Testing of Bob Calf Fecal Swabs for the Presence of *Escherichia coli* O157:H7

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**ABSTRACT**

Rectal swabs were collected from 304 Bob calves (calves under 10 d old) brought to slaughter in the states of Washington (77 swabs), California (127 swabs), and Wisconsin (100 swabs). The swab samples were tested for the presence of *Escherichia coli* O157:H7 by the use of a direct smear method, enrichment method, and the use of the Petrifilm™ Test Kit-HEC-for hemorrhagic *E. coli* O157:H7 (3M Company, St. Paul, MN). The organism was not isolated from any of the samples by any method, though the 3M test kit did give 21 positive signals. Of these positive signals, three were shown to be caused by sorbitol-positive, O157-positive, H7-negative *E. coli*. The cause of the other 18 signals was not determined.

In recent years *Escherichia coli* O157:H7 has emerged as an important enteric pathogen, epidemiologically associated with the consumption of ground beef and raw milk 

Collection of fecal swabs

Samples were collected by U.S. Department of Agriculture, Food Safety and Inspection Service meat inspectors from live animals brought to slaughter. Rectal swabs were collected from Bob calves, placed in Stuart’s transport medium (Difco), and shipped at ambient temperature. Swabs were analyzed within 3 to 5 d from the time of collection. Upon arrival at the laboratory, the swabs were analyzed either immediately or held at 4°C no longer than 48 h before analysis. The samples were collected from California (127 samples), Washington (77 samples), and Wisconsin (100 samples). It was noted that the swabs were very heavily coated with feces.

**MATERIALS AND METHODS**

Collection of fecal swabs

Samples were collected by U.S. Department of Agriculture, Food Safety and Inspection Service meat inspectors from live animals brought to slaughter. Rectal swabs were collected from Bob calves, placed in Stuart’s transport medium (Difco), and shipped at ambient temperature. Swabs were analyzed within 3 to 5 d from the time of collection. Upon arrival at the laboratory, the swabs were analyzed either immediately or held at 4°C no longer than 48 h before analysis. The samples were collected from California (127 samples), Washington (77 samples), and Wisconsin (100 samples). It was noted that the swabs were very heavily coated with feces.

**Isolation techniques**

**Direct streak method.** Sample swabs were used to make dime-sized smears of sample on plates of MacConkey sorbitol agar containing 0.1 g/L of 5-bromo-4-chloro-3-indoxyl-β-D-glucuronic acid cyclohexylammonium salt (MSA-BCIG; 8). A sterile transfer loop was used to streak the sample for isolation. The plates were incubated at 42°C for 24 h. White or translucent colonies (sorbitol negative, β-glucuronidase negative) were picked to a gridded set of plates containing eosin methylene blue (EMB, Difco) plus additional agar for a final concentration of 3% (to prevent *Proteus* swarming) and phenol red sorbitol agar containing 4-methylumbelliferyl-β-D-glucuronide (PRS-MUG; 7). The plates were incubated at 35°C overnight. Those cultures that were typical *E. coli* on EMB, and sorbitol negative and MUG negative on PRS-MUG, were tested directly for the O157 antigen using a latex agglutination test (Oxoid; 4).

**ELISA method.** After the swabs were used for the direct streak method, they were placed in 10 ml of modified EC broth containing 20 mg/L novobiocin (mEC+n; 7) and mixed on a vortex mixer for 1 min. The turbidity in the mEC+n broth was examined visually and appropriate dilutions were made, ranging from 0 to 10⁶ in 9-ml dilution blanks of Butterfield’s phosphate diluent (14). Petrifilm™ Test Kit-HEC-for hemorrhagic *Escherichia*
coli 0157:H7 (3M Company, St. Paul, MN; 9) plates were inoculated with 1 ml of each of the two highest dilutions and the manufacturer's instructions were followed for detection and isolation of the organism. The procedure involved the inoculation of Petrifilm™ E. coli count plates which were incubated at 42°C for 18 h. Small colonies that formed on the guar were tested for the presence of the O157 antigen by a blot ELISA. The colonies producing a positive signal were removed from the guar using the BBL Prompt™ Inoculation System and spread plated on MSA-BCIG plates for isolation. Isolates from this method were tested for the O157 antigen using the latex agglutination test and presumptive positive isolates were inoculated into biochemical tube media and then tested for H7 agglutination. E. coli O157+, H7-isolates were serotyped by the E. coli Reference Center, Pennsylvania State University.

Enrichment method. After dilutions were made from the mEC+n broth containing the swab, the broth was inoculated statically at 35°C. This culture was streaked for isolation on MSA-BCIG at 6 and 24 h. Due to heavy competitive microflora, the 6-h incubation period was included about halfway through the study to increase the probability of isolating E. coli O157:H7. A maximum of 12 typical colonies (white or translucent) were picked to EMB and PRS-MUG plates, and isolation proceeded as performed in the direct smear method.

Preparation of fecal samples inoculated with E. coli O157:H7

A culture of E. coli O157:H7 was inoculated onto a plate count agar slant (Difco) and incubated overnight at 35°C. The cells were removed from the slant using a sterile transfer loop and suspended in 0.85% sterile saline solution to produce a final concentration of 50% T (transmittance) at 520 nm on a Bausch and Lomb Spectronic 21. This routinely produced approximately 7.0 × 10^8 organisms per ml. This suspension was diluted to 10^-5 in Butterfield's phosphate diluent to produce a suspension of approximately 7 × 10^7/ml. A sterile cotton tipped swab was saturated with the diluted cell suspension and placed into a tube containing 10 ml of mEC+n broth and a fecal swab. The sample was agitated on a vortex mixer for 1 min, and the direct smear, ELISA, and enrichment procedures, as previously described, were followed.

RESULTS AND DISCUSSION

To determine the ability of the three isolation methods (direct, ELISA, and enriched) to isolate the target organ-

ism, 5 fecal swabs were inoculated with E. coli O157:H7, and the three procedures were followed. Of the five trials, the target organism was isolated in 3 samples by the ELISA only, in 1 sample by all three methods, and was undetected by any method in 1 sample (Table 1). The 1 sample in which the target organism was isolated with all three methods had few competing organisms.

It appears that the difficulty experienced isolating from the direct smear and enrichment methods was due to the very heavy background microflora of the fecal swabs. The blot ELISA method was better at isolation due to its ability to detect one positive colony on a crowded Petrifilm plate. Furthermore, the recommended method for isolating the organism from the Petrifilm, specifies using spread plates rather than streak plates. Spread plates produce more isolated colonies than are usually obtained by streaking with a loop.

The value of using BCIG in the MSA agar was examined. E. coli O157:H7 is sorbitol negative and β-glucuronidase negative. Therefore, sorbitol in the MSA is not fermented, and there is no β-glucuronidase to turn the colonies blue; consequently, the colonies should remain white. Of the swabs showing typical colonies on MSA-BCIG, only 14.8% were found to be EMB positive, sorbitol negative, and β-glucuronidase negative (Table 2). It was noted that many of the colonies were picked under crowded condi-

### TABLE 1. Comparison of methods for recovering E. coli O157:H7 from inoculated rectal swabs.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Direct swab</th>
<th>mEC+n enrichment at 24 h</th>
<th>3M Blot ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Isolated.
- = Not isolated.

### TABLE 2. Number of Bob calf rectal swabs producing typical colonies.

<table>
<thead>
<tr>
<th>Location</th>
<th>Rectal swabs</th>
<th>Inc. Time</th>
<th>Direct smear and mEC+n enrichment</th>
<th>Swabs with typical &amp; atypical colonies on MSA-BCIG</th>
<th>3M Blot ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Typical</td>
<td>Atypical</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive swabs</td>
<td>O157-</td>
<td>O157+ H7</td>
</tr>
<tr>
<td>WA</td>
<td>77</td>
<td>Direct</td>
<td>40 (52)</td>
<td>5 (6)</td>
<td>7 (9)     5 2</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>6</td>
<td>15 (41)</td>
<td>4 (11)</td>
<td>11 (30)   39 (51)</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>24</td>
<td>47 (61)</td>
<td>8 (10)</td>
<td>39 (31)   17 (14)</td>
</tr>
<tr>
<td>CA</td>
<td>127</td>
<td>Direct</td>
<td>25 (20)</td>
<td>8 (6)</td>
<td>10 (15)   9 (7) 8 1</td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>6</td>
<td>11 (16)</td>
<td>1 (1)</td>
<td>10 (15)   9 (7) 8 1</td>
</tr>
<tr>
<td></td>
<td>127</td>
<td>24</td>
<td>35 (28)</td>
<td>7 (6)</td>
<td>28 (22)   5 (5) 5 0</td>
</tr>
<tr>
<td>WI</td>
<td>100</td>
<td>Direct</td>
<td>34 (34)</td>
<td>1 (1)</td>
<td>33 (33)   5 (5) 5 0</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>6</td>
<td>15 (21)</td>
<td>2 (3)</td>
<td>13 (18)   5 (5) 5 0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>24</td>
<td>28 (28)</td>
<td>1 (1)</td>
<td>27 (27)   5 (5) 5 0</td>
</tr>
</tbody>
</table>

a The 6-h incubation period was included later in the study.

b Typical colonies were white or translucent; ( ) = % of all swabs from specified state.
c Typical colonies were EMB+, PRS-, MUG- (14.8% of swabs from MSA-BCIG showed typical colonies); all typical colonies were O157-.
d Serotype O157+, H7- isolates tested sorbitol positive.
tions. It is probable that this crowding prevented color development, particularly the red color indicating fermentation of the sorbitol.

We tested 304 fecal swabs from Bob calves using all three methods (direct, enriched, ELISA) without finding any positive O157:H7 samples. Where the blot ELISA indicated the presence of the O157 antigen, sorbitol-positive colonies were isolated as well as sorbitol-negative colonies, and these were also tested for the O157 antigen (9). Three of the 21 samples that gave positive ELISA signals were found to contain sorbitol-positive, O157-positive, H7-negative E. coli. The cause of the signal observed in the remaining 18 samples was not determined (Table 2).

It appears from these results that an ELISA, or other noncultural method, has the best chance of detecting O157:H7 in a sample as heavily contaminated as these fecal swabs. The lack of even one positive isolation from 304 Bob calves does not disprove the idea that these young calves are likely to harbor the organism; it only reinforces the observation that it is not very common and is difficult to isolate if it is present.

ACKNOWLEDGMENTS

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REFERENCE