2015

# Distribution of *Escherichia coli* Passaged through Processing Equipment during Ground Beef Production Using Inoculated Trimmings

Mohammad Koohmaraie
*IEH Laboratories and Consulting Group*

Joseph M. Bosilevac
*USDA Meat Animal Research Center*, mick.bosilevac@ars.usda.gov

Michael de la Zerda
*IEH Laboratories and Consulting Group*

Ali Mohseni Motlagh
*American Foods Group*

Mansour Samadpour
*IEH Laboratories and Consulting Group*

*Journal of Food Protection, Vol. 78, No. 2, 2015, Pages 273–280*
doi:10.4315/0362-028X.JFP-14-369

# Distribution of *Escherichia coli* Passaged through Processing Equipment during Ground Beef Production Using Inoculated Trimmings

**MOHAMMAD KOOHMARAIE,[1]* JOSEPH M. BOSILEVAC,[2] MICHAEL DE LA ZERDA,[1] ALI MOHSENI MOTLAGH,[3] AND MANSOUR SAMADPOUR[1]**

[1]*IEH Laboratories and Consulting Group, 15300 Bothell Way N.E., Lake Forest Park, Washington 98155;* [2]*U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933-0166; and* [3]*American Foods Group, LLC, Green Bay, Wisconsin, USA*

MS 14-369: Received 8 August 2014/Accepted 8 September 2014

## ABSTRACT

The contamination of raw ground beef by *Escherichia coli* O157:H7 is not only a public health issue but also an economic concern to meat processors. When *E. coli* O157:H7 is detected in a ground beef sample, the product lots made immediately before and after the lot represented by the positive sample are discarded or diverted to lethality treatment. However, there is little data to base decisions on how much product must be diverted. Therefore, five 2,000-lb (907-kg) combo bins of beef trimmings were processed into 10-lb (4.54-kg) chubs of raw ground beef, wherein the second combo of meat was contaminated with a green fluorescent protein (GFP)–expressing strain of *E. coli*. This was performed at two different commercial ground beef processing facilities, and at a third establishment where ground beef chubs from the second grinding establishment were mechanically split and repackaged into 3-lb (1.36-kg) loaves in trays. The GFP *E. coli* was tracked through the production of 10-lb (4.54-kg) chubs and the strain could not be detected after 26.5% more material (500 lb or 227 kg) and 87.8% more material (1,840 lb or 835 kg) followed the contaminated combo at each establishment, respectively. Three-pound (1.36-kg) loaves were no longer positive after just 8.6% more initially noncontaminated material (72 lb or 33 kg) was processed. The GFP strain could not be detected postprocessing in any residual meat or fat collected from the equipment used in the three trials. These results indicate that diversion to a safe end point (lethality or rendering) of the positive lot of ground beef, plus the lot before and lot after should remove contaminated ground beef, and as such provides support for the current industry practice. Further, the distribution and flow of *E. coli* on beef trimmings through various commercial equipment was different; thus, each establishment needs to consider this data when segregating lots of ground beef and establishing sampling protocols to monitor production.

*Escherichia* coli O157:H7 is of considerable public health concern; it can cause serious infections that lead to chronic conditions and death *(11, 14)*. After a large outbreak of illnesses due to *E. coli* O157:H7 in undercooked hamburger *(2)*, the U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) declared *E. coli* O157:H7 to be an adulterant in raw ground beef under 9 CFR §301.2 *(1)*. Currently, the frequency of detected *E. coli* O157:H7 contamination is less than 1% *(16)*, suggesting that most raw ground beef is not contaminated or contaminated at a level below the sensitivity of current sampling and detection methods. Ground beef is formulated and produced from various types of beef trimmings supplied in 2,000-lb (907-kg) combo bins. A combo bin is typically a container measuring 48 by 40 by 40 in. (122 by 102 by 102 cm) of beef trimmings from multiple carcasses. Current risk assessments of contamination estimate the average 2,000-lb combo bin of beef trimmings contains 13 and 41 *E. coli* O157:H7 organisms during the seasons of low and high prevalence, respectively *(15)*. This implies that 32% of 2,000-lb grinder loads in the low prevalence season and 14% of 2,000-lb grinder loads in the high prevalence season are not contaminated. This risk assessment predicts that in the low prevalence season, between 40% (5th percentile) and 88% (95th percentile) of these grinder loads contained one or more *E. coli* O157:H7. In the high prevalence season, between 61% (5th percentile) and 94% (95th percentile) of grinder loads contained one or more *E. coli* O157:H7 *(15)*.

In spite of a continued investment in research and the implementation of proven interventions *(10)*, *E. coli* O157:H7 continues to present a challenge to food processors, especially the beef industry. While finished product testing has never been and should never be considered as a method for controlling *E. coli* O157:H7, it can be viewed as one last effort to detect and remove contaminated product before it enters commerce, therefore reducing recalls.

While there are a number of finished product testing strategies in use by the ground beef processing sector, we are not aware of a scientific basis for any of these schemes for industrial scale production that consider and identify the

* Author for correspondence. Tel: 206-940-3334; Fax: 206-260-7922; E-mail: mk@iehinc.com

TABLE 1. *Summary of equipment[a] used at each establishment*

| | Est 1 | Est 2 | Est 3 |
|---|---|---|---|
| Blender | Ross Blender<br>M7225<br>Weiler, Whitewater, WI | Cozzini Mixer/Blender<br>CMB6000-CO2<br>Cozzini, Chicago, IL | NA[b] |
| Coarse grinder | Ross Grinder with ½-in. blade<br>1109E<br>Weiler, Whitewater, WI | Ross Grinder with ½-in. blade<br>1109E<br>Weiler, Whitewater, WI | NA |
| Fine grinder | Ross Grinder with ⅛-in. blade<br>1109E<br>Weiler, Whitewater, WI | Ross Grinder with ⅛-in. blade<br>1109E<br>Weiler, Whitewater, WI | NA |
| Chub packager | K-Pak<br>4000<br>Kartridge Pak, Davenport, IA | K-Pak<br>4000<br>Kartridge Pak, Davenport, IA | NA |
| Grinder/former | NA | NA | Vemag<br>HP30/HP15<br>Reiser, Canton, MA |
| Loaf portioner | NA | NA | Vemag MMP220<br>Reiser, Canton, MA |

[a] The make, model number, and manufacturer for each piece of equipment used to process ground beef at each establishment are listed.
[b] NA, not applicable; this piece of equipment was not used at the establishment.

distribution of *E. coli* O157:H7 in production equipment. Most ground beef processors define lots (production and sales units) of product based on a unit of production time, such as 15, 30, or 60 min where the amount of ground beef produced per minute can vary between each processor. Processors sample and test each lot of ground beef for *E. coli* O157:H7. If a ground beef sample is found to be positive, the product from the sampled lot, the lot before, and the lot after are discarded or subjected to lethality treatment. This practice is based on the assumption that as more material flows through the processing equipment, contaminating bacteria move with the contaminated material and do not linger in the equipment to further contaminate all subsequent lots. Therefore, it is important for ground beef processors to determine the distribution and flow of a contamination event by identifying the length of time required for *E. coli* O157:H7 to be no longer detectable and presumably removed from their system. To answer this question, a marked strain of *E. coli* was tracked through ground beef production. The procedures described here can be used to support recommendations for product disposition when a positive ground beef sample has been identified.

## MATERIALS AND METHODS

**Design.** Five combo bins of beef trimmings were processed into approximately 1,000 10-lb (4.5-kg) chubs (sealed tube packaging). Combo bins were loaded sequentially with only the second combo bin containing a bolus (approximately $1 \times 10^7$ CFU) of green fluorescent protein (GFP)–expressing *E. coli,* acting as a surrogate for *E. coli* O157:H7. Samples of ground beef were collected from every other 10-lb chub for analysis. This procedure was performed in two different establishments using different blending, grinding, and packaging equipment (Table 1). Then the ground beef chubs from the second processing establishment that had not been opened for sample collection were shipped to a third establishment where they were sorted and sequentially processed into 3-lb (1.4-kg) ground beef loaves then again sampled and analyzed (Fig. 1). Analysis consisted of a combination of culture isolation of the GFP *E. coli* and PCR detection of GFP *E. coli* gene marker.

**Bacterial strain.** Plasmid pMRP9-1 *(4)* (a gift of E. Peter Greenberg's laboratory, University of Washington) contains the *GFP* gene from *Aequorea victoria (3)* under control of the *lac* promoter and the *T7* phage gene 10 ribosomal binding site *(4).* The plasmid constitutively expresses GFP and confers resistance to ampicillin and carbenicillin. pMRP9-1 was transformed into TOP10 chemically competent *E. coli* (Invitrogen, Carlsbad, CA) and the resulting pMRP9-1 transformant strain was maintained on Difco tryptic soy agar (BD, Sparks, MD) supplemented with 100 μg/ml carbenicillin (Teknova, Hollister, CA). GFP has an excitation maximum of 481 nm and an emission maximum of 507 nm; therefore colonies of *E. coli* expressing GFP can be easily viewed and identified under long-wave UV light as green fluorescing colonies. Further growth characterizations of this strain showed that neither growth at 42°C for 12 h, nor storage at 4°C for 48 and 72 h significantly affected ($P > 0.05$) bacterial viability or loss of the GFP tag (data not shown). Inoculation studies identified an average minimum level of detection of the GFP *E. coli* of 5 CFU/375 g of ground beef (data not shown).

**Inoculation of trimmings.** Purge (the liquid that settles at the bottom of combo bin from beef) was collected from several combo bins of beef trim to use as diluent of an overnight culture of the GFP *E. coli* strain that was grown in *E. coli* enrichment media (IEH, Lake Forest Park, WA) containing 100 μg/ml carbenicillin. The final concentration of the overnight culture was determined by plating 1:10 serial dilutions prepared in Difco buffered peptone water (BD) onto PetriFilm *E. coli*/Coliform Count Plates (3M Microbiology, Minneapolis, MN) that were then incubated and counted according to the manufacturers recommendations. The overnight GFP *E. coli* culture was diluted into 100 ml of purge (at approximately $1 \times 10^5$ CFU/ml) to use as inoculum. Four 7.6-gal (28.8-liter) utility tote boxes (Newell-Rubbermaid, Winchester, VA) measuring 21.5 by 17.75 by 7 in. (54.6 by 45 by 17.8 cm) were used to hold 25 lb (11.3 kg) of trimmings each, and then 100 ml of inoculum, 25 ml per box, was added and mixed manually for 2 min. The inoculated trimmings were then held at

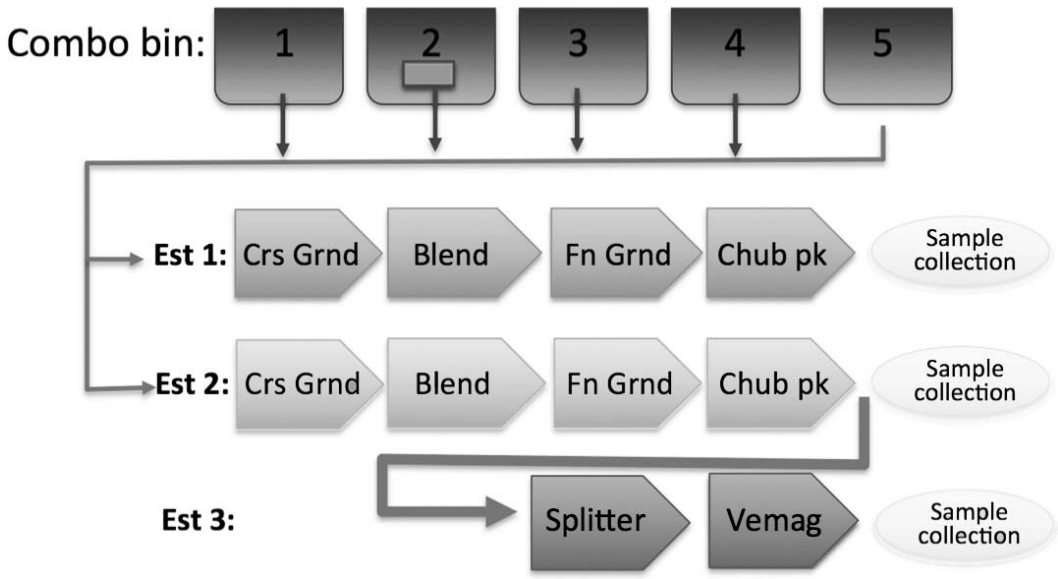


FIGURE 1. *Schematic representation of experimental design. Combo bins 1 through 5 were processed into ground beef sequentially, with combo bin 2 containing a bolus of GFP* E. coli. *Establishments 1 (Est 1) and 2 (Est 2) processed 10-lb chubs, and establishment 3 (Est 3) processed 3-lb loaves made from 10-lb chubs from Est 2. The steps in production before sample collection are indicated. Crs Grnd, coarse ground; Fn Grnd, fine ground; Chub Pk, chub packaging.*

4°C for 1 h to allow attachment to the beef trimmings surfaces *(9)*. The 100 lb of inoculated trimmings were added to a combo bin that was half filled (1,000 lb) with trimmings. Then another 900 lb (408 kg) of trimmings was added to surround and cover the inoculated trimmings. The level of GFP *E. coli* present in the inoculated trimmings was 250 CFU/g, which is a final concentration of 12.5 CFU/g in the inoculated combo bin.

**Ground beef processing: production of ground beef chubs.** Ground beef chubs were produced at two different establishments (Est 1 and Est 2) using the equipment listed in Table 1. The first combo bin (noninoculated) was dumped into the coarse grinder and sent through the establishments' standard process of ground beef production (Fig. 1). The trimmings were then sequentially coarse ground, blended (at this stage $CO_2$/dry ice was added to control the temperature), fine ground, and then transferred via gondolas to a chub packager where 10-lb (4.54-kg) chubs were produced. Once material from the first combo bin cleared the coarse grinder and was in the blender, the second (inoculated) combo bin was introduced into the coarse grinder. After the material from combo bin 1 cleared the blender and was in the fine grinder, then the material from combo bin 2 entered the blender and combo bin 3 was placed in the coarse grinder. Combo bins 4 and 5 were introduced through the coarse grinder sequentially after each of the previous combo bins had cleared through the blender and fine grinders. At the end of the processing and before cleaning or sanitation steps, any meat and fat remaining in or on the equipment was collected for testing. The meat was placed in Whirl-Pak bags (Nasco, Fort Atkinson, WI), chilled, and transported to the laboratory for analysis with ground beef samples (as described below). The amounts and locations of all postprocessing meat samples were recorded.

**Ground beef processing: production of ground beef loaves.** Ground beef chubs produced at the second ground beef production establishment were stored at 2 to 5°C overnight and then boxed, placed on pallets, and transported by refrigerated (2 to 6°C) truck to a third processing establishment (Est 3) where they were processed into 3-lb (1.36-kg) ground beef loaves using equipment summarized in Table 1. Upon arrival, chubs were sorted and placed in sequential order according to production information printed on their labels. All chubs produced from combo bins 1, 2, 3, 4, and 5 were grouped onto individual pallets for transport to the production room of the third establishment. Pallet number 1 (noninoculated chubs from combo bin 1) was used to start production of ground beef loaves. Chubs were placed on the chub splitting line, split mechanically, and dumped into a gondola that was next dumped into the horn of the loaf former. Loaves were produced and removed from the line at a rate of every third loaf. Chubs of pallet 2 containing the inoculated ground beef were split and prepared as described for pallet 1. The ground beef from pallet 2 was input to the line after the horn of the loaf former had emptied of chubs from pallet number 1. This process was repeated for ground beef from pallets 3 through 5. The collected loaves were sequentially numbered, placed in plastic collection trays, and maintained under refrigeration in preparation for sampling. At the completion of loaf forming, ground beef, and/or purge that remained in the interlock belt, interlock belt catch pan, chub opening station catch pan, the auger and the auger housing were collected in sample bags for analysis. The amounts and locations of all postprocessing meat samples were recorded and the samples transported to the laboratory for analysis with ground beef samples (as described below).

**Sample collection: ground beef chubs.** Starting with the first packaged chub, every other chub was opened along the longitudinal axis by using a chemically sanitized knife. Ground beef was aseptically sampled along the entire length of the chub until 375 g were collected from each chub. Samples were placed in prenumbered bags, which corresponded to the numbered chubs, placed in ice chests with ice packs, and moved to refrigerated storage. The next day, the ice chests of samples were shipped via overnight delivery to the laboratory for analysis.

**Sample collection: ground beef loaves.** Samples were collected by removing the plastic overwrap from the loaf tray and taking a 375-g sample from the tray. Samples were placed in prenumbered bags, which corresponded to the numbered loaves, placed in ice chests with ice packs, and moved to refrigerated

storage. The next day, the ice chests of samples were shipped via overnight delivery to the laboratory for analysis.

**Detection of GFP *E. coli*.** Each 375-g sample was stomached in 750 ml of *E. coli* enrichment media (IEH) containing 100 μg/ml carbenicillin and incubated for 12 ± 0.5 h at 42°C. From each enrichment 100 μl was streaked for isolation on a tryptic soy agar plate containing 100 μg/ml carbenicillin. Following incubation for 24 h at 37°C, the plates were viewed under a hand-held UV light (UVP, Upland, CA) to determine the presence or absence of GFP *E. coli* colonies. Concurrently, 2 μl of enrichment was mixed with 50 μl of Rhodia lysis buffer (17 mM Tris, pH 8.5; 2 mM $MgCl_2$; 1.5% IGEPAL CA-630; Solvay, Brussels, Belgium) containing 0.5 μl of 20 mg/ml proteinase K (IBI Scientific, Peosta, IA). The lysis was incubated at 37°C for 20 min, at 95°C for 10 min, and then held at 4°C. One microliter of lysis was added to 24 μl of PCR reaction buffer, containing 0.28 μM forward GFP primer 5′-TGTCCACACAATCTGCCCTTTC-3′, 0.28 μM reverse GFP primer 5′-ATGCCATGTGTAATCCCAGCAG-3′, and 1 U of *Taq* polymerase in 0.2-ml reaction tubes. Samples were mixed and briefly centrifuged to spin the reaction contents to the bottom of the tubes. PCR was performed using an Eppendorf Mastercycler (Eppendorf NA, Hauppauge. NY) under the following conditions: 4°C for 2 min, 95°C for 2 min, followed by 32 cycles at 95°C for 10 s, 65°C for 30 s, and 72°C for 20 s. After 72°C for 4 min, the reactions were held at 4°C. PCR products were loaded into a 1% agarose gel in a gel apparatus (Owl Separations; Thermo Fisher Scientific, Waltham, MA) containing 0.5 × Tris-borate-EDTA and 100 μg of ethidium bromide for electrophoresis (30 min, 235 V), and then gel images were captured for analysis using a UV transilluminator and an EDAS 290 gel documentation system (Kodak, Rochester NY). Lanes exhibiting a product of the proper target size (97 bp) with appropriate positive and negative control results were considered GFP *E. coli* positive. Replicate serial dilutions were performed to determine the lowest limit of detection for the GFP *E. coli* using PCR and streak plating following enrichment. The lowest limit was found to be 5 CFU/375 g sample. Comparison between plating results and PCR detection showed the number of GFP *E. coli*–positive samples was not different ($P > 0.05$) within a combo bin (data not shown). Therefore, for speed and accuracy of results, PCR for the *GFP* gene was performed and reported as the indicator of contamination.

## RESULTS

To determine how long *E. coli* persists in a processing line after a contamination event occurs, one combo bin of noninoculated trim was ground (combo bin 1), followed by combo bin 2 of trimmings inoculated with a GFP-producing *E. coli* strain, followed by combo bins 3, 4, and 5 of noninoculated trimmings, with samples taken and analyzed after every 20 lb of production (every other 10-lb chub). If the inoculated 100-lb (45-kg) bolus was to be evenly mixed into the 2,000-lb combo, the inoculation level for combo bin 2 in its entirety would be 12.5 CFU/g.

In the first establishment where this procedure was conducted, none of the samples taken from combo bin 1 were positive. When combo bin 2 was processed, 81.4% of the samples collected for that combo bin ($n = 86$) representing 1,730 lb (785 kg) of product were positive for the GFP marker (Table 2). In sequential samples from this combo bin of material, intermittent samples throughout were positive ranging from 80 to 95% positive over a moving 20-sample window (Fig. 2). When material from combo bin 3 was analyzed ($n = 94$) the prevalence rate of GFP *E. coli* was 11.7%, and the positive samples were only present in the initial 500 lb (227 kg) or 26.4% of that combo bin. The remaining 73.4% of combo bin 3 as well as combo bins 4 and 5 were negative for GFP *E. coli*. When material from combo bin 3 entered the system, the positive rate over a 20-sample window steadily decreased from about 40 to 0% (Fig. 2). After ground beef production at establishment 1, neither the leftover meat in the system, nor the fat collected from the grinder head was positive for the GFP *E. coli* (data not shown).

Establishment 2 used different grinding equipment for the production of 10-lb chubs (Table 1). For the trial at establishment 2, individual sample test results followed the trend recorded at establishment 1. At establishment 2, all samples correlating to combo bin 1 were negative (Table 2). Samples collected from ground beef produced from combo bin 2 ($n = 96$) were 62.5% positive for GFP *E. coli*. The distribution of the GFP *E. coli* in the ground beef produced from combo bin 2 at this establishment was more variable than at establishment 1. At establishment 2 the GFP *E. coli* ranged from 40 to 90% over a 20-sample moving window of the ground beef processed from combo bin 2 (Fig. 2). When Combo bin 3 was processed, 12 (11.7%) of 103 samples correlating to combo bin 3 were positive, representing the first 1,840 (835 kg) of 2,050 lb (930 kg) packaged from this combo bin or 87.8% of the ground beef made from this combo bin. As the material from combo bin 3 was processed, the 20-sample moving average showed sporadic positive samples with averages ranging from 0 to 30% until near the end of the combo bin where the final positive sample was detected. All samples from ground beef processed from combo bins 4 and 5 were negative for the presence of GFP *E. coli*. Neither the leftover meat in the system nor the fat collected from the grinder head was positive for the GFP *E. coli* (data not shown).

Production of 3-lb loaves at establishment 3 used the remaining ground beef from the trial at establishment 2 (Table 2). Individual sample results for ground beef loaf production at the third establishment tracked GFP *E. coli* contamination and identified 39 (41.5%) of 94 of the 3-lb ground beef loaf samples from combo bin 2 as positive for GFP *E. coli*. Only one of the first nine samples taken from combo bin 3 was positive ($n = 93$; 1.1%), representing the first 72 (33 kg) of 840 lb (381 kg) packaged from this combo, or about 8.6% of the following combos material. Finally, after all product was processed through the end of the fifth combo, residual ground beef samples were collected from the equipment. Ground beef recovered from the interlock belt, interlock belt catch pan, chub opening station catch pan, and the Vemag equipment was negative for the GFP *E. coli,* as was the fat from the Vemag auger and the purge and fat from inside the Vemag auger housing (data not shown).

## DISCUSSION

While contamination of beef trimmings by *E. coli* O157:H7 might occur as a point source due to the presence

TABLE 2. *Distribution of GFP* E. coli *in ground beef production by sequential combo bins of starting materials at each establishment*

| Establishment | | Values for each combo bin | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 GFP | 3 | 4 | 5 |
| Est 1 | *n* sampled[a] | 95 | 86 | 94 | 98 | 86 |
| | lb packaged[b] | 1,890 | 1,740 | 1,890 | 1,970 | 1,730 |
| | % positive overall[c] | 0.0 | 81.4 | 11.7 | 0.0 | 0.0 |
| | % material to clear contamination[d] | NA[e] | 100 | 26.5 | 0 | 0 |
| Est 2 | *n* sampled | 96 | 96 | 103 | 98 | 104 |
| | lb packaged | 1,910 | 1,910 | 2,050 | 1,950 | 2,070 |
| | % positive | 0.0 | 62.5 | 11.7 | 0.0 | 0.0 |
| | % material to clear contamination | NA | 100 | 87.8 | 0 | 0 |
| Est 3 | *n* sampled | 89 | 94 | 93 | 94 | 94 |
| | lb packaged | 795 | 830 | 840 | 867 | 849 |
| | % positive | 0.0 | 41.5 | 1.1 | 0.0 | 0.0 |
| | % material to clear contamination | NA | 100 | 8.6 | 0 | 0 |

[a] Values represent the number of samples collected and tested from each combo bin. At Est 1 and Est 2, samples were 10-lb chubs. At Est 3, samples were 3-lb loaves.
[b] Values represent the number of pounds of ground beef packaged from each combo.
[c] Values represent the percent positive for GFP *E. coli* of all samples for each combo bin.
[d] Values represent the percentage of material of each combo bin required to pass through equipment to reach undetectable levels of GFP *E. coli* at each establishment.
[e] NA, not applicable.

of fecal material, the process of manufacturing raw ground beef disperses this contamination into the batch of trimming being ground. The physical contamination of the processing equipment itself is thus a concern because this would lead to the adulteration of subsequently processed batches of raw ground beef. To determine the distribution of the passage of contamination caused by such an event, trimmings were inoculated with an *E. coli* O157:H7 surrogate that could be easily enriched for in media containing carbenicillin and easily detected by either direct plating because it produces GFP or by PCR for the unique *GFP* gene.

Our detection test took advantage of a laboratory-developed strain of *E. coli* carrying GFP- and carbenicillin-resistance markers. This provided sensitive and reliable enrichment for rapid PCR detection results. This surrogate strain could also be direct plated for detection as well if PCR is not a feasible method of detection. Our comparison of PCR and plating detection methods showed no statistical difference between the two methods, therefore in our studies we relied on the more rapid PCR detection method.

The surrogate GFP *E. coli* was generated in commercially available competent TOP10 *E coli*. TOP10 *E. coli* is a commercial name for the substrain DH10B of *E coli* K-12. A laboratory strain such as this is arguably not as robust as a wild-type *E. coil* strain. For instance, it is known that DH10B require leucine for growth due to the loss of the *leuLABCD* operon. DH10B also contains two alleles (*relA1* and *spoT1*) that cause sensitivity to shifts in nutrients and lower rates of growth compared with wild-type *E. coli (5).* However, recent sequencing shows that DH10B contains unexpected wild-type alleles such as *deoR (5),* thus contradicting its arguable weaknesses as a suitable surrogate for *E. coli* O157:H7. Considering the addition of the selective factors of GFP expression and carbenicillin resistance in our strain, it is as detectable as equal levels of *E. coli* O157:H7 using immunomagnetic concentration and selective media.

These procedures were carried out at three commercial establishments, two producing 10-lb chubs of ground beef and one establishment producing 3-lb loaves using the ground beef remaining from the trial in the second establishment. The three trials followed routine ground beef production protocols, without modification, in three establishments inspected by the FSIS. Therefore, it would be difficult to replicate these actual conditions and equipment outside of each individual establishment. However, because we observed routine industry protocols, the observations reported here should be generally repeatable in other establishments using comparable equipment.

By inoculating a 100-lb bolus of trimmings to a level of 250 CFU/g with GFP *E. coli,* and then mixing that bolus into a combo bin with a final weight of 2,000 lb, each 375-g sample taken from the inoculated 2,000-lb lot would be estimated to contain about 4,700 target cells assuming the bolus was evenly mixed throughout the combo bin. This level would be expected to decrease as additional uncontaminated combo bins were processed through the grinding system. The results of the 10-lb (5-kg) chub trial in the first establishment (Table 2) suggest that after a volume of approximately 26.5% of the following combo bin has been processed, the level of *E. coli* is reduced to a level below the limit of detection, while at establishment 2, 87.8% of the following combo bin is required to reduce GFP *E. coli* to below the limit of detection.

The results of the trials at establishments 1 and 2 demonstrate a different distribution pattern of *E. coli* in the contaminated combo bin (Fig. 2). At establishment 1, 70 (81.4%) of 86 of the 10-lb ground beef chub samples from combo bin 2 were positive for GFP *E. coli* as opposed to 60 (63.2%) of 95 of the 10-lb ground beef chubs produced

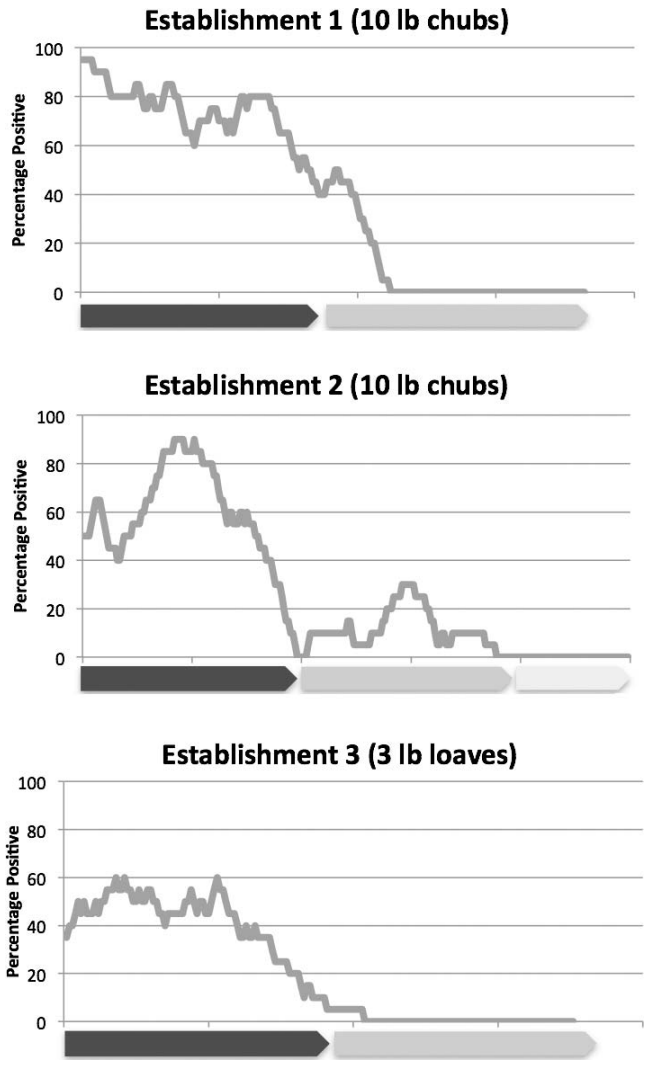


FIGURE 2. *Percentage of GFP-positive samples in a 20-sample moving window for combo bins 2, 3, and 4. Combo bin 2 (inoculated): dark bar; combo bin 3 (noninoculated): medium bar; combo bin 4 (noninoculated): light bar, establishment 2 only.*

from combo bin 2 at establishment 2. Examining the prevalence of the GFP *E. coli* at establishment 1 over a 20-sample window (Fig. 2) showed rates of positive samples that ranged from 60 to 94% through the first 1,500 lb (680 kg) of combo bin 2, and then became lower as combo bin 3 was introduced. The pattern observed over the 20-sample window at establishment 2 showed the contaminated center of the combo bin was not uniformly distributed; rather the GFP *E. coli* prevalence started at approximately 50%, rose to 90%, then fell back toward 50%. The prevalence of the GFP *E. coli* in combo bin 2 then dropped to near zero at the end of its run as combo bin 3 was added. The 20-sample window then shows the material of combo bin 3 pulling the remaining GFP *E. coli* still in the equipment through in a similar wave of contamination.

The pattern of contamination of samples collected from ground beef produced from combo bin 3 was different between establishments 1 and 2. While the actual number of positives was not different between the two establishments—11 (11.7%) of 94 samples at establishment 1 and 12 (11.7%) of 103 samples at establishment 2—the positive samples from combo bin 3 at establishment 1 occurred in the first 500 lb, while the complimentary set of samples at establishment 2 represented the first 1,840 lb. The combo bin 3–positive samples from establishment 2 were detected in a sporadic pattern. This illustrates that, even though the inoculation levels from the spiked combo bins were the same, it was possible to detect *E. coli* in ground beef samples taken much later in production from combo bin 3 at establishment 2. This again may be due to differences in grinding equipment not uniformly blending the product in combo bin 2. The nonuniform distribution carried over to the following combo bin 3.

Because we anticipated there to be substantial differences in contamination results between establishments 1 and 2 due to general equipment differences, we planned to further process the remaining ground beef chubs from establishment 2 at establishment 3, and repackage the ground beef into 3-lb loaves in overwrapped trays. This is not an unusual industry practice, as 10-lb ground beef chubs are used as material to fill customer orders for items such as different sized loaves and patties. Comparing the results from establishment 2 (the 10-lb chubs) with the results from establishment 3 (the 3-lb loaves), the level of *E. coli* detected decreased in the 3-lb loaves. Whereas 60 (62.5%) of 96 of the 10-lb ground beef chub samples from combo bin 2 from establishment 2 were positive, only 39 (41.5%) of 94 of the 3-lb ground beef loaf samples were positive. Further, in samples taken from combo bin 3, 12 (11.7%) of the 103 samples from establishment 2 were positive, representing the first 1,840 lb packaged following combo bin 2, while only 1 (1.1%) of the 93 samples at establishment 3 was positive, representing the first 72 of the 840 lb packaged.

The reduction in *E. coli*–positive samples from establishment 2 to establishment 3 may have a number of explanations. One may be the injury of bacteria during transport and handling; however our initial viability and detection characterization of our GFP *E. coli* strain suggests this is not the case. Another similar explanation is that the physical process of passing through the new loaf forming and packaging equipment caused stress and or injury to the *E. coli* that reduced its ability to proliferate; however we measured the presence of the high copy number *GFP* gene, therefore even slowly growing injured cells should have been detected. A final explanation is that these results are from different samples that came from a redistributed volume of previously packaged ground beef that may have resulted in more sparsely distributed organism.

Others have shown in studies of inoculated beef trimming that grinding results in random and nonrandom distributions of the inoculated *E. coli (13).* Since the inoculum level was the same for each of our trial, the model and make of grinding equipment plays a significant role in the distribution of the *E. coli* (Table 1). Equipment in the second establishment was less efficient at evenly mixing the bolus spiked into the middle of the combo bin 2 than the model and make of equipment at establishment 1.

In laboratory grinding studies using laboratory-scale and mid-size commercial grinding equipment *(7, 8)* it was

reported that although a linear relationship can be observed between inoculation and prevalence of *E. coli* O157:H7 in ground beef, the distribution patterns showed that the *E. coli* used in the experiments was not detectable in 3 to 43% of the ground beef produced from trimmings inoculated with 4 to 5 log CFU/g *E. coli* O157:H7. Also in the laboratory studies, the *E. coli* O157:H7 was found to persist in the grinders at the attachment point between the blade and die. The distribution of *E. coli* observed in our data was much greater than previous reports, most likely due to the fact that our study followed commercial practices of coarse grinding beef trimming then blending, before final grinding. This process more evenly distributes the *E. coli* compared with the direct grinding of trimmings in the previous studies. Indeed when other laboratory studies were performed using a table-top bowl cutter *(6)* the distribution of inoculated *E. coli* O157:H7 was reported to be more uniform and not significantly different throughout the ground beef produced. The studies reporting the use of the table-top bowl cutter followed the contaminated batch with a noncontaminated batch of beef trimming and found it to be thoroughly contaminated as well after passing through the equipment. No attempts in that study were made to process additional batches of trimming to determine how long the contamination persisted.

Our study was performed to provide information on the distribution and passage of *E. coli* O157:H7 through commercial ground beef processing equipment. We demonstrate a distribution in the establishments involved similar to that reported for a natural *E. coli* O157:H7 contamination event in a beef patty production facility *(12)*. In that report, most of the contaminated product was identified using culture isolation to be limited to an 80-min window of production, while additional testing using PCR methods identified subsequent sporadic positive samples. The passage of that contamination event through the equipment showed that like our inoculation study the contaminating *E. coli* passes through the equipment with the contaminated beef trimmings, as long as no additional rework of material occurs.

In summary, the differences in the detection patterns between the first and the second establishments indicate that at least a minimum of production from a full combo bin of beef trimming should be sent to lethality treatment at these establishments after the previous combo bin has tested positive for *E. coli* O157:H7. The results described here support decision making at establishments when a ground beef sample tests positive for pathogenic *E. coli*. Though it is desirable to replicate such a study several times in every establishment, such experiments are prohibitively costly and require expertise not commonly available. Thus, reliability on studies such as this are central to documenting established performance standards and actions. Our results support the current industry practice, after an *E. coli*–positive result is obtained, of sending to lethality treatment material produced from the identified combo bin and the immediate preceding and following combo bins of material. Because of the significance of the subject matter (detecting contaminated ground beef with *E. coli* O157:H7), we recommend that each ground beef–producing establishment conduct their own in-plant studies using the design described in this study. In the event that for whatever reasons, the establishments are unable to conduct their own in-plant validation study, it is highly recommended that following a contamination event, extensive sampling and testing of the combo bins (ground beef lots) before and after the contaminated lot be performed to provide support for the establishment decision-making process following a contamination event. It will be prudent to hold all products while intensive investigating is underway.

## ACKNOWLEDGMENTS

The authors thank Laura Vandehey, Christine Vandehey, Robert Schneider, Daniel Fix, and Dustin Sykora all of an IEH satellite laboratory for the technical assistance they provided. They also thank Drs. Gerald Zinstein for his editorial assistance and Sam Myoda for his technical assistance.

## REFERENCES

1. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2004. Characterization of O157:H7 and other *Escherichia coli* isolates recovered from cattle hides, feces, and carcasses. *J. Food Prot.* 67: 993–998.
2. Centers for Disease Control and Prevention. 1993. Preliminary report: Foodborne outbreak of *Escherichia coli* O157:H7 infections from hamburgers—western United States. *Morb. Mortal. Wkly. Rep.* 42: 85–86.
3. Cormack, B. P., R. H. Valdivia, and S. Falkow. 1996. FACS-optimized mutants of the green fluorescent protein (GFP). *Gene* 173: 33–38.
4. Davies, D. G., M. R. Parsek, J. P. Pearson, B. H. Iglewski, J. W. Costerton, and E. P. Greenberg. 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 280:295–298.
5. Durfee, T., R. Nelson, S. Baldwin, G. Plunkett, V. Burland, B. Mau, J. F. Petrosino, X. Qin, D. M. Muzny, M. Ayele, R. A. Gibbs, B. Csörgo, G. Pósfai, G. M. Weinstock, and F. R. Blattner. 2008. The complete genome sequence of *Escherichia coli* DH10B: insights into the biology of a laboratory workhorse. *J. Bacteriol.* 190:2597–2606.
6. Flores, R. A. 2004. Distribution of *Escherichia coli* O157:H7 in beef processed in a table-top bowl cutter. *J. Food Prot.* 67:246–251.
7. Flores, R. A., and T. E. Stewart. 2004. Empirical distribution models for *Escherichia coli* O157:H7 in ground beef produced by a mid-size commercial grinder. *J. Food Sci.* 69:M121–M126.
8. Flores, R. A., and M. L. Tamplin. 2002. Distribution patterns of *E. coli* O157:H7 in ground beef produced by a laboratory scale grinder. *J. Food Prot.* 12:1894–1902.
9. Kalchayanand, N., T. M. Arthur, J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2009. Effectiveness of 1,3-dibromo-5,5 dimethylhydantoin on reduction of *Escherichia coli* O157:H7- and *Salmonella*-inoculated fresh meat. *J Food Prot.* 72:151–156.
10. Koohmaraie, M., T. M. Arthur, J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S. D. Shackelford, and T. L. Wheeler. 2007. Interventions to reduce/eliminate *Escherichia coli* O157:H7 in ground beef. *Meat Sci.* 77:90–96.
11. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–625.
12. Pruett, W. P., Jr., T. Biela, C. P. Lattuada, P. M. Mrozinski, W. M. Barbour, R. S. Flowers, W. Osborne, J. O. Reagan, D. Theno, V. Cook, A. M. McNamara, and B. Rose. 2002. Incidence of *Escherichia coli* O157:H7 in frozen beef patties produced over an 8-hour shift. *J. Food Prot.* 65:1363–1370.

13. Reinders, R. D., R. De Jonge, and E. G. Evers. 2003. A statistical method to determine whether micro-organisms are randomly distributed in a food matrix, applied to coliforms and *Escherichia coli* O157 in minced beef. *Food Microbiol.* 20:297–303.
14. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17:7–15.
15. U.S. Department of Agriculture, Food Safety and Inspection Service. 2001. Risk assessment of the public health impact of *Escherichia coli* O157:H7 in ground beef. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/00-023N/00-023NReport.pdf. Accessed 1 June 2014.
16. U.S. Department of Agriculture, Food Safety and Inspection Service. 2014. Microbiological testing program for *Escherichia coli* O157:H7: individual positive results for raw ground beef (RGB) and RGB components. Available at: http://www.fsis.usda.gov/wps/portal/fsis/topics/data-collection-and-reports/microbiology/ec/testing-program-for-e-coli-o157h7-and-non-o157-stec. Accessed 1 June 2014.