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Research Note

Prevalence of Shiga Toxin–Producing *Escherichia coli* in Ground Beef and Cattle Feces from King County, Washington

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ABSTRACT

Shiga toxin–producing *Escherichia coli* (STEC) is increasingly recognized as a common cause of diarrhea. STEC infection is a major public health threat because of its ability to cause serious and potentially life-threatening illnesses. The main reservoirs of STEC are believed to be the intestinal tracts of animals. Several studies have investigated the prevalence of STEC in various food items. The objective of this study was to determine the prevalence of STEC in the Seattle ground beef supply. In addition, the relative amount of STEC contamination between stores was compared, and possible differences between types of ground beef based on fat content (9, 16, and 23%) were investigated. A survey of *Stx*-I and/or *Stx*-II genes in fecal samples from cattle at a local slaughterhouse was also conducted. Of 296 ground beef samples tested from area retail grocery stores, 16.8% were positive for the presence of the toxin genes. Our data showed that there was no statistically significant difference ($P > 0.05$) in the prevalence of STEC between the ground beef samples of different fat contents and between grocery store chains. Of the 103 cattle fecal samples tested, 19 (18.4%) were found positive for the presence of *Stx*-I and/or *Stx*-II genes. The presence of a rather high percentage of STEC in the food supply in the absence of large number of cases suggests that not all STEC lineages are pathogenic for humans.

Infection with Shiga toxin–producing *Escherichia coli* (STEC) serotype O157:H7 can result in a wide range of outcomes, including asymptomatic infection, mild diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura *(10, 13).* Several studies have been performed to determine the reservoirs of STEC and its frequency in many food products, animal feces, and environmental samples *(4–6, 18, 23, 29, 31).* Farm animals are believed to be the main reservoir for STEC. Although STEC infections are mostly foodborne, there have also been several waterborne outbreaks of *E. coli* O157:H7 *(2, 16, 33).* STEC infections have been linked to a variety of products, including foods of animal origin, apple juice, apple cider, sprouts, and lettuce *(1, 3, 4, 7, 12, 17, 19–23, 26). Stx* production has been shown in *E. coli* strains from a large number of serotypes, isolated from a wide variety of animals, foods, and environmental samples *(4, 6, 8, 18, 22, 27, 29, 30, 32, 35).* A study in 1987 by Doyle and Schoeni *(8)* found that 3.7% of 164 ground beef samples tested positive for *E. coli* O157:H7 by an antigen-antibody–based assay. Since 1987, several other studies have investigated the prevalence of STEC in food products using several detection methods *(8, 15, 22, 24, 27, 30, 32, 34, 35).* These studies have demonstrated variable levels of STEC in beef-related products, ranging from 3.7% *(8)* to as high as 11% *(15)* and 38% *(22).* A previous study from our laboratory found that 23% of beef samples tested were positive for STEC *(27).* The variation in prevalence rates is partly due to the detection method used, as well as to actual differences in the prevalence of STEC in the various study locations and times.

In 1985, Mohammed et al. *(18)* found that 28 of 172 (16.3%) cattle fecal samples were positive for STEC by a technique that directly measured toxin production. A 1990 study performed by Samadpour et al. *(26)* using DNA probes for *Stx*-I and -II genes found that 9 of 28 (32%) calf fecal samples were positive for STEC. Additionally, several studies have investigated the prevalence of STEC in various animal feces *(5, 24, 31, 36).* These studies demonstrated the presence of STEC gene pools in farm animals and in the food supply.

The objective of the present study was to determine the prevalence of STEC in the Seattle ground beef supply. The relative amount of STEC contamination between stores was compared, and possible differences between the fat content of meat samples were explored. Sampling at the grocery stores is a logical step in assessing the potential exposure of the general population to STEC. During each sampling episode, samples were collected from display cases in selected store outlets. Each type of ground beef, on the basis of fat content (9, 16, and 23%), was sampled to allow for possible differences between grades. A lower limit of detection of STEC for the method used in this study was previously determined to be 13 organisms per 10-g

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sample *(26).* In addition, a survey of STEC prevalence in fecal samples from cattle at a slaughterhouse was conducted. Fecal samples from cattle at an area slaughterhouse were collected to establish the prevalence of STEC in cattle that enter the slaughter process.

MATERIALS AND METHODS

Sample collection. Ground beef samples were collected from display cases in selected store outlets. Each type of ground beef, on the basis of fat content (9, 16, and 23%), was sampled to allow for possible differences between grades. The samples were transported to the laboratory on ice and were processed within 3 h of sampling. Cattle fecal samples were collected by filling a sterile 50-ml tube with fecal matter using a sterile disposable wooden spatula. Whenever possible, samples were taken from freshly deposited feces in areas where the type of cattle, dairy or beef breeds that shed the feces, could be determined. Samples were transported to the laboratory on ice.

Ground beef samples, enrichment, and colony isolation. Methods used in this study are a modification of methods developed previously *(8, 14, 26, 27).* Ten grams of a ground beef or fecal sample was dispensed aseptically into 90 ml modified Trypticase soy broth (MTSB). The MTSB contained 30 g TSB base (Becton Dickinson, Cockeysville, Md.), 1.5 g bile salts no. 3 (Difco Laboratories, Detroit, Mich.), and 1.5 g dibasic sodium phosphate (J. T. Baker, Phillipsburg, N.J.) per liter of distilled water. In addition, 100 μl novobiocin (Sigma Chemical Co., St. Louis, Mo.) solution was added to each of the 90-ml MTSB mixtures. The antibiotic solution was prepared by adding 1 g novobiocin in 10 ml distilled water and then filtering the solution through a 0.2-μm pore syringe filter (Corning, Acton, Mass.). The MTSB enrichment cultures were incubated overnight at 37°C in a shaking incubator at 150 rpm. Twenty-four hours later, the enrichment cultures were serially diluted in 0.1% peptone solution (Difco). Dilutions of 10^{-4}, 10^{-5}, and 10^{-6} were plated onto modified Trypticase soy agar plates, which were identical to MTSB except for the addition of 1.5% agar. The plates were incubated at 37°C overnight. Two dilutions of each enrichment with the greatest number of isolated colonies were selected for further analysis. Colonies were transferred to Whatman 541 filter paper (Whatman, Inc., Clifton, N.J.) by placing the filter on the surface of Trypticase soy agar plates. The plates were then stored at 4°C for up to 2 weeks to allow for isolation of positive colonies, whereas the Whatman filters were processed further.

Colony hybridization. Intact bacteria cells on Whatman 541 filter paper were lysed by immersing the filter papers in a 1.5 N NaOH and 1.5 N NaCl solution and heating the solution in a microwave for 1 min. The filter papers were then transferred into a pH 7 solution of 1.5 N Tris and 1.5 N NaCl solution for 10 min to stabilize the DNA, cell debris, and filter components. The filters were air dried. Filters remain stable, even when stored for many years, and can be probed, stripped, and reprobed multiple times *(9).*

The bacterial genome attached to the filter was probed with *Stx*-I and -II gene fragments as described previously *(26).* Briefly, a 1,142-bp *Taq*I-*Hin*cII fragment of the *Stx*-IA and *Stx*-IB subunits and an 842-bp *Sma*I-*Psi*I fragment of the *Stx*-IIA subunit were purified. The *Stx* gene fragments were labeled with [α-^{32}P]dCTP (Dupont, Boston, Mass.) as follows: equal amounts of each of the *Stx*-I and -II gene fragments were added to a mixture of nonradioactive nucleotides (dATP, dGTP, and dTTP) (Promega, Madison, Wis.), buffer (Promega), random hexanucleotide primer (Life Technologies, Rockville, Md.), and DNA Polymerase (Klenow fragment, GIBCO-BRL, Grand Island, N.Y.). This reaction was allowed to process for 4 h. The labeled DNA probe was ethanol precipitated, dried, and resuspended in Tris-EDTA buffer, and was then boiled, iced, and added to a solution of 50% formamide (Fisher Scientific, Fair Lawn, N.J.), 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% sodium dodecyl sulfate (SDS; Fisher), 1 mM EDTA (Sigma), and 100 μg boiled, sonicated salmon sperm DNA (Sigma) with an excess of blocking reagent (Roch Diagnostics Corp., Indianapolis, Ind.). The filters were added one at a time to the hybridization solution. The reaction was incubated overnight at room temperature with gentle agitation. The next day, filters were washed with 0.1× SSC and 0.1% SDS for 20 to 30 min at 70°C to remove nonspecific binding material. The filters were then dried and exposed to x-ray film (Eastman Kodak Co., Rochester, N.Y.) in the presence of an intensifying screen.

Colonies that were positive for *Stx*-I and -II displayed as dark patches on the film. Positive colonies were streaked for isolation onto MacConkey agar plates and incubated overnight at 37°C. Isolated lactose-positive colonies were then transferred to two Trypticase soy agar plates marked with premade grids, 48 colonies to each plate. The colony hybridization process was repeated, and positive strains were taken from the duplicate plate for storage and later analysis.

Statistical analysis. The data were analyzed by the chi-square test to examine the possible differences in levels of STEC contamination of ground beef between stores and fat content, beef versus dairy, at the 0.05 significance level.

RESULTS AND DISCUSSION

Three supermarket chains in the Seattle area were sampled for ground beef in sufficient number for statistical analysis (253 samples). Additionally, 43 samples were taken from five independent grocers in the area.

The overall prevalence of STEC in ground beef was 50 of 296 (16.8%). The prevalence values for STEC, from stores 1 to 3, were 21 of 93 (22.6%), 15 of 76 (19.7%), and 8 of 84 (9.5%), respectively. Analysis of these data by the chi-square test revealed that the differences seen between stores were not significant at the 0.05 level for the size of samples tested. The prevalence of STEC reported in our current investigation (16.8%) falls within the range of an earlier study performed in our laboratory (23%) *(27)* and, in other studies, 28 and 38% *(22),* 10.7% *(30),* and 9% *(32).*

Of the 296 total ground beef samples, 165 of the samples were grouped into one of three fat content categories. As for the rest of the samples, either their labels did not provide percentage fat information, or they had different levels of fat content, and the number of samples in each fat content category was too few for data analysis. The prevalence of STEC in meat products with a fat content of 9, 16, and 23% was 14% (7 of 51), 37% (7 of 19), and 17% (16 of 95), respectively. Chi-square analysis of the data showed that the observed differences between the three types of ground beef (with regard to fat content) were not significant at the 0.05 level. However, this may be an artifact of the uneven sample numbers in the three categories and the sampling plan.

Fifteen percent (8 of 52) of the beef cattle fecal samples were positive for STEC, while 22% (11 of 51) of dairy cattle fecal samples were positive; thus, the overall prevalence of STEC in cattle fecal samples was 18.4% (19 of 103).

A 1990 study by Samadpour et al. *(26),* measuring the percentage of STEC in a small number of calf fecal samples, found 9 of 28 (30%) to be positive for STEC, which was slightly higher than the results obtained by Mohammed et al. in 1985 *(18),* with 28 of 172 (16.3%) cattle fecal samples testing positive for STEC by a technique that directly measured toxin production. In this study, the overall prevalence of STEC in cattle fecal samples was found to be 18.4% (19 of 103). This difference in STEC prevalence in beef cattle versus dairy cows was not statistically significant.

More than 100 serotypes of *E. coli* have been linked to *Stx* production *(11),* and several of the serotypes have been shown to cause severe illness and deaths in humans. This makes STEC a potential challenge for the food industry and for the public health system.

Although *E. coli* O157:H7 appears to be the most frequently encountered STEC in the United States, the general consensus is that there may be a higher level of underreporting for the other serotypes of STEC, mainly because of the difficulties in their isolation and identification *(11, 28).* However, it is important to note that mere possession of the *Stx* toxin gene is not sufficient to convert a nonpathogenic *E. coli* strain into a pathogen. Many nonclinical isolates of STEC strains lack the *eae* gene and the *hly* genes *(25).* Although the prevalence of STEC in retail ground beef is much higher than that of *E. coli* O157:H7, which is a member of the group *(27),* it does not follow that the public health impact of the group is higher by the same magnitude.

Because most food safety policies are in response to foodborne outbreaks, there is an inherent danger that a major outbreak caused by an STEC would result in excessive regulation that would encompass a broad group of nonpathogenic STEC. There is clearly a need to study the pathogenicity of STEC in order to be able to separate the avirulent STEC from the virulent lineages.

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