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Isolation of *Escherichia coli* O157:H7 from Retail Fresh Meats and Poultry

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A total of 896 samples of retail fresh meats and poultry was assayed for *Escherichia coli* serogroup O157:H7 by a hydrophobic grid membrane filter-immunoblot procedure developed specifically to isolate the organism from foods. The procedure involves several steps, including selective enrichment, filtration of enrichment culture through hydrophobic grid membrane filters, incubation of each filter on nitrocellulose paper on selective agar, preparation of an immunoblot (by using antiserum to *E. coli* O157:H7 culture filtrate) of each nitrocellulose paper, selection from the filters of colonies which corresponded to immunopositive sites on blots, screening of isolates by a Biken test for precipitin lines from metabolites and antiserum to *E. coli* O157:H7 culture filtrate, and confirmation of isolates as Vero cell cytotoxic *E. coli* O157:H7 by biochemical, serological, and Vero cell cytotoxicity tests. *E. coli* O157:H7 was isolated from 6 (3.7%) of 164 beef, 4 (1.5%) of 264 pork, 4 (1.5%) of 263 poultry, and 4 (2.0%) of 205 lamb samples. One of 14 pork samples and 5 of 17 beef samples contaminated with the organism were from Calgary, Alberta, Canada, grocery stores, whereas all other contaminated samples were from Madison, Wis., retail outlets. This is the first report of the isolation of *E. coli* O157:H7 from food other than ground beef, and results indicate that the organism is not a rare contaminant of fresh meats and poultry.

Several outbreaks and cases of hemorrhagic colitis and hemolytic uremic syndrome caused by *Escherichia coli* serogroup O157:H7 have been linked epidemiologically to consumption of ground beef (4, 6–9); however, the organism reportedly has been isolated only once from food. This isolation was made from a frozen ground-beef patty obtained from a meat processor that supplied beef patties to restaurants associated with an outbreak of *E. coli* O157:H7 hemorrhagic colitis (10). Dairy cattle recently have been identified as a reservoir of *E. coli* O157:H7, with the organism isolated on two occasions from the feces of young animals of herds associated with cases of hemolytic uremic syndrome in children following consumption of raw milk (2, 5). Studies by Beery et al. (1) revealed that *E. coli* O157:H7 can readily colonize the ceca of chickens and be excreted in the feces for several months, suggesting that chickens may also be a reservoir of the organism.

The purposes of this study were to develop a procedure that could specifically isolate *E. coli* O157:H7 from foods and to use this procedure to determine the prevalence of the organism in retail meats and poultry.

MATERIALS AND METHODS

Meat and poultry samples. A total of 147 ground-beef, 250 pork, 257 poultry, and 200 lamb retail samples was obtained from the refrigerated-meats sections of several Madison-area grocery stores from June 1985 to July 1986. All meat and poultry samples were fresh, uncooked, packaged, and on display for consumer selection. Usually, 20 to 25 samples were purchased on each day of testing. The samples were immediately (within 1 h) brought to the laboratory and assayed for *E. coli* O157:H7.

Additionally, a total of 17 ground-beef, 14 pork, 6 poultry, and 5 lamb retail samples was obtained from several grocery stores in the Calgary, Alberta, Canada, area during June 1985. These samples were frozen (−29°C) after purchase and sent on dry ice to the Food Research Institute. Once received, samples were held at −70°C and tested for *E. coli* O157:H7 within 3 weeks of receipt.

Enrichment. A 25-g portion of food specimen was aseptically removed from each meat or poultry sample and added to 225 ml of modified Trypticase soy broth (mTSB) in a 1-liter Erlenmeyer flask. The mTSB enrichment medium was composed of (per liter) Trypticase soy broth (TSB; 30 g; BBL Microbiology Systems, Cockeysville, Md.), bile salts 3 (1.5 g; Difco Laboratories, Detroit, Mich.), dipotassium phosphate (1.5 g), and novobiocin (20 mg; Sigma Chemical Co., St. Louis, Mo.). The enrichment medium without novobiocin was autoclaved and allowed to cool to room temperature before filter-sterilized novobiocin was added. The pH of the mTSB was 7.4. Food specimens were added to the mTSB soon after the addition of novobiocin. The enrichment cultures were then incubated with agitation (100 gyrations per min) at 37°C for 18 to 24 h.

Filtration. After incubation, the enrichment culture was diluted (for raw meats, usually 10^{-6} and 10^{-7}) in 0.01 M phosphate-buffered saline, pH 7.5, and 1 ml of each dilution in 10 ml of PBS was filtered individually through hydrophobic grid membrane filters (HGMF; ISO-GRID; QA Laboratories, Toronto, Ontario, Canada). The appropriate dilutions of enrichment culture were determined on the basis of colony development on HGMF, with 10 to 500 colonies per HGMF being an acceptable range. Each HGMF was placed over an equivalent-size (6- by 6-cm) wetted (sterile mTSB) nitrocellulose paper (0.45-μm pore size; Schleicher & Schuell, Keene, N.H.) on the surface of a plate of modified Trypticase soy agar (mTSA). mTSA is composed of the same ingredients as mTSB plus 1.5% agar. The plates were incubated at 37°C for 18 to 24 h.

Immunoblots. After incubation, the HGMF was removed from the nitrocellulose paper, placed on a fresh plate of mTSA, and refrigerated for later use. The nitrocellulose paper was then treated by using an immunoblot procedure to

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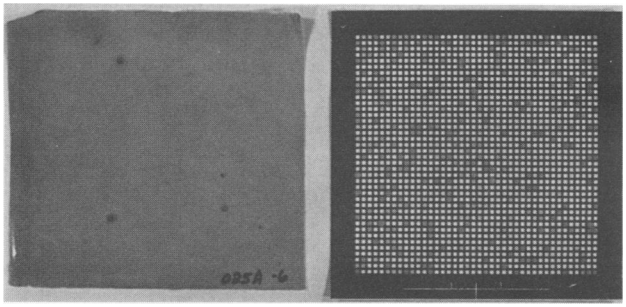

FIG. 1. HGMF (right) with bacterial colonies from a 10^{-6} dilution of enrichment culture of ground-beef specimen (25 g) inoculated with *E. coli* O157:H7 (1.5 CFU/g). Immunoblot (left) of nitrocellulose paper held in contact with HGMF during incubation on mTSA. Dark spots indicate areas that reacted with antiserum to O157CF.

detect Vero cell cytotoxin(s) produced by bacterial colonies developing on the HGMF. To inactivate any free microbial peroxidase, the nitrocellulose paper was treated with 0.02 M periodic acid (37°C, 15 min), washed twice in salts-PBS (S-PBS) solution (composed of 121 g of NaCl, 15.5 g of KCl, 12.7 g of $MgCl_2$, 10.2 g of $CaCl_2 \cdot 2H_2O$, 2.0 g of $NaH_2PO_4 \cdot H_2O$, and 3.9 g of $Na_2HPO_4 \cdot 7H_2O$ per liter, adjusted to pH 7.4), treated with 0.012 M sodium borohydride (30 min), and again washed three times in S-PBS. The nitrocellulose paper was treated with 1% bovine serum albumin (BSA) in S-PBS for 1 h with agitation (50 gyrations per min) at 37°C and suspended in rabbit antiserum to *E. coli* O157:H7 culture filtrate (O157CF; 1:5,000 dilution) in 1% BSA for 1 h with agitation at 37°C. The rabbit antiserum was prepared by intravenously inoculating (ear vein) New Zealand White rabbits with formalinized culture filtrate (treated with 0.5% Formalin for 2 weeks at 37°C) of *E. coli* 932 serogroup O157:H7 grown in TSB at 37°C for 18 h. A total of five inoculations was given (0.5, 1.0, 2.0, 4.0, and 4.0 ml), one each at 5-day intervals. The antiserum was heated at 56°C for 30 min and then was absorbed six times with ca. 10^{12} cells of heat-treated (121°C, 1 h) *E. coli* O157:H7 (1 ml of packed cells per 10 ml of antiserum) before use.

The nitrocellulose paper was washed twice in 1% BSA in S-PBS and then held for 10 min at 37°C in fresh 1% BSA in S-PBS. This procedure was repeated three times. The nitrocellulose paper was suspended in 1:1,000 goat anti-rabbit immunoglobulin G conjugated with horseradish peroxidase (ICN Immunobiologicals, Lisle, Ill.) for 1 h with agitation at 37°C and then washed three times and incubated in S-PBS (without BSA) by the procedure described above. The nitrocellulose paper was placed in 4-chloro-1-naphthol solution (0.25 g of 4-chloro-1-naphthol [Sigma] dissolved in 80 ml of methanol and added to 500 ml of Tris hydrochloride, pH 7.4, and 2.5 ml of 3% H_2O_2) at room temperature for about 5 min and washed in deionized, distilled water.

Selection of colonies. Each nitrocellulose paper with blue areas representing the possible presence of *E. coli* O157:H7 Vero cell cytotoxin(s) was aligned with its respective HGMF (Fig. 1), and colonies corresponding with each blue area were selected and grown on Trypticase soy agar (TSA) for further characterization.

Biken tests. Each isolate was assayed by a Biken test (3) to determine if the isolate was likely to be producing *E. coli* O157:H7 Vero cell cytotoxin(s). Biken plates with 8 to 10 ml of mTSA (0.75% agar instead of 1.5%) per petri dish (90 by 15 mm) were prepared by using the wide end of a sterile Pasteur pipette to punch a 6-mm well at each of the four quadrants of the plate. Three isolates and an *E. coli* O157:H7 culture (control) were spot inoculated in a square cluster approximately equidistant from each other and about 0.6 cm away from each well. Hence, 12 isolates were tested per Biken plate.

The plates were incubated for 18 to 24 h at 37°C to allow development of colonies, and then 1 drop (about 20 μl) of rabbit antiserum to O157CF, appropriately diluted based on precipitin line formation with control isolates of *E. coli* O157:H7, was added to each well. Plates were again incubated at 37°C for 18 to 24 h and observed for precipitin lines between the isolates and the central wells of antiserum. Isolates producing precipitin lines were selected for further characterization, whereas the other isolates were discarded.

Vero cell cytotoxicity testing. A continuous monkey kidney (Vero) cell line was maintained by weekly trypsinization of confluent monolayers grown in Medium 199 (GIBCO Laboratories, Grand Island, N.Y.) containing 5% fetal calf serum and 0.005% gentamicin. After trypsinization, 10^5 cells were suspended in 1 ml of growth medium in each well of a 24-well polystyrene, flat-bottom tissue culture plate (Becton Dickinson Labware, Oxnard, Calif.). The cells were allowed to grow for 2 days, at which time fresh growth medium (1 ml per well) was added. Culture filtrate (0.1 ml) of individual isolates selected from HGMF was added to duplicate wells with confluent monolayers. Culture filtrate was obtained by growing each isolate in TSB at 37°C for 18 to 24 h, removing the cells by centrifugation (5,000 × *g*, 15 min), and filtering the supernatant fluid through a 0.2-μm-pore-size Acrodisc membrane filter (Gelman Sciences, Inc., Ann Arbor, Mich.). A portion of culture filtrate (0.3 ml) was treated with rabbit antiserum (0.3 ml) to O157CF and incubated at 37°C with agitation (50 gyrations per min) for 30 min, and 0.1 ml was added to duplicate wells of Vero cells to determine if toxicity could be neutralized. After 48 h, detached Vero cells were removed by vigorous shaking and the remaining cells were washed three times with 0.01 M PBS, treated for 30 min with methanol, again washed three times with 0.01 M PBS, stained with 10% Giemsa, and rinsed with deionized, distilled water. Duplicate wells of each plate were treated with 0.1-ml portions of O157CF to be used as controls. Vero cells treated with O157CF were completely detached, and plates were visibly clear after staining. Culture filtrates of isolates producing an equivalent toxicity (no evidence of purple-stained Vero cells on plates) and neutralized by antiserum to O157CF were considered to contain Vero cell cytotoxin(s) like that of *E. coli* O157:H7.

Confirmation of isolates. Isolates determined to produce *E. coli* O157:H7-like Vero cell cytotoxin(s) were confirmed as enterohemorrhagic *E. coli* O157:H7 by identification based on the API 20E miniaturized diagnostic kit (Analytab Products, Plainview, N.Y.), by serology with O157 and H7 antisera (Difco; *E. coli* Reference Center, Pennsylvania State University, University Park), and by inability to produce acid from sorbitol (10) within 24 h at 37°C on MacConkey agar base (Difco) plus 1% sorbitol.

Inoculation and recovery tests. Four 100-g portions of ground beef (aerobic plate count, ca. 10^5 CFU/g) with no detectable *E. coli* O157:H7 were each inoculated with a different level (ca. 1.5, 2.5, 4.5, or 10 cells per g) of a nalidixic acid-resistant strain of *E. coli* O157:H7 (1). For each level of inoculum, a 25-g portion of meat was added to duplicate flasks of the enrichment medium described above. Enrichments and *E. coli* O157:H7 isolations were done by the procedure described above, and the number of *E. coli* O157:H7 in each culture after enrichment was determined by

plating dilutions of enrichment culture on MacConkey agar with 30 μg of nalidixic acid per ml. Isolates from plates inoculated with the highest dilution were randomly selected and confirmed as *E. coli* O157:H7 by the API 20E diagnostic kit and serology. This study was done in duplicate.

RESULTS AND DISCUSSION

Isolation of *E. coli* O157:H7 from inoculated samples. Inoculation studies revealed that, by using the HGMF-immunoblot procedure, *E. coli* O157:H7 could be isolated from ground beef that had been inoculated with as few as 1.5 *E. coli* O157:H7 cells per g and that had an aerobic plate count of 10^5 CFU/g. *E. coli* O157:H7 grew well in enrichment cultures held at 37°C for 18 h, with cell populations developing to 2×10^6 to 4×10^6 CFU/ml in cultures that had been inoculated with 1.5 to 10 *E. coli* O157:H7 cells per g of ground beef, respectively. Plate counts of enrichment cultures on TSA at 35°C for 48 h ranged from 2×10^9 to 5×10^9 CFU/ml; hence *E. coli* O157:H7 was present in considerably lower numbers than the predominant microflora of the enrichment cultures.

Although this procedure can effectively isolate Vero cell cytotoxic *E. coli* O157:H7 from raw meats inoculated with low numbers of the organism, the method is not amenable to routine testing because of its complexity and extensive need for personnel time. An additional drawback of the procedure was that many isolates selected from the HGMF for further testing were not the organism of concern because the antiserum used for the immunoblots was not strictly specific for *E. coli* O157:H7 Vero cell cytotoxins. More-specific antisera for these Vero cell cytotoxins have recently been produced in rabbits and will greatly improve the procedure by reducing the number of false-positive spots on immunoblots. However, a more-rapid procedure that can easily be used for routine testing of foods is definitely needed.

Isolation of *E. coli* O157:H7 from retail meats and poultry. A total of 164 ground-beef, 264 pork, 263 poultry, and 205 lamb samples was assayed for *E. coli* O157:H7. The organism was isolated from 6 beef (3.7%), 4 pork (1.5%), 4 poultry (1.5%), and 4 lamb (2.0%) samples, indicating that the bacterium is associated with foods of animal origin and not specifically with beef. The organism was isolated from a large percentage of Calgary meats, i.e., 5 (31%) of 17 beef and 1 (7.1%) of 14 pork samples, which correlates with the unusually high incidence of *E. coli* O157:H7 infections reported in the Calgary area (6). Perhaps the high prevalence of the organism in Calgary meats is responsible for the high incidence of infection in that location.

The types of meat and poultry specimens from which *E. coli* O157:H7 was isolated included ground beef, pork chop, pork loin, pork hock, ground pork, chicken leg, turkey drumstick, lamb riblet, lamb loin chop, lamb shoulder blade chop, and lamb leg. Additionally, we have isolated the organism from specimens of foods associated with outbreaks of hemorrhagic colitis or hemolytic uremic syndrome. These foods included unpasteurized milk from the bulk tank of a farm at which *E. coli* O157:H7 was isolated from the feces of a heifer (5), a frozen chicken nugget obtained from an opened box from which a sitter of children with *E. coli* O157:H7 infection periodically removed pieces for meals, and ground beef from a restaurant epidemiologically linked to an outbreak of hemorrhagic colitis. These results suggest that foods, particularly foods of animal origin, may be an important source of *E. coli* O157:H7 infections.

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