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Occurrence of Shiga-Like Toxin-Producing *Escherichia coli* in Retail Fresh Seafood, Beef, Lamb, Pork, and Poultry from Grocery Stores in Seattle, Washington

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Fresh meat, poultry, and seafood purchased from Seattle area grocery stores were investigated for the presence of Shiga-like toxin-producing *Escherichia coli* by using DNA probes for Shiga-like toxin (SLT) genes I and II. Of the 294 food samples tested, 17% had colonies with sequence homology to SLT I and/or SLT II genes.

Since 1982, several foodborne outbreaks of hemorrhagic colitis (HC) have been reported in which clinical spectra of symptoms range from asymptomatic infection and mild diarrhea to the more severe HC and the microangiopathic hemolytic anemias hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (7). Shiga-like toxin-producing *Escherichia coli* (SLTEC) have been recovered from food incriminated in HC outbreaks, with the predominant serotype isolated being O157:H7, and there have been a few reports of other serotypes (O145:H and O111:H). The SLTEC serogroups associated with human disease are numerous and include O1, O2, O4, O5, O6, O22, O23, O26, O38, O45, O48, O50, O55, O73, O75, O91, O100, O103, O104, O105, O111, O113, O114, O115, O117, O118, O119, O121, O125, O126, O128ab, O132, O145, O153, O163, O165, and O166, as well as untypeable isolates (1, 6, 7). The possibility exists that the predominance of O157:H7 may result from its ease of isolation based on its inability to ferment sorbitol, as compared with other serotypes (7). Aside from this study, there have been two studies to determine the occurrence of SLTECs in the food supply (meat and poultry) in the United States of America. Doyle and Schoeni (2) recovered *E. coli* O157:H7 from six (3.7%) of 164 beef samples, four (1.51%) of 264 pork samples, four (1.5%) of 263 poultry samples, and four (2.0%) of 205 lamb samples obtained from retail outlets from Madison, Wis., and Calgary, Alberta, Canada. We have also detected SLTEC (non-O157:H7) in surimi-based delicatessen salad, goat milk, and blueberries (12).

SLTEC O157:H7 is one of the pathogens recovered most frequently from diarrheal stool samples at the Children's Hospital and Medical Center in Seattle (13). A high frequency of isolation of *E. coli* O157:H7 has also been reported by a large health maintenance organization serving the greater Puget Sound area (9) and from neighboring Vancouver, British Columbia, Canada (5).

Epidemiological studies of HUS and HC in Seattle have failed to identify the source of the infectious agents in sporadic cases. The purpose of this study was to investigate the presence of SLTEC in foods in the Seattle area and to determine whether any are of *E. coli* serotype O157:H7, which might explain the frequent occurrence of enteric infections with this pathogen in this region.

All food samples were obtained from the shelves of local grocery stores in the Seattle area, kept on ice, and transported to the laboratory, where they were refrigerated immediately and analyzed within 8 h. The food samples were prepackaged or were packaged at the time of purchase.

Enrichment cultures from the food samples were processed as previously described (12). Briefly, 100 μl of the 10^{-4}, 10^{-5}, and 10^{-6} dilutions of enrichment cultures (in peptone water) was spread plated on modified Trypticase soy agar (12) plates. After overnight incubation at 37°C, the dilutions with the highest number of isolated colonies were selected for colony hybridization. A piece of Whatman 541 filter paper (Whatman Inc., Clifton, N.J.) was labeled and marked asymmetrically with three dots and placed on the colonies. The dot locations were copied on the back of each plate. The filters were then lifted and placed colony side up on a tray lined with Whatman no. 1 paper saturated with 0.5 N NaOH and 1.5 N NaCl. The tray was covered and moved into a steamer for 10 min, after which the filters were immersed in a solution of 0.5 N Tris (pH 8)–1.5 M NaCl for 10 min and then air dried. The plates were stored in a sealed plastic bag at 4°C after recovery of positive colonies.

DNA probes (11) were prepared and labeled as previously described (3, 12). DNA hybridization was performed under conditions of high stringency (10). Cytotoxicity was assayed by the method of Gentry and Dalrymple (4), in which Vero cells were substituted for HeLa cells. Isoenzyme analysis was performed as previously described (14).

Of the 294 food samples that were analyzed for the presence of SLTEC with the SLT DNA probes, 51 (17%) had bacterial colonies with DNA sequences homologous to the SLT structural genes. Of the 51 SLT probe-positive samples, 5 (10%) were positive only with the SLT I probe, 34 (67%) were positive only with the SLT II probe, and 12 (24%) were positive with both SLT I and SLT II probes (Table 1).

When enrichment cultures of 60 beef samples were analyzed with SLT probes, 14 (23%) were positive. Two (3%) were positive only with the SLT I probe, 7 (12%) were positive only

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TABLE 1. Results of O serotyping and analysis of isoenzyme patterns (electrophoretic typing) of SLTEC strains isolated from various food samples which were purchased from different grocery stores in the Seattle area

Strain no. and source	Source of sample[a]	O serotype[b]	Electrophoretic typing result[b]
1 Ground beef	A/I	O6*	ET-11
2 Ground beef	A/II	O113*	ET-6*
3 Ground beef	A/III	O163*	ET-12
4 Ground beef	A/II	O−	ET-8
5 Ground lamb	A/II	O−	ET-9
6 Ground veal	B/I	X-3	ET-10
7 Ground beef	B/I	X-3	ET-10
8 Ground beef	C/I	O153*	ET-6*
9 Lamb	A/II	O−	ET-5
10 Lamb	A/II	O−	ET-8
11 Lamb	C/I	O8	ET-13
12 Pork	A/III	O91*	ET-7

[a] Grocery chain/store number.
[b] Asterisks indicate serotypes or ETs associated with known enterohemorrhagic *E. coli*.

with the SLT II probe, and 5 (8%) were positive with both SLT I and SLT II probes.

Of the 51 pork samples tested, 2 (4%) were positive only with the SLT I probe, 6 (12%) were positive only with the SLT II probe, and 1 (2%) was positive with both SLT I and SLT II probes.

Five (24%) of the 21 lamb samples tested were positive only with the SLT II probe, and 5 (24%) were positive with both SLT I and SLT II probes.

Of the eight veal samples tested, four (50%) were positive only with the SLT II probe and one (13%) was positive with both SLT I and SLT II probes.

Four (12%) of the 33 chicken samples and 1 (7%) of the 15 turkey samples tested positive with the SLT II probe. All of the poultry samples were negative with the SLT I probe.

Of the 62 fish samples tested, 1 (2%) was positive only with the SLT I probe and 5 (8%) were positive with the SLT II probe.

Two (5%) of the 44 shellfish samples were positive only with the SLT II probe. Of the probe-positive fish and shellfish samples tested, none were positive with both SLT I and SLT II genes.

Overall, 14 (23%) of 60 beef samples, 9 (18%) of 51 pork samples, 10 (48%) of 21 lamb samples, 5 (63%) of 8 veal samples, 4 (12%) of 33 chicken samples, 1 (7%) of 15 turkey samples, 6 (10%) of 62 fish samples, and 2 (5%) of 44 shellfish samples were positive with SLT probe(s).

Positive colonies from 12 of the samples were recovered, all of which were found to produce SLT(s). The results of serotyping and isoenzyme analysis are shown in Table 1. Although none of the recovered positive strains were of the O157 serogroup and the isolates were of different serotypes, comparison of the electrophoretic typing patterns of the isolates to that of a collection of SLTECs isolated from humans and animals with disease showed close relationships. Two of the isolates, strains 2 and 8, are both of electropherotype (ET) ET-6, which has a single allele difference from strain CL-3, an O113:H21 SLTEC from a human source (8). It is interesting to note that although isolates 2 and 8 have the same ET, they belong to two different serotypes (O113 and O153). Strain 12 (serotype O91) is ET-7, which has a single allele difference from Centers for Disease Control strain 48368-C3, a human SLTEC isolate of serotype O146:H21. Most of the SLTEC food isolates fall into a cluster and are distantly related to the O157:H7 clone.

Five of the SLTECs isolated from food are of the serotypes which have been associated with diarrhea, HC, or HUS. These include serotypes O6, O91, O113, O153, and O163 (7). Of the other seven strains, four are O negative, two are X-3, and one is of serotype O8.

The 12 SLTEC strains were isolated from 12 food samples that were purchased from five stores belonging to three different grocery chain supermarkets (Table 1). Of the five SLTEC strains which were isolated from food samples obtained from grocery store A/II (chain A, store number 2), two were isolated from ground beef samples which were purchased at different times (Table 1, strains 2 and 4). These two strains had different ETs (ET-6 and ET-8), and one belonged to serogroup O113, whereas the other one was O-antigen nontypeable. The other three strains (strains 5, 9, and 10, Table 1) were isolated from three lamb samples purchased from the same store at different times. These strains were all O-antigen nontypeable; however, they belonged to three different ETs (ET-5, ET-8, and ET-9), indicating that they are different strains. Two strains (strains 3 and 12, Table 1) were isolated from two different types of food (ground beef and pork) purchased at the same time from grocery store A/III. These two strains had different serotypes (O163 and O91) and different ETs (ET-12 and ER-7). Strain 1 (Table 1), which was isolated from a ground beef sample from grocery store A/I, which belongs to the same grocery chain, is distinct in ET and serotype from the rest of the strains isolated from the other A chain stores. In summary, eight different SLTEC strains were isolated from food samples obtained from three different grocery stores which belong to the same chain store. This result suggests that SLTECs may enter the food chain at many independent points and that there is not a single common source of contamination within the grocery stores. Strains 6 and 7 (Table 1) were isolated from a ground beef sample and a ground veal sample, respectively, obtained from the same grocery store (B/I) at the same time. Both strains belong to the same serogroup (X-3) and have the same ET. However, these two strains could be distinguished by restriction fragment length polymorphisms detected with the SLT gene probes (unpublished data). This result further supports the previous observation that the meat supply can be contaminated at different points through the production line.

The fact that none of our isolates recovered from food are of the O157 serotype and the higher frequency of detection of SLTECs in our study compared with that of Doyle and Schoeni (2) may reflect the higher frequency of SLTECs of other serotypes in the food supply. Although several serotypes of non-O157 SLTECs have been recovered from symptomatic humans and animals, the extent of the contribution of non-O157 SLTECs to disease is not well established. Without a simple detectable phenotype such as the sorbitol reaction, most cases involving the non-O157 SLTECs are overlooked in clinical laboratories. Clearly, more work is needed to determine the range and scope of distribution of SLT genes among *E. coli* serotypes, the ease of transfer of these genes among *E. coli* strains in the environment, their distribution in nature, their mode of entry into the food chain, and their potential pathogenicity.

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