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 cellexime 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 cellexime 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 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 × 10³ 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 agar (8, 17), or feces may be selectively enriched in broth followed by plating onto selective, differential agar (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...
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, Cockeyville, Md.) at 37°C were used to produce a standard inoculum in sterile normal saline solution to produce the inoculum for the tests. 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 (REM E. coli O157:H7 Latex Test Kit; Remel, Lenexa, Kans.) to ascertain their O157:H7 status. If the REM E. coli O157:H7 latex test was positive, the suspect bacterial colony was subcultured onto an 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 REM 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, AccucMed 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 then serially passaged in TSB at 37°C for 24 h, at which time sorbitol-negative colonies were picked for identification (see the protocol described above under Identification of E. coli O157:H7 colonies); the SMAC plates were reincubated for 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). 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 TSB (containing 50 mg/ml of ceftaxime, 150 mg/ml of cefixime, 25 μg/ml of polymyxin B, 150 mg/ml of polymyxin E, and 1 mg/ml of vancomycin) and was evenly spread. The SMAC plates were incubated for 18 to 24 h at 37°C. If the O157:H7 colonies were identified after thorough mixing, the broth was reincubated for an additional 24 h, at which time additional colonies were picked, if present, for screening as described above under Identification of E. coli O157:H7 colonies.

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.

Experiment 3. Experiment 3 was done to evaluate a second, different broth enrichment called mECnov (DiFCO Laboratories) by using the protocol from NAHMS, 1996 (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 ceftaxime and polymyxin B sulfate (SmAC) followed by staining 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 (HAIJNA formulation) with novobiocin (GNHAINAnov) for their relative abilities to detect E. coli O157:H7 in the feces of culled 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 culled 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.

Experiments. Experiments 1, 2A, 2B, 4, and 6 used fecal samples from culled 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 to test adult dairy cattle.

(i) Experiment 1. Experiment 1 was used to determine the 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). One gram of feces was added to 9 ml of TSB (containing 50 mg/ml of ceftaxime, 150 mg/ml of cefixime, 25 μg/ml of polymyxin B, 150 mg/ml of polymyxin E, and 1 mg/ml of vancomycin) and was evenly spread. The SMAC plates were incubated for 18 to 24 h at 37°C. If the O157:H7 colonies were identified after thorough mixing, the broth was reincubated for an additional 24 h, at which time additional colonies were picked, if present, for screening as described above under Identification of E. coli O157:H7 colonies.

(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 E. coli 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 TSB (containing 50 mg/ml of ceftaxime, 150 mg/ml of cefixime, 25 μg/ml of polymyxin B, 150 mg/ml of polymyxin E, and 1 mg/ml of vancomycin) and was evenly spread. The SMAC plates were incubated for 18 to 24 h at 37°C. If the O157:H7 colonies were identified after thorough mixing, the broth was reincubated for an additional 24 h, at which time additional colonies were picked, if present, for screening as described 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 NAHMS, 1996 (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 following enrichment broths were used: the new broth, broth for gram-negative bacteria in frozen bovine feces (DiFCO NAHMS), had successfully used selective enrichment in mod-
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^−3 and the 10^−4 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.

(vi) 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 the 10−3 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 tested after having been frozen for 7 months; this sub-set 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 that 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 36% 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 10^0 to 10^3 CFU/ml) in mECnov, when mixed with fresh cull 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 broth 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

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**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**

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>No. of cull dairy cows in field study</th>
<th>Experimentally contaminated dairy cow feces used (no. positive/no. negative)</th>
<th>Type of treatment used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,668 (fresh)</td>
<td>No (—/—)</td>
<td>DIR°</td>
</tr>
<tr>
<td>2A</td>
<td>116 (frozen)</td>
<td>No (—/—)</td>
<td>TSBcv</td>
</tr>
<tr>
<td>2B</td>
<td>176 (frozen)</td>
<td>Yes (5/—)</td>
<td>mECnov</td>
</tr>
<tr>
<td>3</td>
<td>210 (fresh)</td>
<td>Yes (6/14)</td>
<td>GNHAJNAnov</td>
</tr>
<tr>
<td>4</td>
<td>88 (fresh)</td>
<td>Yes (24/—)</td>
<td></td>
</tr>
</tbody>
</table>

° DIR, direct plating without enrichment broth onto plain SMAC agar.

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

<table>
<thead>
<tr>
<th>No. of specimens</th>
<th>Initial preenrichment level of O157:H7 (CFU/ml)</th>
<th>No. of positive specimens detected/no. tested with the following enrichment broth type:</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>None (control)</td>
<td>0/14</td>
</tr>
<tr>
<td>3</td>
<td>1.5 × 10^2</td>
<td>1/3</td>
</tr>
<tr>
<td>2</td>
<td>1.5 × 10^3</td>
<td>1/2</td>
</tr>
<tr>
<td>1</td>
<td>1.5 × 10^4</td>
<td>1/1</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>No. (type) of specimens</th>
<th>Initial preenrichment level of O157:H7 (CFU/ml)</th>
<th>No. of positive specimens detected/no. tested with the following enrichment broth type:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DIR*</td>
</tr>
<tr>
<td>88 (cull cow feces)</td>
<td>Unknown</td>
<td>1/88</td>
</tr>
<tr>
<td>8 (experimental adult cow feces)</td>
<td>$1.5 \times 10^4$</td>
<td>0/8</td>
</tr>
<tr>
<td>8 (experimental adult cow feces)</td>
<td>$1.5 \times 10^3$</td>
<td>0/8</td>
</tr>
<tr>
<td>8 (experimental adult cow feces)</td>
<td>$1.5 \times 10^2$</td>
<td>0/8</td>
</tr>
<tr>
<td>Total (experimental controls)</td>
<td></td>
<td>0/24</td>
</tr>
</tbody>
</table>

* DIR, direct plating without enrichment broth onto plain SMAC agar.

The 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. Prevalence 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-
tations of 100 CFU/ml. This was corroborated in experiment 4 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 GNHAJANov as another alternative to the more commonly used TSBNev and mEConv. 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 mEConv. While the results for experiment 5 would not convince a clinical microbiologist to use GNHAJANov 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 SMACeCt; 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 SMACeCt 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) SMACeCt 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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