A Research Note

Low Specificity of the HEC O157™ ELISA in Screening Ground Beef for Escherichia coli O157:H7

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ABSTRACT

During January-August, 1991, 74 retail ground beef samples were tested for the presence of Escherichia coli O157:H7 using the commercial HEC O157™ ELISA kit. A total of 17 samples (23%) were presumptively positive for E. coli O157:H7. Twenty-nine isolates were taken from colonies corresponding to positive spots on the immunoreactive discs and 13 from colonies on sorbitol MacConkey agar No. 3 plates. Of these isolates, 30 were positive when retested as pure cultures with the ELISA (enzyme-linked immunoabsorbent assay). Only 15 of these 30 isolates were E. coli, 6 were identified as Hafnia alvei, and 9 were not identifiable. None of the E. coli isolates were serotype O157:H7. These results confirm that: a) the HEC O157™ ELISA method for detecting E. coli O157:H7 has low specificity, and b) use of the HEC O157™ ELISA necessitates thorough confirmatory testing.

Outbreaks of foodborne illness have been associated with the presence of Escherichia coli O157:H7 in undercooked meats (1,10), and in many cases the popular press has referred to hemolytic uremic syndrome caused by E. coli O157:H7 as "hamburger disease." As a result, the meat industry and food regulators in Canada are concerned about the frequency of contamination of ground meat by E. coli O157:H7 and other verocytotoxigenic strains of E. coli.

The advantages and disadvantages of several E. coli O157:H7 detection methods were recently reviewed (1). The HEC O157™ ELISA method (3M Canada Inc., London, Ontario) is designed to serve as a rapid negative screen for E. coli O157:H7. A protocol is included in the kit for use in analyzing raw ground beef. When positive test results are obtained using the kit, it is recommended that confirmatory biochemical and serological tests be done (4). According to Doyle (1), the use of the O157 polyclonal antibody in this kit may lead to cross-species positive reactions that may be regarded as false presumptive positive results. The objective of our study was to determine the frequency of false presumptive positive results when the HEC O157™ ELISA kit was used to test retail ground beef samples in Saskatoon, Saskatchewan, Canada, for E. coli O157:H7.

MATERIALS AND METHODS

Samples

A total of 74 samples were purchased from eight Saskatoon supermarkets during January-August 1991. Samples were transported to the laboratory within 30 min and held at 4°C for no more than 24 h prior to analysis.

Sample preparation and analyses

In order to estimate the microbiological quality of the hamburger samples, aerobic bacteria and coliforms were enumerated in an 11.0-g sample aseptically removed from a randomly selected location in the ground beef. This sample was homogenized for 120 s in 99 ml of 0.1% (wt/vol) peptone (Difco Laboratories, Detroit, MI) diluent using a LabBlender 400 Stomacher (A.J. Seward, Bury St. Edmunds, United Kingdom). Serial dilutions were made using the same diluent and plated in duplicate on Petrifilm™ Aerobic Count Petrifilms™ and Petrifilm™ Coliform Count Petrifilms™ (3M Canada Inc., Detroit, MI) diluent using a LabBlender 400 Stomacher. The films were incubated at 35°C for 48 ± 2 h. Colonies on the Petrifilms™ were counted according to the manufacturer’s instructions. Because transformed microbiological data approximate a normal distribution (3), log10 CFU/g was calculated for each sample.

For the HEC O157™ ELISA method, a total of 25 g of ground beef was aseptically removed from five sites in the meat mass. For ground beef patties or ground beef packaged on a styrofoam tray, the five sampling sites were the center and four sites along the perimeter of the meat. When the sample was ground beef packaged in a cylindrical shape, the five sites were randomly selected from throughout the meat. The sample was then mixed for 120 s with 225 ml of modified EC broth (4) using a LabBlender 400 Stomacher. This enrichment homogenate was then incubated at 36°C for 6 h on a LabLine Model 3545 Orbital Shaker Bath (Melrose Park, IL) with shaker speed of 100 revolutions per min. Following enrichment, 1:10 and 1:100 dilutions of the enrichment broth in Butterfield’s phosphate diluent (9) were plated on Petrifilm™ E. coli Count films (3M Canada Inc., London, Ontario), and the films were incubated at 42°C for 18 h. After incubation, all E. coli Count Petrifilms™ were analyzed....

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Identification of presumptively positive isolates

For the first seven presumptively positive samples, colonies corresponding to the dark grey/black spots on the immunoreactive discs were streaked on nutrient agar (Difco) plates. Following 48 h incubation at 35°C, individual colonies were transferred to Sorbitol MacConkey agar No. 3 (Oxoid) and incubated 48 h at 35°C to test for sorbitol fermentation. The same colonies were retested using the HEC O157™ ELISA to confirm the positive ELISA result. The API 20E test strip system (API Laboratory Products Ltd., St. Laurent, Quebec) was used to determine colony identity. Colonies that were identified as Escherichia coli were subsequently tested for reactivity with E. coli O157 and H7 antisera (Difco) using the tube agglutination method suggested by the manufacturer.

In an attempt to simplify and improve recovery of presumptively positive organisms, a different method was used for the other 10 presumptively positive samples. The original modified EC enrichment broth was reincubated at 36°C (after the inoculation of the Petrifilm™ E. coli count films) without shaking for 16-18 h while the remainder of the ELISA test was conducted. Serial dilutions in Butteryfield’s phosphate diluent were then spread plated on Sorbitol MacConkey agar No. 3 and incubated at 42°C for 18-22 h. Colonies negative for sorbitol fermentation were selected for retesting with the HEC O157™ ELISA and the API 20 E test strip systems. Colonies identified as E. coli were serologically tested as described above.

Statistical analysis

A statistical analysis was done to determine if samples having presumptively positive HEC O157™ ELISA result contained larger numbers of aerobic bacteria and coliforms. The means of log₁₀ CFU/g for aerobic bacteria and coliforms were compared for samples having presumptive positive HEC O157™ ELISA results and those with negative results. The t-test for samples of unequal sizes (8) with a significance level of 5% was used.

RESULTS AND DISCUSSION

Microbiological quality of samples

The 74 samples of retail ground beef had a mean aerobic bacterial count of log₁₀ 5.7 CFU/g (standard deviation = 0.9) and a mean coliform count of log₁₀ 2.9 CFU/g (standard deviation = 0.8). The mean log₁₀ CFU/g values for aerobic bacteria and coliforms were compared on samples testing presumptively positive in the HEC O157 ELISA (6.1 and 2.9, respectively) and samples testing negative (5.6 and 2.9, respectively) were not significantly different at the 5% level.

Seventeen of the 74 samples tested presumptively positive in the HEC O157™ ELISA. Twenty-nine isolates from the Petrifilm™ E. coli films and 13 from Sorbitol MacConkey agar No. 3 were retested with the HEC O157™ ELISA. Of the 29 isolates from the Petrifilms™, 24 were positive on retesting. Only 6 of the 13 isolates from the Sorbitol MacConkey agar No. 3 were positive upon retesting.

The 30 isolates that were positive upon retesting with the ELISA were identified as shown in Table 1. Fifteen of the isolates were identified as E. coli, but none were serotype O157:H7. Six isolates were identified as Hafnia alvei, and nine were not identifiable.

### TABLE 1. Identification of isolates with false presumptive results in the HEC O157™ ELISA for E. coli O157:H7 in retail ground beef sold in Saskatoon, Saskatchewan, Canada, in January-August, 1991.

<table>
<thead>
<tr>
<th>Source of isolate</th>
<th>ECP*</th>
<th>SMac*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-O157:H7 E. coli</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Hafnia alvei</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Unidentified</td>
<td>5</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>6</td>
<td>30</td>
</tr>
</tbody>
</table>

*The source of the isolates was either Petrifilm™ E. coli Count films (ECP) used in the HEC O157™ ELISA for E. coli O157:H7, or Sorbitol MacConkey agar Number 3 (SMac) plates inoculated with the preenrichment broth used in the ELISA.

Although the sample size in this study was small, the high percentage (23%) of samples having false-positive results is of concern. As stated by Doyle (1), the polyclonal O157 antibody used in the HEC O157™ ELISA lacks sufficient specificity. A recent paper by Padhye and Doyle (5) describes an improved ELISA method for the detection of E. coli O157:H7 in food. This method uses a polyclonal antibody specific for E. coli O157 antigen as the capture antibody and a monoclonal antibody specific for E. coli serotypes O157:H7 and 026:H11 as the detection antibody.

Our failure to detect E. coli O157:H7 in any of the 74 samples is not inconsistent with other published results. A survey in Manitoba, Canada, found that only 2.4% of retail ground beef samples contained type O157 verocytotoxigenic E. coli (7). Doyle and Shoeni (2) found E. coli O157:H7 in 3.7% of retail ground beef samples from Wisconsin and Alberta. Read et al. (6) found that 10.4% of southwestern Ontario beef samples contained vero-cytotoxigenic E. coli, but none of the verocytotoxigenic isolates were serotype O157:H7.

We conclude from this study that: a) the HEC O157™ ELISA has low specificity for E. coli O157:H7, b) thorough confirmatory testing of presumptively positive isolates obtained using this method is very important, and c) retail ground beef in Saskatoon, Saskatchewan, Canada, is infrequently contaminated with E. coli O157:H7.

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REFERENCES

LOW SPECIFICITY OF E. COLI O157:H7 ELISA


