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Incidence of *Escherichia coli* O157:H7 in Frozen Beef Patties Produced over an 8-Hour Shift†

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ABSTRACT

A ground beef patty processor detected *Escherichia coli* O157:H7 in five production lots during routine testing with polymerase chain reaction (PCR) technology. This finding stimulated research to determine the incidence and potential entry points of the pathogen during processing. One of these lots (53,960 kg) was divided into 71 pallets (760 kg each) of food service ground beef patties. Ten cartons (19 kg each) were removed from each pallet, for a total of 710 cartons. Four patties were taken from each carton and subdivided to provide comparable samples for *E. coli* O157:H7 analyses by three different laboratories. Two laboratories employed different immunoassay tests, and one used PCR to screen samples. One sample set was analyzed for aerobic plate, coliform, and *E. coli* Biotype I counts to determine if any relationship existed between these microbial groups and the incidence of *E. coli* O157:H7. For 73 samples, presumptive positive results for *E. coli* O157:H7 were obtained by one or more methods. For 48 of these 73 samples, positive results for the pathogen were culture confirmed. The largest number (29) of culture-confirmed positive *E. coli* O157:H7 results were detected by PCR. Most positive results were obtained during a short segment of processing. All culture-confirmed *E. coli* O157:H7 strains were further characterized by two genetic subtyping techniques, resulting in two to four different patterns, depending on the subtyping procedure employed. For any sample tested, the aerobic plate count was <3.0 log CFU/g, and coliform and *E. coli* Biotype I counts were ≤ 1.00 log CFU/g. The results of this study suggest that most positive samples were associated with a contaminated batch of raw material introduced just before the 1725- to 1844-h processing segment. These results also indicate that more aggressive sampling plans and genetic screening technologies such as PCR may be used to better detect low levels of *E. coli* O157:H7 in ground beef products.

Escherichia coli O157:H7 is recognized as a significant foodborne pathogen. Hemorrhagic colitis caused by *E. coli* O157:H7 can lead to complications such as hemolytic uremic syndrome, which may be fatal to young children. The most common disease manifestation of *E. coli* O157:H7 in elderly people is thrombotic thrombocytopenic purpura. Cases of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura have been associated with the consumption of undercooked ground beef contaminated with *E. coli* O157:H7. Only a few cells are necessary to cause illness *(13). E. coli* O157:H7 levels of 0.9 to 4.3 CFU/g were reported for samples tested from lots of ground beef that had been implicated in a 1993 outbreak of hemolytic uremic syndrome in humans *(7, 16).*

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† Reference to a brand name or firm does not constitute endorsement of a product by the U.S. Department of Agriculture, Food Safety and Inspection Service.

‡ Deceased.

The commercial processing of ground beef includes grinding, mixing, regrinding, forming (into patties), freezing, packaging, and frozen storage (Fig. 1). The beef production industry has established testing and control regimens to protect the public from contaminated ground beef. The strategy for such regimens often includes the testing of raw material and/or processed ground product for *E. coli* O157:H7. Even with extensive testing, the distribution and movement of contaminated material through a production lot is not well understood. Such contamination can lead to expensive cleanup and redirection, or even destruction of the product.

Immunoassay methods that yield a presumptive positive or negative screening result in ≤ 24 h are available for *E. coli* O157:H7 testing. Typical sensitivities of immunoassays are approximately 10^6 CFU/ml, postenrichment. Lower levels could result in false-negative results and preclude confirmation testing. Confirmation failures may also result when the levels of competing microbial flora reach $\geq 10^5$ CFU/g in the enrichment broth *(12),* a common sit-

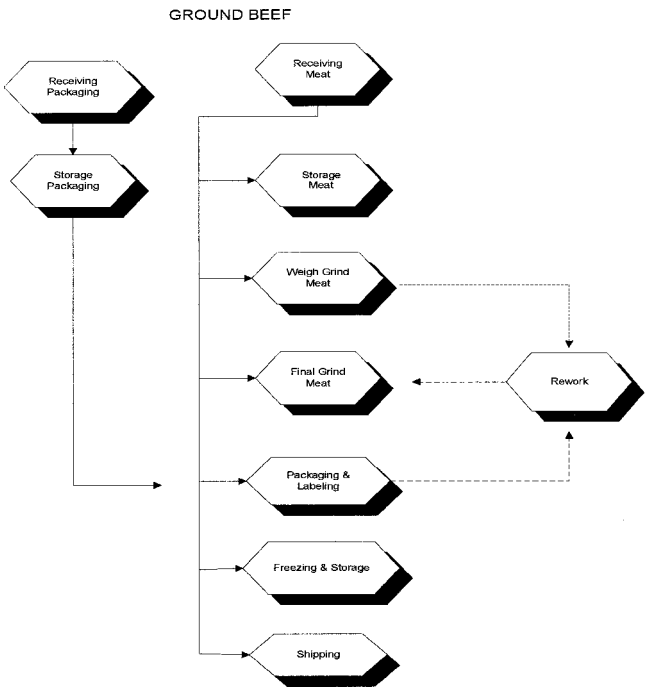

FIGURE 1. *Flow diagram for commercial ground beef processing.*

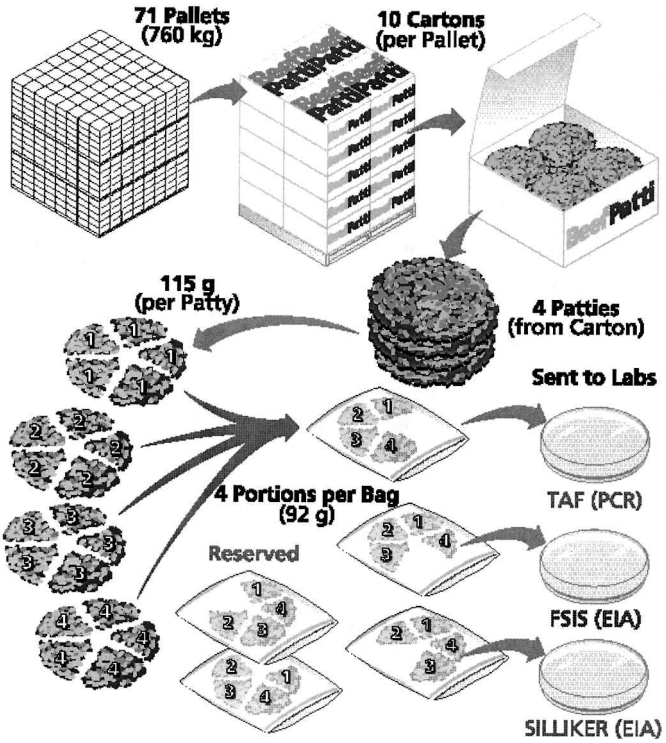

FIGURE 2. *Sampling procedure used to detect* E. coli *O157:H7 in frozen ground beef patties.*

uation for ground beef. In the past, the screening of large numbers of ground beef samples has resulted in high percentages of unconfirmed positive results. These unconfirmed results could indicate either insufficient sensitivity of the isolation procedure or nonspecificity of the immunoassay *(3, 9, 12).* Polymerase chain reaction (PCR) technologies may offer greater sensitivity, rapid response, and results that are more definitive, since they are based on genetic information *(2, 15).*

Over a 6-week period, a ground beef patty processor detected *E. coli* O157:H7 in five production lots using PCR. This finding prompted a study to determine the incidence and potential entry points of *E. coli* O157:H7 during processing. One of these lots (53,960 kg) was divided into 71 pallets (760 kg each) of food service ground beef patties, which were set aside for additional observation.

This study was designed to determine (i) if the processor's routine on-line sampling was sufficient to consistently detect *E. coli* O157:H7 in ground beef batches, (ii) if a more aggressive sampling plan (a larger analytical unit and more samples) would significantly increase the chances of detecting the pathogen in pallets of finished product, and (iii) if other microbial populations could be correlated with the incidence of *E. coli* O157:H7.

MATERIALS AND METHODS

Sampling procedure. Frozen patty samples were obtained from 71 pallets produced during a second-shift (1600 to 0054 h) process (Fig. 2). Sampling was conducted by using a random-numbers table. Number assignments were made across pallets and pallet cartons. Ten cartons from each pallet were sampled. Four frozen patties (115 g each) were obtained from each carton.

Each frozen patty was split into five equal portions (each ~23 g) with a sterile metal punch device. Each patty portion was then placed in a separate sterile bag. This procedure was repeated for each of the four patties removed from each carton. Therefore, each bag, representing one of five split sample sets from each carton, contained four frozen patty sections and weighed approximately 92 g. For each of the five split samples, the total weight of each pallet sample of patty portions was 920 g, which included 10 bags with four frozen patty sections in each bag. This sampling procedure allowed a 650-g composite sample or a total of ten 65-g samples to be obtained from each pallet.

Sample analysis. Sample sets were analyzed for *E. coli* O157:H7 by Texas American Foodservice (TAF), Fort Worth, Tex.; by the Special Projects and Outbreak Support Laboratory, Food Safety and Inspection Service (FSIS), Athens, Ga.; and by Silliker Laboratories, Grand Prairie, Tex. The ten 65-g patty samples from each pallet were tested as individual analytical units by each laboratory. Figure 3 outlines the analytical procedures used in the study.

The FSIS and Silliker laboratories employed immunoassay methods to detect *E. coli* O157:H7. The TAF laboratory used PCR to screen for the pathogen. The manufacturer's directions were followed for each of the screening tests. TAF sent all screen-positive sample enrichments to Qualicon, Inc., for confirmation testing. The specific screening tests used were the Reveal immunoassay (Neogen Corporation, Lansing, Mich.) (immunoassay A), the Assurance Polyclonal Enzyme Immunoassay (BioControl, Bellevue, Wash.) (immunoassay B), and BAX for Screening/*E. coli* O157:H7 (Qualicon, Inc., Wilmington, Del.) (PCR). Immunoassay A, immunoassay B, and PCR were employed by the FSIS, Silliker, and TAF laboratories, respectively.

Confirmation protocols. Broth cultures suspected to contain *E. coli* O157:H7 on the basis of immunoassay screening were confirmed according to the protocol outlined in the U.S. Department of Agriculture (USDA) FSIS *Microbiology Laboratory Guidebook (18)* or by a procedure incorporating immunomagnetic

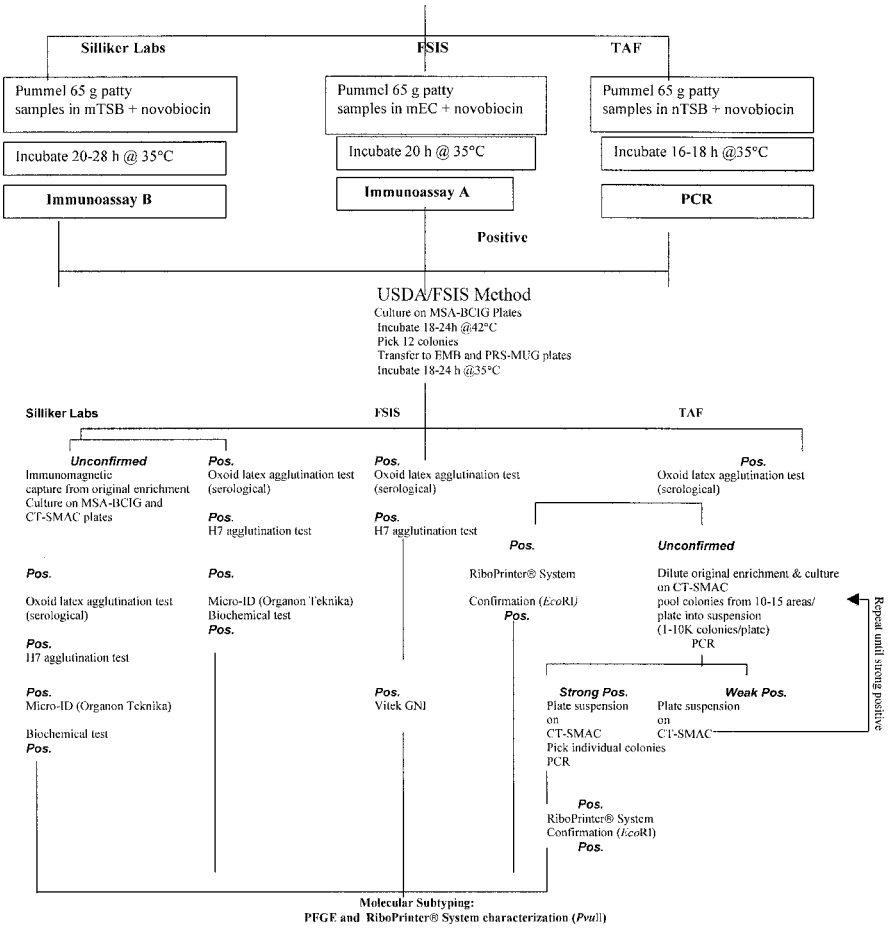

FIGURE 3. *Summary of analytical procedures used for the detection of* E. coli *O157:H7 in frozen ground beef patties.*

beads *(1)*. For the USDA-FSIS protocol, presumptive enrichment broths were diluted in Butterfield's phosphate diluent to obtain 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} dilutions. From each dilution, 0.1-ml portions were spread onto MacConkey sorbitol agar (MSA; Difco Laboratories, Detroit, Mich.) with 5-bromo-4-chloro-3-indolyl-β-D-glucuronide sodium salt (BCIG; Sigma Chemical Co., St. Louis, Mo.) plates by using sterile bent glass rods. Inoculated MSA-BCIG plates were incubated for 18 to 24 h at 42°C. Twelve typical colonies from each MSA-BCIG plate were transferred to eosin methylene blue and phenol red sorbitol-4-methylumbelliferyl-β-D-glucuronic acid plates that were incubated for 18 to 24 h at 35°C. Typical colonies from these plates were confirmed by each laboratory as outlined in Figure 3.

When samples could not be confirmed by the USDA-FSIS procedure, Silliker Laboratories used a slightly modified immunomagnetic bead capture protocol *(1)* to obtain isolated colonies for cultural confirmation. One milliliter of enrichment broth was pipetted into a capture tube containing 20 μl of Dynabeads (Dynal, Lake Success, N.Y.). The tubes were capped and inverted several times for 10 min. After mixing, the tubes were inserted into a holder containing a magnetic strip and slowly inverted several times for 3 min to capture and concentrate beads on the walls of the tubes. With the magnetic strip still in place, the liquid content of each tube was removed without disturbing the beads. One milliliter of buffered wash solution was added to each tube, and then the tubes were closed and the magnetic strip was removed. The tubes were inverted several times for 10 min to wash the beads, after which the magnetic strip was replaced and the capture-concentration and liquid removal steps were repeated. Finally, the magnet was removed, the beads were resuspended with 200 μl of buffer (versus the 100 μl indicated in the referenced protocol), and the tubes were mixed several times. The final suspensions were serially diluted and spread onto MSA-BCIG plates and MSA containing cefixime (Sigma) and potassium tellurite (Sigma) (CT-SMAC). Typical colonies on these agar plates were confirmed by USDA-FSIS procedures *(18)*.

Qualicon attempted to confirm all PCR-positive samples by the FSIS-recommended recovery method *(18)* with one modification: The FSIS method uses petri plates (150 by 15 mm) of MSA-BCIG. In these experiments, standard laboratory plates (100 by 15 mm) were used.

Samples that remained unconfirmed by the FSIS method were subjected to the Qualicon isolation protocol. PCR-positive enrichments were diluted to 10^{-2} to 10^{-3} in 0.1% peptone water and were then plated onto MSA, MSA-BCIG, and CT-SMAC. Disposable inoculating loops (1 μl) were used to pool colonies from 10 to 15 separate areas of the agar plates containing approximately 1,000 colonies, such that the pools collected represented the entire plate. A loopful of the mixed colonies from each pooled area was suspended in 1 ml of 0.1% peptone water, generating a suspension of approximately 10^8 CFU/ml. Each pooled suspension was directly tested for the presence of *E. coli* O157:H7 by the standard PCR protocol. PCR-positive suspensions were diluted to 10^{-3} to 10^{-5} in 0.1% peptone water and plated onto CT-SMAC. The plates were incubated at 37°C for 24 h. Plates with approximately 100 colonies were selected, and typical colonies on these plates were confirmed according to procedures outlined in Figure 3.

Ribotyping. The RiboPrinter Microbial Characterization System (Qualicon, Inc.) was used to generate a standardized digital RiboPrint pattern for the confirmed *E. coli* O157:H7 isolates

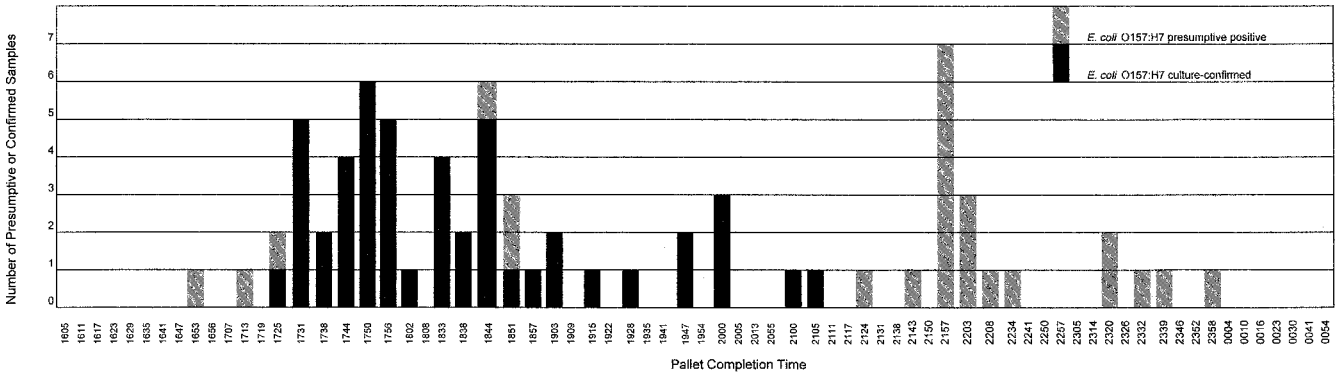

FIGURE 4. *Distribution of presumptive positive (gray bars) and culture-confirmed (solid bars)* E. coli *O157:H7 isolates in a frozen ground beef patty lot. Completion times for 71 pallets of product are shown on the horizontal axis. Completion times without bars represent sampling intervals that were negative for* E. coli *O157:H7.*

(4–6, 14, 17). Single *Eco*RI identification RiboPrint patterns were obtained for all *E. coli* O157:H7 isolates. All samples that were confirmed to be *E. coli* O157:H7 with *Eco*RI were further characterized with *Pvu*II, whose utility in segregating *E. coli* O157:H7 strains has been demonstrated *(11). Pvu*II replaced *Eco*RI in the automated processing protocol of the system.

PFGE. The FSIS laboratory used pulsed-field gel electrophoresis (PFGE) to genetically subtype *E. coli* O157:H7 isolates recovered by the three laboratories involved in this study. A Centers for Disease Control and prevention (CDC) standardized method described by the Foodborne and Diarrheal Diseases Branch *(8)* was used. *E. coli* O157:H7 cells were suspended in agarose, lysed, and then restricted with approximately 50 U of *Xba*I (Boehringer Mannheim, St. Louis, Mo.). Gels were subjected to 19 h of electrophoresis with a CHEF Mapper XA (BioRad Laboratories, Hercules, Calif.) set to auto-algorithm 30- to 600-kbp default values.

Quantitative microbial analyses. Silliker Laboratories also analyzed the split samples for aerobic, coliform, and *E. coli* Biotype I plate counts. A 50-g portion from each sample bag (see "Sampling Procedure" above) was diluted 1:10 in Butterfield's phosphate diluent and pummeled in a stomacher (Seward Medical, London, UK) for 2 min. Serial dilutions of the 1:10 mixture were prepared with Butterfield's phosphate diluent. Aerobic plate counts (APC) were obtained by pour plating serial dilutions with Standard Methods Agar (SMA; Difco). SMA pour plates were incubated for 48 h at 35°C and then evaluated for APC. Coliform and *E. coli* Biotype I counts were obtained by transferring serial dilutions to Coliform/*E. coli* Petrifilm (3M, Minneapolis, Minn.) plates. Petrifilm plates were incubated for 24 h at 35°C and then evaluated for coliform and *E. coli* Biotype I populations.

RESULTS AND DISCUSSION

Figure 4 graphically depicts the distribution of presumptive positive and culture-confirmed *E. coli* O157:H7 samples for the second shift's production of frozen ground beef patties. Table 1 shows the total number of and temporal relationship between presumptive positive and culture-confirmed *E. coli* O157:H7 results observed for each production time interval for which any presumptive positive samples were obtained.

Although culture-confirmed *E. coli* O157:H7 isolates were detected for the period from 1725 to 2105 h (approximately 3 h 40 min of production), the largest number of positive results occurred between 1725 and 1844 h (approximately 1 h 20 min of production), suggesting that a raw material source introduced into the formulation just before the production of these patties likely contained substantial levels of the pathogen. PCR presumptive positive results were obtained for the period from 1653 to 2358 h (approximately 7 h of production).

Of the 710 samples tested, presumptive positive results were obtained for 73 (about 10% of all samples) by at least one of the screening methods (Table 1). Forty-eight of the 73 presumptive positive isolates (66% of the presumptive positive samples, or about 7% of all samples) were confirmed to be *E. coli* O157:H7 by cultural procedures. A control set of seven 3.5-g samples, representing the *E. coli* O157:H7 test portion routinely sampled by TAF (25 g/5,448 kg or 6.25 g/1,362 kg) and originally testing positive by PCR, was found to test negative by PCR in this study (data not shown). The control samples were obtained at the same time as the 710 study samples.

These results demonstrate the high probability of heterogeneous distribution and low levels of *E. coli* O157:H7 not only in the lot but also in any given patty; only seven of the samples were presumptive positive by two or more methods (Table 1). To verify that the distribution and low contamination levels of *E. coli* O157:H7 caused disparities in results among the three screening and culture methods, eight additional patties were obtained from the only cartons that tested positive by at least two of the three screening methods. These patties were divided into 40 equal pieces (five from each patty), and each piece was tested for the presence of *E. coli* O157:H7 by PCR. The number of positive pieces per patty ranged from 0 to 5, with only one patty having all five pieces test positive. These data confirm that an aggressive sampling plan may detect low levels of *E. coli* O157:H7 in lot samples in which the distribution of the pathogen is heterogeneous.

All isolates were analyzed by PFGE and ribotyping to determine the relationship between genetic subtypes for each method and the potential for a common source of contamination. Subtyping by PFGE revealed four *E. coli* O157:H7 pattern types (Fig. 5), and ribotyping revealed two pattern types (Fig. 6). The PFGE types were arbitrarily designated A (40 isolates), B (7 isolates), C (3 isolates), and

TABLE 1. *Incidence of* E. coli *O157:H7 in ground beef patties*[a]

Time (h)/sampling order[b]	No. of presumptive positive and culture-confirmed samples with:			Total[c]	Corrected[d]
	PCR	Immunoassay A	Immunoassay B		
1653/28	1 (0)			1 (0)	1 (0)
1713/29	1 (0)			1 (0)	1 (0)
1725/41	2 (1)			2 (1)	2 (1)
1731/43	4 (4)	1 (1)		5 (5)	5 (5)
1738/19	2 (2)			2 (2)	2 (2)
1744/22[e]	3 (3)	4 (3)		7 (6)	**4 (4)**
1750/17[e]	4 (3)	4 (4)	1 (1)	9 (8)	**6 (6)**
1756/16	3 (3)	2 (2)		5 (5)	5 (5)
1802/25	1 (1)			1 (1)	1 (1)
1833/26[e]	3 (3)	2 (2)		5 (5)	**4 (4)**
1838/24		2 (2)		2 (2)	2 (2)
1844/21	2 (2)	2 (1)	2 (2)	6 (5)	6 (5)
1851/23		1 (0)	2 (1)	3 (1)	3 (1)
1857/18	1 (1)			1 (1)	1 (1)
1903/14	2 (2)			2 (2)	2 (2)
1915/39		1 (1)		1 (1)	1 (1)
1928/27			1 (1)	1 (1)	1 (1)
1947/35	1 (1)	1 (1)		2 (2)	2 (2)
2000/12	1 (1)	2 (2)		3 (3)	3 (3)
2100/8	1 (1)			1 (1)	1 (1)
2105/6	1 (1)			1 (1)	1 (1)
2124/4	1 (0)			1 (0)	1 (0)
2143/6	1 (0)			1 (0)	1 (0)
2157/4	6 (0)	1 (0)		7 (0)	7 (0)
2203/5	2 (0)	1 (0)		3 (0)	3 (0)
2208/8	1 (0)			1 (0)	1 (0)
2234/13	1 (0)			1 (0)	1 (0)
2320/9	2 (0)			2 (0)	2 (0)
2332/10	1 (0)			1 (0)	1 (0)
2339/6	1 (0)			1 (0)	1 (0)
2358/16	1 (0)			1 (0)	1 (0)
Total	50 (29)	24 (19)	6 (5)	80 (53)	73 (48)
% culture confirmed	58	79	83	66	66

[a] Confirmed results are in parentheses.

[b] Time of pallet completion/sampling order of pallets from second-shift production lot.

[c] Total numbers of presumptive and confirmed results considering all method outcomes (regardless of method "overlap" for a given sample).

[d] Actual number of presumptive positive or culture-confirmed samples, corrected for individual samples testing positive by more than one method. "Corrected" values differing from "total" values are in bold.

[e] For these times there were individual samples for which *E. coli* O157:H7 was detected by more than one method. This method "overlap" is seen by the values in the "total" and "corrected" columns.

D (3 isolates). PFGE types A and B differed by one band, suggesting a possible common source for these pulsotypes. The ribotypes were classified into one of two *Pvu*II ribogroups: ribogroups a (50 isolates) and b (3 isolates). Isolates designated PFGE types A, B, and C all corresponded to the isolates in ribogroup a. The three isolates designated PFGE type D all corresponded to isolates in ribogroup b.

Subtypes were randomly distributed among samples taken from the 71 pallets (Table 2). Based on the observation of more than one *E. coli* O157:H7 genetic subtype, one could surmise that contamination of the ground beef patty production lot was due to multiple-source contamination from the raw materials (fresh lean, fatty trim, and frozen beef) used. However, an examination of raw-material records suggests that a fresh trim lot from one supplier could have introduced *E. coli* O157:H7 (all genetic subtypes) into the ground beef patty production lot during the period for which the largest number of positive samples was obtained (1725 to 1844 h). Thus, contrary to what would be expected, the raw-material records from this study do not support the contamination of ground beef patties from multiple sources. The introduction of rework (Fig. 1) back into the process most likely explains the lower incidence of *E. coli* O157:H7 genetic subtypes observed after 1844 h (Fig. 4).

Levels of indicator organisms recovered from patty samples were very low for a raw ground beef product. For comparison, in the nationwide raw ground beef survey con-

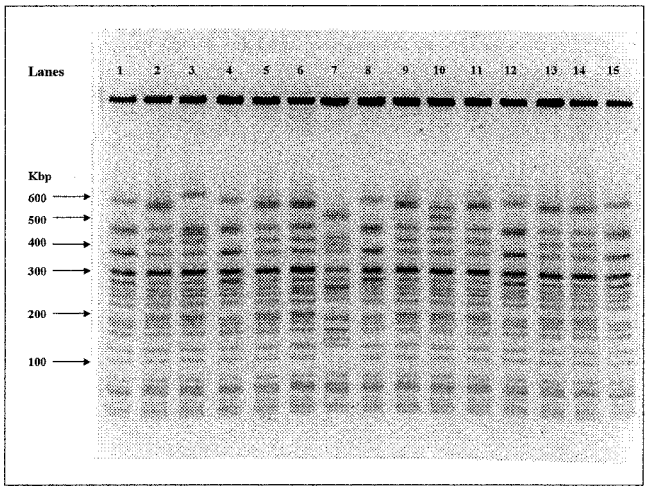

FIGURE 5. *Representative PFGE images of* E. coli *O157:H7 pulsotypes A through D recovered from frozen ground beef patties. Lanes 1, 4, 8, 12, and 15, CDC standard strain G5244 (control); lanes 2, 5, 6, 9, 11, 13, and 14, pulsotype A; lane 3, pulsotype C; lane 7, pulsotype D; lane 10, pulsotype B.*

ducted by the USDA from 1993 to 1994, aerobic, coliform, and *E. coli* Biotype I counts averaged 3.90, 1.98, and 1.73 log CFU/g, respectively *(19).* In our study, APC ranged from <2.00 to 2.85 log CFU/g, and coliform and *E. coli* Biotype I levels were not detectable (<1.00 log CFU/g) in most cases.

No trends could be discerned relating the concentrations of any indicator population to the incidence of *E. coli* O157:H7. Consequently, this study demonstrated that negative or low *E. coli* Biotype I populations did not correlate with the absence of viable *E. coli* O157:H7.

The comparison of methods was not a primary objective of this study because of the limitations imposed by the format of the study. Although efforts were made to subdivide samples from common patties, the disseminated samples from each period were not necessarily equivalent because of the heterogeneous distribution and low levels of the *E. coli* O157:H7 in the patties. Furthermore, each laboratory used a different screening procedure and a different culture method to detect and confirm the presence of viable *E. coli* O157:H7 in presumptive positive samples. Despite these differences, analysis of the data revealed that some comparative conclusions were appropriate. To reach such conclusions, however, further discussion of the data and methods is necessary.

For samples from the production period from 2000 to 0054 h, the number of presumptive positive PCRs (20) was much larger than number of presumptive positive immunoassay reactions (4) (Table 1). In addition, only five (20%) of the presumptive positive samples for this period were culture confirmed. These results are very different from those observed for the production period from 1605 to 1954 h, for which 30 presumptive positive PCR results were reported, compared with 19 presumptive positive results for immunoassay A and 6 for immunoassay B. Of the presumptive positive reactions for this period, 85% were culture confirmed.

TABLE 2. *Distribution and number of* E. coli *O157:H7 PFGE subtypes (A, B, C, and D) and ribotypes (a and b) by production time and sampling order*

Time (h)/ sampling order[a]	No. of subtypes[b]			
	A (a)	B (a)	C (a)	D (b)
1725/41	1			
1731/43	5			
1738/19	2			
1744/22	4	2		
1750/17	4	2	1	
1756/16	6			
1802/25	1			1
1833/26	3	1	1	
1838/24	2			
1844/21	1	1	1	
1851/23	1			
1857/18	1			
1903/14	2			
1915/39	1			
1928/27		1		
1947/35	1			1
2000/12	3			
2100/8	1			1
2105/6	1			
Total	40	7	3	3

[a] Time of pallet completion/sampling order of pallets from second-shift production lot.

[b] Lowercase letters in parentheses represent ribotype designations equivalent to PFGE subtypes. There were 50 a isolates and 3 b isolates.

This discrepancy can be explained by the mishandling of the samples from the 2000- to 0054-h production period. The samples from this period were the first ones analyzed in the study. The shipping of these samples to the FSIS was delayed. Consequently, they were temperature abused before immunoassay analysis. Since the PCR analysis was carried out at TAF, shipping was not necessary, and thus there was no temperature abuse before PCR analysis. However, the same delay and temperature abuse occurred when PCR presumptive positive sample broths were shipped to Qualicon for culture confirmation.

The shipping protocol was improved for the remainder of the study. The sample mishandling could explain both the discrepancy between PCR and immunoassay presumptive positive results and the low rate of culture confirmation of presumptive positive samples for the production period from 2000 to 0054 h.

There is often a hesitancy to accept positive PCR results because of the potential detection of DNA from nonviable organisms, especially when the PCR positive result cannot be culture confirmed. Although the detection of DNA from nonviable organisms is theoretically possible, in practice, it is not a concern. The probability of detecting DNA from nonviable organisms is a direct function of the starting concentration of nonviable organisms in the original sample. At least 20 DNA copies in the 5-μl subsample taken from the enrichment broth for analysis are required

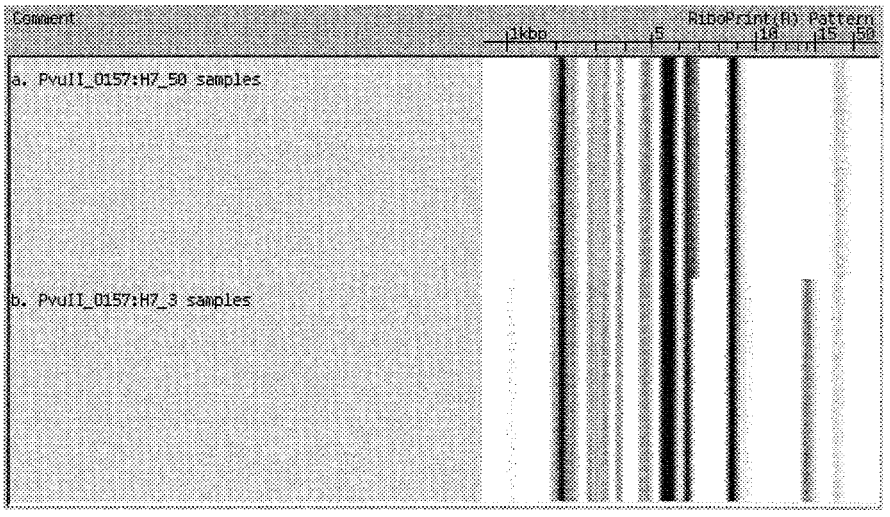

FIGURE 6. *Images of* E. coli *O157:H7 ribotypes a (top) and b recovered from frozen ground beef patties.*

to consistently obtain a positive result with PCR. This amount corresponds to a concentration of 4-log nonviable cells per ml of enrichment broth or 5-log nonviable cells per g in the original sample. At lower starting concentrations, the probability of obtaining a positive result with PCR falls dramatically.

Figure 7 shows the probability of obtaining a positive PCR result as a function of nonviable cell concentration. For concentrations of <4 log cells per g, the detection of nonviable cells is not a concern. Because the highest reported APC in this study was 2.85 log CFU/g, a high starting concentration of nonviable *E. coli* O157:H7 cells in any sample was very unlikely.

It was clear that PCR produced a substantially larger number of presumptive positive and culture-confirmed results than did either of the two immunoassay procedures used in this study (Table 1). Nevertheless, test sensitivity, specificity, and false-positive and -negative rates could not be assessed for the assays employed in this study.

Procedures used by the laboratories for viable culture confirmation of presumptive positive results appeared to vary significantly in terms of effectiveness. In order to better confirm the presumptive positive results obtained with PCR, the Qualicon laboratory employed a more exhaustive confirmation procedure (growth from selective plates was pooled, tested by PCR, and, if found to be positive, replated) than did the Silliker and FSIS laboratories. Although this procedure required more effort and time than the procedures used by the other two laboratories, it was clear that the additional effort confirmed the presence of viable *E. coli* O157:H7 in several additional samples. This potential for significant differences in culture method performance is further illustrated by the recent implementation of an improved USDA-FSIS *E. coli* O157:H7 culture detection method *(10)* in which an immunomagnetic bead capture step has been included. The enhanced procedure *(10),* implemented to supplant the USDA-FSIS method *(18)* used in this study, has demonstrated a minimum fourfold increase in the number of raw ground beef samples confirmed to be contaminated by viable *E. coli* O157:H7/NM.

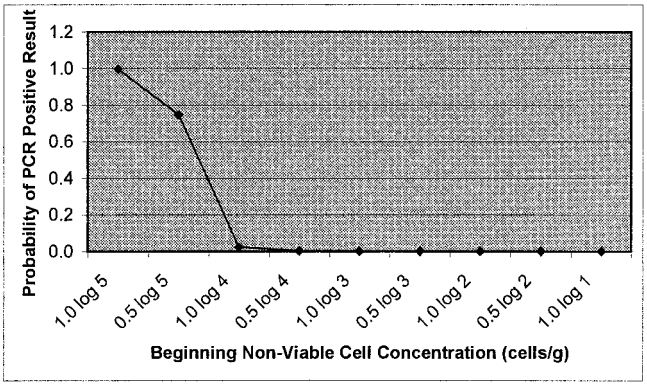

FIGURE 7. *The probability of obtaining a positive PCR result due to the detection of DNA from nonviable* E. coli *O157:H7 cells. This plot is based on the sample preparation protocol used in this study.*

Although the original routine sampling and testing program was sufficient to detect *E. coli* O157:H7 in the study lot, when it was repeated (control sample set for this research), the pathogen was not detected. Our study suggests that sensitive screening technologies (such as the PCR method) used in tandem with an aggressive sampling plan (i.e., the testing of larger analytical units and more samples, as in this study) may be a highly effective means of screening ground beef for the potential presence of *E. coli* O157:H7. Furthermore, our research indicates that negative or small *E coli* Biotype I populations may not correlate with the absence of viable *E. coli* O157:H7.

DEDICATION

In memory of Dr. Charles (Chuck) Lattuada.

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