1.5×10^3 CFU/ml, and eight tubes with a concentration of 1.5×10^4 CFU/ml; after 1 g of negative dairy cow feces was added to each positive control broth tube, they were treated blindly as if they were real cull cow feces. Thus, each enrichment broth type was tested against 88 fresh cull cow fecal samples and 24 positive control experimental fecal samples, and all 112 (88 + 24) fecal samples were directly plated onto SMACct plates for comparisons. All tubes were tested blindly by the investigators.

RESULTS

Experimental protocols. Table 1 presents an overview of the protocols for culturing of feces in each of the six experiments.

Experiment 1. Direct plating during August and September of fresh cull cow feces onto 100-mm plain SMAC agar plates with no additives resulted in the finding of *E. coli* O157:H7 in 16 of 1,668 specimens (0.96% prevalence).

Experiment 2A. In experiment 2A feces from 116 animals were retested after having been frozen for 7 months; this subset of the original 1,668 fecal specimens included 10 that were previously positive for O157:H7 in experiment 1. This testing detected O157:H7 in nine fecal specimens, including eight previously positive by direct plating (which is an 80% recovery rate on retesting, having missed two specimens previously shown to be positive), plus one new positive specimen not detected when it was tested in experiment 1.

Experiment 2B. As in experiment 2A, experiment 2B used 176 cull cow fecal specimens that had previously been tested directly, except that the specimens the had been frozen for 8 months; they included 11 previously positive specimens from experiment 1 and 1 specimen that was positive in experiment 2A. This study detected 12 specimens positive for *E. coli* O157:H7 isolates, i.e., only 6 of the 11 previously positive fecal specimens from experiment 1 (which is a 58% recovery rate on retesting), the 1 positive fecal specimen included from experiment 2A, plus 5 new positive specimens not previously detected.

Experiment 3. Experiment 3 was of a simple design to evaluate NVSL's protocol by using mECnov. The results showed that *E. coli* O157:H7 (at various preenrichment concentrations ranging from 10^6 to 10^1 CFU/ml) in mECnov, when mixed with fresh cow feces in five replicates, was recovered even from the highest dilution of broth in five of five experiments; i.e., we could detect O157:H7 in broths whose preenrichment concentrations were as low as 10^2 CFU/ml.

Experiment 4. Experiment 4 was a field test of the Washington State protocol used in experiments 2A and 2B. Of 210 fresh fecal samples from cull dairy cows, 4 were positive, for a prevalence of 4 of 210 (1.90%).

Experiment 5. Experiment 5 was performed mainly to evaluate the potential of using GNHAJNAnov as another alternative to the more commonly used protocols with TSBcv and mECnov. Table 2 contains the results for this experiment. Known negative and experimentally contaminated positive fecal specimens were used. GNHAJNAnov detected *E. coli* O157:H7 in only a single specimen containing the intermediate

TABLE 2. Comparison of GNHAJNAnov enrichment broth versus TSBcv and mECnov for detection of *E. coli* O157:H7 in experimentally contaminated dairy cow feces

No. of specimens	Initial preenrichment level of O157:H7 (CFU/ml)	No. of positive specimens detected/no. tested with the following enrichment broth type:		
		TSBcv	mECnov	GNHAJNAnov
14	None (control)	0/14	0/14	0/14
3	1.5×10^2	1/3	0/3	0/3
2	1.5×10^3	1/2	1/2	1/2
1	1.5×10^4	1/1	1/1	0/1

TABLE 3. Comparison of TSBcv, mECnov, and GNHAJNAnov and direct plating for detection of *E. coli* O157:H7 in cull dairy cow feces and in experimentally contaminated adult dairy cow feces

No. (type) of specimens	Initial preenrichment level of O157:H7 (CFU/ml)	No. of positive specimens detected/no. tested with the following enrichment broth type:			
		DIR[a]	TSBcv	mECnov	GNHAJNAnov
88 (cull cow feces)	Unknown	1/88	2/88	2/88	1/88
8 (experimental adult cow feces)	1.5×10^4	0/8	8/8	6/8	7/8
8 (experimental adult cow feces)	1.5×10^3	0/8	8/8	8/8	8/8
8 (experimental adult cow feces)	1.5×10^2	0/8	4/8	6/8	3/8
Total (experimental controls)		0/24	20/24	20/24	18/24

[a] DIR, direct plating without enrichment broth onto plain SMAC agar.

preenrichment level of 10^3 CFU/ml. It is noteworthy that all broths except TSBcv failed to detect organisms in specimens with the lowest level of preenrichment contamination with O157:H7 (i.e., 10^2 CFU/ml); TSBcv detected the organism in only one of three specimens with the lowest level of contamination. Because this study used small numbers of specimens and was done as a prelude to further studies, no statistical evaluation of the results was performed.

Experiment 6. Experiment 6 was the final comparison of three protocols with primary enrichment broths versus direct plating of specimens (Table 3). Among the 88 new and fresh cull cow samples from the packing plant, 3 O157:H7 isolates were detected by using data from all three broth enrichment protocols (prevalence, 3 of 88 [3.4%]). It was noteworthy that all three enrichment broth protocols resulted in similar rates of recovery of *E. coli* O157:H7, even for specimens with the lowest levels of broth preenrichment contamination. Also, direct plating of feces resulted in poor overall recovery of *E. coli* O157:H7 but did manage to recovery O157:H7 from an actual cull cow fecal specimen.

Confirmatory testing of *E. coli* isolates. All of the original 16 cull cow isolates (experiment 1), in addition to the six new isolates from the retesting of frozen feces (experiments 2A and 2B), were tested by PCR according to the manufacturer's directions by using the BAX O157:H7 Pathogen Detecting System and were confirmed to be O157:H7.

DISCUSSION

With an organism such as *E. coli* O157:H7, which reportedly has a low prevalence of shedding from the bovine animal, one would expect to have difficulty designing studies of bacterial culture protocols that used only field specimens. Thus, experimentally contaminated feces were included in some of the present study protocols (experiments 3, 5, and 6). Another difficulty is specimen acquisition; i.e., the retrieval of specimens from packing plants is a complicated process that requires the coordinated efforts of an entire team of people working in concert and the tolerance of the packing plant staff whose pace of work is usually disrupted by researchers who are retrieving specimens.

Because of what was perceived as perhaps a low point prevalence (0.96%) of *E. coli* O157:H7 shedding for cull cows found by using only direct plating in experiment 1, we felt that it was necessary to look at other, perhaps more sensitive, culture methods to evaluate whether the prevalence of *E. coli* O157:H7 in the northeastern U.S. region was similar to those in other U.S. regions. Thus, in experiments 2A and 2B the published Washington State protocol (with TSBcv and SMACct) was used as a proven method for evaluation of a subset of frozen samples from experiment 1. The incorporation of broth enrichment step (TSBcv) and then plating of the enrichment broth onto selective or differential agar (SMACct) with a larger surface areas for the picking of colonies (i.e., 150 mm in diameter versus the direct method's 100 mm in diameter) resulted in the detection of 6 new specimens positive for O157:H7. Thus, while the original prevalence of *E. coli* O157:H7 was 16 of 1,668, or 0.96%, the new prevalence was 16 original direct test-positive specimens plus 6 new positive specimens, which is equal to 22 of 1,668 specimens, or a 1.3% prevalence. For the clinical microbiologist, we were also concerned about the effects of the freezing of specimens on recovery of bacteria; the 80% recovery and the 58% recovery on retesting of frozen samples previously positive for O157:H7 confirmed our fears of the loss of viability of *E. coli* from freezing. As a result, when possible, only fresh specimens should be used to search for O157:H7 by culture. It is possible that had broth enrichment been used for the original 1,668 fresh fecal specimens, our results may have shown a point prevalence higher than 1.3% for New York cull dairy cattle.

E. coli O157:H7 has been found to be widespread in the United States and Canada, but with a consistently low prevalence of shedding in the feces of adult cows (5). The new overall prevalence of 1.3% found after the retesting done in experiments 2A and 2B was similar to that found in other studies with single sampling designs. Prevalences in surveys have ranged from 0.28% in 3,570 dairy cattle in the northwestern United States (12), to 1.6% of pen-floor samples which represented samples from 11,881 feedlot cattle in 100 feedlots and 13 states as part of the NAHMS Cattle on Feed Evaluation study (2), to a high of 4% of 2,103 cattle at an abattoir in South Yorkshire, England (7). In the most recent U.S. survey, NAHMS Dairy '96, the prevalence of *E. coli* O157:H7 shedding in feces from cows on farms in 21 major dairy states was 0.9% among 3,600 dairy cows on 91 farms, 2.8% among 600 cows to be culled in 7 days, and 1.8% among 2,200 culled dairy cows at 97 markets (4). Thus, our results were within the realm of what has been reported in other U.S. studies and support, on a single-sample basis, the claim that the prevalence of fecal shedding of *E. coli* O157:H7 in cows entering the slaughter process is relatively low by current detection methods. The laboratories in the U.S. studies mentioned above (2, 4, 12) used selective broth enrichment followed by subculture onto selective differential agar; and the European studies (7) used direct plating onto selective differential agar without prior enrichment in selective broth.

Experiment 3 was a brief preliminary study to learn more about NVSL's protocol (with mECnov and SMACct) which was used in the NAHMS studies throughout the United States. This protocol resulted in good analytical sensitivity even at the low end of our sampling protocol, i.e., with organism concen-

trations of 100 CFU/ml. This was corroborated in experiment 6 when the NVSL method performed as well as the Washington State protocol.

The 1.9% prevalence of shedding detected in experiment 4 was indeed higher than the 0.96% prevalence among the directly plated samples in experiment 1 and higher than the revised prevalence of 1.3% after the inclusion of data from the retesting of broth-enriched samples in experiments 2A and 2B.

Experiment 5 was the other preliminary study that used GNHAJNAnov as another alternative to the more commonly used TSBcv and mECnov. In our laboratory GNHAJNA is a commonly used enrichment broth for enteric specimens; it was a likely broth for adaptation to O157:H7 enrichment with the addition of novobiocin, as had been done with mECnov. While the results for experiment 5 would not convince a clinical microbiologist to use GNHAJNAnov for O157:H7 culture, this was probably because of the small number of specimens used in that experiment. In contrast, the results of a larger-scale experiment (experiment 6) showed that this enrichment broth shows promise for use in clinical laboratories attempting to isolate *E. coli* O157:H7 bacteria.

Experiment 6 showed that the three different broth enrichments, when used in the same subculturing protocol, were all essentially as effective as each other for the recovery of *E. coli* O157:H7 bacteria. It is noteworthy that the protocols for direct plating were different for experiment 1, which used plain SMAC agar, than for experiment 6, which used SMACct; despite this change, there was no great improvement in experiment 6 for the recovery of *E. coli* O157:H7 by direct plating; perhaps this is indirect evidence, too, that broth enrichment is the more important step for an improved rate of recovery of O157:H7. In our experience we felt that sorbitol-negative colonies were more easily observed on SMACct than on plain SMAC agar.

In summary, this project has established that for the optimal recovery of *E. coli* O157:H7 from feces by culture, it is preferable to use a broth enrichment step rather than direct plating of specimens; most broth enrichment protocols suggest the plating out of at least two dilutions of the broth onto large (150-mm) SMACct plates and the reading of those plates after 24 and 48 h of incubation. Undoubtedly, there will continue to be improvements in the laboratory's ability to sensitively and rapidly detect *E. coli* O157:H7 in clinical specimens. However, we must also be concerned about the non-O157:H7 VTEC bacteria that may be within patient samples and must also be aware that sorbitol-fermenting *E. coli* O157:H7 strains are being found (15). Sensitive methods for the detection of Shiga-like toxins by immunoassay and by PCR techniques will become increasingly important tools for the clinical microbiologist.

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