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# Inactivation of Shiga Toxin–Producing O157:H7 and Non-O157:H7 Shiga Toxin–Producing *Escherichia coli* in Brine-Injected, Gas-Grilled Steaks†‡

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## ABSTRACT

We quantified translocation of *Escherichia coli* O157:H7 (ECOH) and non-O157:H7 verocytotoxigenic *E. coli* (STEC) into beef subprimals after brine injection and subsequently monitored their viability after cooking steaks cut therefrom. Beef subprimals were inoculated on the lean side with ca. 6.0 log CFU/g of a five-strain cocktail of rifampin-resistant ECOH or kanamycin-resistant STEC, and then passed once through an automatic brine-injector tenderizer, with the lean side facing upward. Brine solutions (9.9% ± 0.3% over fresh weight) consisted of 3.3% (wt/vol) of sodium tripolyphosphate and 3.3% (wt/vol) of sodium chloride, prepared both with ($Lac^+$, pH = 6.76) and without ($Lac^-$, pH = 8.02) a 25% (vol/vol) solution of a 60% potassium lactate–sodium diacetate syrup. For all samples injected with $Lac^-$ or $Lac^+$ brine, levels of ECOH or STEC recovered from the topmost 1 cm (i.e., segment 1) of a core sample obtained from tenderized subprimals ranged from ca. 4.7 to 6.3 log CFU/g; however, it was possible to recover ECOH or STEC from all six segments of all cores tested. Next, brine-injected steaks from tenderized subprimals were cooked on a commercial open-flame gas grill to internal endpoint temperatures of either 37.8°C (100°F), 48.8°C (120°F), 60°C (140°F), or 71.1°C (160°F). Regardless of brine formulation or temperature, cooking achieved reductions (expressed as log CFU per gram) of 0.3 to 4.1 of ECOH and 0.5 to 3.6 of STEC. However, fortuitous survivors were recovered even at 71.1°C (160°F) for ECOH and for STEC. Thus, ECOH and STEC behaved similarly, relative to translocation and thermal destruction: Tenderization via brine injection transferred both pathogens throughout subprimals and cooking highly contaminated, brine-injected steaks on a commercial gas grill at 71.1°C (160°F) did not kill all cells due, primarily, to nonuniform heating (i.e., cold spots) within the meat.

Over the past 30 years undercooked ground beef has quite arguably been the food vehicle most commonly attributable to illness from verocytotoxigenic *Escherichia coli*; however, since the 1990s, among meat products, mechanically and/or chemically tenderized beef (i.e., nonintact beef) has also been more commonly associated with human illness *(2, 3, 8, 9, 11, 20, 31, 40, 42)*. Illnesses attributed to contamination of foods, especially meat, with ECOH are well documented *(27, 33)*. In contrast, of some 14 outbreaks attributed to non-O157:H7 verocytotoxigenic *E. coli* (STEC) since 1990, only 5 were associated with a food vehicle, and none involved beef *(27)*. That being said, it is noteworthy that in August 2010, a Pennsylvania slaughtering and processing facility recalled some 8,500 lb (3,855.5 kg) of ground beef because of possible contamination with serotype O26 STEC *(26)* and its association with a cluster of illnesses in Maine and New York, thus making this the first reported outbreak attributed to a non-O157 serotype of *E. coli* in beef.

A wealth of general information has been published on diarrheagenic *E. coli (4, 29, 33)*, and considerable information exits for characterization and control of ECOH in foods *(5)*, including in tenderized–enhanced beef *(2, 3, 38)*, but there have been far fewer such studies published for STEC *(6, 7, 27)*. As is true for ECOH, any cells of STEC that might be present on the surface of whole-muscle meats could potentially be transferred into deeper tissue by tenderization. To date, a few studies have addressed and/or quantified internalization of ECOH, but not STEC, from the surface into the interior of beef subprimals after blade tenderization or chemical injection and/or monitored their subsequent viability after storage *(12, 25, 39, 45)*. Several investigators have also quantified thermal destruction of

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ECOH, but not STEC, in ground beef *(4, 17, 18, 28, 34)*, and fewer studies have been published on thermal inactivation of ECOH in mechanically or chemically tenderized beef *(13, 22, 32, 37, 39, 45)*. However, there have been relatively few, if any, publications on the comparative translocation of ECOH and STEC into blade- or chemically tenderized steaks and/or their fates after proper cooking.

Careful scrutiny of the available literature reveals that among the handful of illness-related recalls linked to nonintact beef, the incriminated products were most often linked with marinated or brine-injected products *(1, 31)*. Considering that about 18% of beef products sold at retail are mechanically tenderized–enhanced *(2)*, and that such products might be perceived by some individuals as being more like steaks (i.e., "intact") than like ground beef (i.e., "nonintact") and thus may not be properly cooked, there could be a potential threat to public health from undercooked tenderized–enhanced beef, especially since both Schmidt et al. *(36)* and Cox et al. *(10)* reported that between 40 and 58% of consumers ordered their steaks medium rare (60 to 62.8°C) to rare (54.4 to 57.2°C). Thus, a greater understanding of how beef is processed, that being tenderized versus injected versus marinated versus tumbled, as well as how it should be cooked, will lead to a more focused, comprehensive, and meaningful comparative risk assessment of intact and nonintact beef. Sufficient data have not been published, however, to conclusively state whether there is a greater risk from ECOH compared with STEC in nonintact beef products, and/or whether the method used for enhancement, namely injection versus mechanical tenderization, appreciably affects the safety of nonintact beef. Thus, the objective of this research was to comparatively and comprehensively fill data voids related to the translocation of ECOH and STEC into beef subprimals after enhancement via chemical injection and to quantify the subsequent lethality of Shiga toxin–producing cells of *E. coli* within steaks prepared from injected–inoculated subprimals after cooking on a commercial open-flame gas grill.

## MATERIALS AND METHODS

**Bacterial strains.** The five rifampin-resistant (100 μg/ml; Sigma Chemical Co., St. Louis, MO) strains of ECOH (USDA-FSIS 011-82, ATCC 43888, ATCC 43889, ATCC 43890, and USDA-FSIS 45756) and the five kanamycin-resistant (100 μg/ml; Sigma Chemical Co.) strains of STEC (B395 [serotype O111:H7], CDC 96-3285 [serotype O45], CDC 90-3128 [serotype O103:H2], CDC 97-3068 [serotype O121], and 83-75 [serotype O145:HNM]) used in this study were confirmed, cultured, and maintained as described previously *(22, 25)*. Of note, the kanamycin-resistant STEC strains were generated specifically for the purposes of the present study, whereas the rifampin-resistant ECOH strains were generated specifically for/in our previous study *(22)*.

**Inoculation and tenderization of subprimals.** Vacuum-packaged top butt beef subprimals (U.S. Department of Agriculture Institutional Meat Purchase Specifications no. 184; ca. 7 to 9 kg [15 to 20 lb] each) were obtained from a local wholesale distributor and stored at 4°C for up 7 days. Each subprimal was inoculated essentially as described previously *(22, 25)*. In brief, each subprimal was inoculated by pipetting 10 ml of either the ECOH or STEC bacterial suspensions over the lean-side surface of the subprimal to a target concentration of ca. 6.0 log CFU/g. The opening of each bag was then sealed with tape, and the inoculated subprimals were stored with the inoculated surface facing down for at least 30 min at 4°C to allow the weight of the subprimal to distribute the inoculum over the surface and to promote attachment of the cells to the meat. Next, one set of subprimals was passed once through an automatic brine injector–tenderizer (Koch/Gunther Injectamatic PI-21, Koch Equipment, Kansas City, MO), with the lean side facing upward. Another set of inoculated subprimals not chemically injected served as positive controls. Brine solutions were formulated as follows: (i) 3.3% (wt/vol) of sodium tripolyphosphate (Brifisol STP New, B.K. Giulini Corp., Simi Valley, CA) and 3.3% (wt/vol) of sodium chloride (Culinox 999 food-grade salt, Morton International, Inc., Chicago, IL) ($Lac^-$), or (ii) 3.3% of sodium tripolyphosphate (Brifisol STP New), 3.3% (wt/vol) of sodium chloride (Culinox 999), and 25% (vol/vol) of a 60% solution consisting of 56% potassium lactate and 4% sodium diacetate on a dry-solids basis (wt/wt; UltraLac KL-564, Hawkins, Inc., Minneapolis, MN) ($Lac^+$). After injection to a target level of ca. 10% over total weight, up to six core samples were obtained from each of the subprimals and cut into five or six consecutive segments, starting from the inoculated surface: Segments 1 to 4 comprised the top 4 cm, and segments 5 and 6 comprised the deepest 4 to 8 cm (Fig. 1A and 1B). Two trials were conducted for each pathogen cocktail, with a single trial consisting of two tenderized subprimals and two nontenderized subprimals (positive controls). For some experiments, tenderized subprimals were vacuum sealed and held at 4°C for up to 15 days to determine the effect of brine and refrigerated storage on the fate of ECOH and STEC. For the translocation matrix, 1 inoculation level × 2 brine formulations × 6 core samples per formulation × 2 trials per formulation × 2 pathogen types × 2 sampling days were tested, for a sum of 96 core samples tested.

**Cooking of chemically tenderized steaks.** Vacuum-packaged top butt beef subprimals were inoculated (ca. 6.0 log CFU/g) with either ECOH or STEC and chemically injected as described above. Steaks were cut from each inoculated, tenderized beef subprimal to a thickness of ca. 2.54 cm (1 in.) and stored for 0 or 15 days at 4°C. The thickness of the steak was selected based on our related publication *(25)*, wherein we reported that the thickness of steaks (2.54 versus 3.18 cm) did not significantly affect the extent of thermal inactivation of ECOH or STEC in blade-tenderized beef, and also because most people prefer steaks of medium thickness, that being 2.54 cm. Next, chemically injected steaks were cooked on a commercial open-flame gas grill (model XXE-4, Bakers Pride, New Rochelle, NY) to instantaneous internal endpoint temperatures of either 37.8°C (100°F), 48.8°C (120°F), 60°C (140°F), or 71.1°C (160°F). Beefsteaks were flipped at the approximate midpoint between the initial and target endpoint temperature. Two calibrated, stainless steel thermocouple probes (type T, model HQTQIN-116-18, Omega Engineering, Inc., Stamford, CT) were inserted into the approximate geometric center of each steak and used to measure the internal temperature of the beefsteaks during cooking; two additional type T thermocouples were used to monitor the temperature of the surface of the grill and the surrounding air, respectively. Steaks were removed from the grill when both thermocouples within a steak reached the target end temperature. The temperature of the steaks, the surface of the grill, and the ambient air ca. 30 cm above the grill grates were continuously monitored with an eight-channel thermocouple data logger (model OM-CP-OCTTEMP, Omega

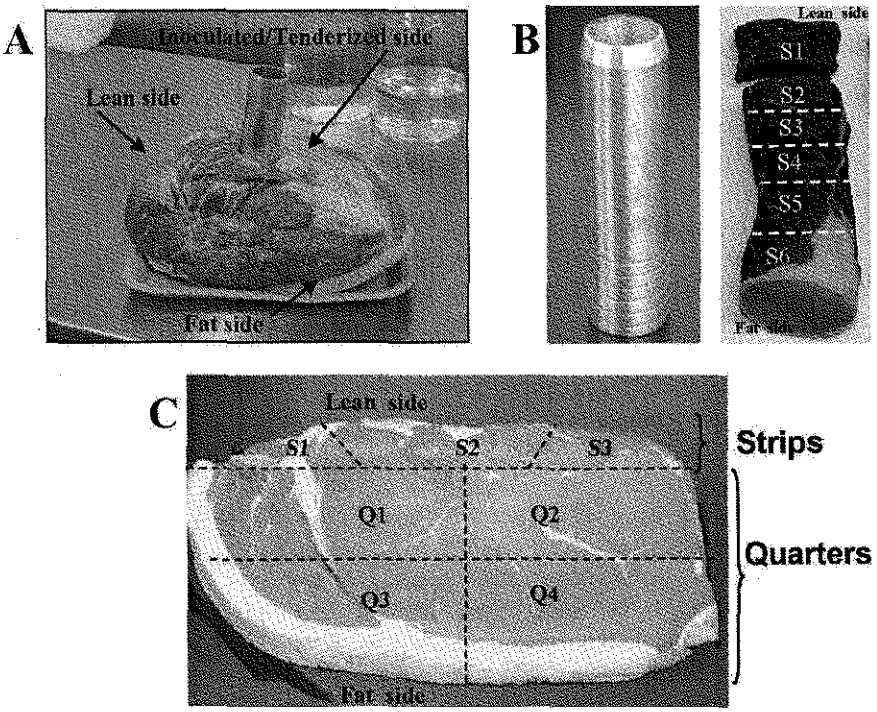


FIGURE 1. *(A) Coring of a beef subprimal. (B) Core apparatus and segmentation of a core sample into six consecutive segments. (C) Segmentation of a brine-injected steak into strips and quarters.*

Engineering, Inc.) at 5-s intervals. Inoculated subprimals that were not injected or cooked served as positive controls. To quantify thermal destruction, as shown in Figure 1C, both cooked and uncooked steaks were portioned into three strips (S1, S2, and S3), each about 1 to 2 cm in depth, and the remaining portion of the steak was cut into four approximately equal quarters (Q1, Q2, Q3, and Q4). Upon removal of a steak from the grill, a calibrated, handheld digital thermometer (model AccuTuff 340, Atkins Technical, Inc., Gainesville, FL) was used to obtain up to eight additional temperature readings from the strips, quarters, and geometric center of each steak. More specifically, when both thermocouples within a steak achieved the desired target temperature, the steak was removed from the grill and placed on a polystyrene foam packaging tray (Koch Supplies, Kansas City, MO), and temperature readings were taken from lean or fat portions of each strip and quarter, as well as from the approximate geometric center, of each steak. Three steaks were individually cooked at each target temperature, and three steaks were not cooked (positive controls). Each of the two trials consisted of 1 inoculation level × 2 brine formulations × 4 cooking temperatures × 3 steaks per temperature × 2 trials per formulation × 2 pathogen types × 2 sampling days, for a total of 192 steaks cooked.

**Microbiological analyses.** To quantify translocation, each of the five or six segments cut from core samples obtained from tenderized subprimals was weighed separately, diluted in 0.1% peptone water (Difco, BD, Sparks, MD), and macerated for 30 s by using a blender, as described previously *(25)*. The slurry was serially diluted in 0.1% peptone water and surface plated onto sorbitol MacConkey agar (Difco, BD) plates plus rifampin (100 μg/ml [SMACR]; Sigma Chemical Co.) or sorbitol MacConkey agar (Difco, BD) plates plus kanamycin (100 μg/ml [SMACK]; Sigma Chemical Co.) for ECOH and STEC, respectively, as described elsewhere *(22, 25)*. Plates were incubated at 37°C for 24 h, and surviving cells were enumerated. When negative for the pathogen by direct plating, samples were enriched as described before *(22, 25)*. The strips and quarters were weighed separately, macerated in a blender, and subsequently plated, with and without prior dilution in sterile 0.1% peptone water, onto SMACR and SMACK for ECOH and STEC, respectively, essentially as described previously *(22)*. Plates were incubated at 37°C for 24 h. When negative for the pathogen by direct plating, samples were enriched as done before *(25)*.

**Statistical analyses.** For phase I of the study, as performed previously *(22, 25)*, transfer of ECOH and STEC cells into the deeper tissues of subprimals via chemical tenderization was expressed (in percent) as the number of cells (CFU per gram) recovered separately from each of the five or six segments obtained from chemically tenderized subprimal cores, divided separately by the number of cells (CFU per gram) recovered from segment 1 of the cores obtained from the nontenderized, positive-control subprimals. The means and standard deviations for the levels of the pathogen recovered from each of the five or six segments and the cumulative totals recovered from core samples were calculated with the statistical function option provided with Excel 2003 software (Microsoft Corp., Redmond, WA). Analysis of variance (ANOVA) was used to determine the effects and interactions of the factors on the log translocation values. Differences in translocation observed for each brine formulation, storage day, sample type, and/or combinations thereof were considered significant by using the least significant difference (LSD) technique at a significance level of $P \leq 0.05$. For phase II of this study, the SAS system (version 9.2, SAS Institute Inc., Cary, NC) was used to determine statistically significant differences among pathogen viability during storage of subprimals or steaks, cooking temperatures, and sample types (i.e., strips versus quarters). Means and standard deviations in the cooking experiments were calculated from individual sets of data for each of the two separate trials at each of the four temperatures tested by using triplicate samples at each time interval. ANOVA was used to determine the effects and interactions of the factors on the log reduction values. Differences in lethality observed for each temperature, sample type, and/or combinations thereof were considered significant, using the LSD technique, with $P \leq 0.05$.

## RESULTS

**Translocation and distribution of ECOH and STEC in beef subprimals after tenderization by chemical injection.** The brine formulations tested contained salt and phosphate, both with ($Lac^+$ = pH 6.76 ± 0.07) and without ($Lac^-$ = pH 8.02 ± 0.25) lactate and diacetate. Brine was delivered at 9.92% ± 0.33% over the fresh, green weight of subprimals. The results validated that tenderization by chemical injection transfers cells of *E. coli* throughout the interior of beef subprimals, with the majority of the cells of ECOH (3.0 to 93.3%) and STEC (25.5 to 82.2%) remaining in the topmost 1 cm (Table 1). These results are in agreement with our prior work on blade tenderization *(23, 24),* wherein we also reported that the majority of cells of ECOH remained in the topmost 1 cm after tenderization. In general, there were no discernible ($P \geq 0.05$) differences in pathogen viability or in translocation of ECOH or STEC cells related to the presence or absence of lactate-diacetate in the brine, either within a couple of hours after injection or after refrigerated storage for up to 15 days. Although, there was no significant ($P \geq 0.05$) effect of refrigerated storage on pathogen viability in chemically injected steaks, there were generally lower numbers of both ECOH and STEC remaining after 15 days of refrigerated storage compared with starting levels.

Regardless of brine formulation or storage time, in general, there were no significant ($P \geq 0.05$) differences in the levels of ECOH or STEC recovered from segment 1 of the tenderized subprimals compared with levels of these pathogens recovered from segment 1 of the core samples obtained from nontenderized, positive-control subprimals. Levels of ECOH or STEC (Table 1) recovered from segment 1 ranged from about 4.7 to 6.3 and 5.5 to 6.2 CFU/g, respectively. For subprimals injected with $Lac^+$ or $Lac^-$ brine, the percentages of cells of ECOH or STEC in segment 2 were ca. 5.6- to 23.2-fold or 7.3- to 15.3-fold lower, respectively, than the percentages of cells recovered from segment 1. A significant ($P \leq 0.05$) linear decrease in pathogen levels was observed from segments 2 through 6, but it was possible to recover cells of ECOH and STEC from all six segments of all cores tested. Total levels of ECOH and STEC transferred into all six segments ranged from 4.1 to >100% and 30.6 to 99.6%, respectively. Levels of ECOH or STEC recovered from all six segments of all cores tested ranged from about 5.1 to 6.4 and 5.6 to 6.2 CFU/g, respectively. No appreciable difference between ECOH and STEC in overall translocation was observed, but lesser levels of ECOH and STEC were internalized into the deeper interior tissues of the meat (segments 2 through 6), compared with the surface (segment 1). Experiments are in progress to evaluate additional brine formulations for potential effects on ECOH and STEC during subsequent storage and/or cooking of nonintact beef.

**Thermal inactivation of ECOH and STEC in chemically tenderized beefsteaks after cooking on a gas grill.** The average come-up times required to reach target internal temperatures of 37.8, 48.9, 60.0, and 71.1°C in brine-injected steaks from tenderized subprimals were ca. 4.7 ± 0.7, 6.3 ± 0.9, 11.0 ± 1.20, and 17.4 ± 2.5 min, respectively. Likewise, the average grill and air temperatures (total of 14,108 readings) were ca. 193.1 ± 18.8°C and 98.1 ± 12.2°C, respectively. Regardless of brine formulation or storage time, as expected, the level of inactivation for ECOH and STEC increased significantly ($P \leq 0.05$) with increasing cooking temperatures between 37.8 and 71.1°C. In addition, regardless of brine formulation, storage time, or cooking temperatures, there were no statistical ($P \geq 0.05$) differences in lethality between ECOH and STEC. In general, for a given formulation and given storage time, regardless of the cooking temperature, no statistical ($P \geq 0.05$) differences were observed among the three strips or among the four quarters of steaks with respect to the extent of thermal inactivation of ECOH or STEC (data not shown). For a given cooking temperature and storage time, with the exception of strips (topmost 1 cm; S1 plus S2 plus S3) from steaks cooked on day 0 to a target internal temperature of 71.1°C, brine formulation did not ($P \geq 0.05$) appreciably affect lethality of ECOH for strips (S1 plus S2 plus S3), or for quarters (Q1 plus Q2 plus Q3 plus Q4), or for total steaks (all strips plus all quarters) (Table 2). Similarly, for a given cooking temperature and storage time or formulation, with the exception of quarters from steaks injected with $Lac^+$ brine that were stored at 4°C for 15 days and cooked at 60.0°C, no statistical differences ($P \geq 0.05$) in the extent of thermal inactivation of STEC were observed for strips (S1 plus S2 plus S3), for quarters (Q1 plus Q2 plus Q3 plus Q4), or for the summation of both strips and quarters for steaks injected with $Lac^+$ or $Lac^-$ brine that were subsequently stored refrigerated for 2 weeks and then cooked (Table 3). In addition, for a given cooking temperature and formulation, although there were generally lower numbers of ECOH (Table 2) and STEC (Table 3) remaining after 15 days of refrigerated storage compared with starting levels, no significant ($P \geq 0.05$) effect of storage on lethality of ECOH and STEC was observed for strips (S1 plus S2 plus S3), for quarters (Q1 plus Q2 plus Q3 plus Q4), or for total steaks (all strips plus all quarters) that were stored for up to 15 days at 4°C.

Storage of steaks injected with $Lac^+$ and $Lac^-$ brine for 15 days at 4°C reduced the levels of ECOH by 0.7 and 1.1 log CFU/g, respectively, whereas the levels of STEC increased slightly by 0.1 and 0.3 log CFU/g. In addition, regardless of storage time, brine formulation, or cooking temperatures, average total reductions ranged from 0.3 to 4.1 log CFU/g for ECOH and from 0.5 to 3.6 log CFU/g for STEC. Although appreciably more cells of ECOH and STEC were recovered from steaks cooked to lower target internal temperatures (37.8 or 48.9°C) compared with those that were cooked to higher target internal temperatures (60.0 or 71.1°C), it was possible to recover cells of ECOH and STEC either by direct plating or by enrichment at all temperatures tested (Tables 4 and 5). It was possible to recover fortuitous survivors from chemically injected steaks after cooking, most likely because of the existence of cold spots (nonhomogeneous heating) within strips or quarters of some steaks. Evidence in support of this contention was

TABLE 1. *Recovery of ECOH and STEC (ca. 6.0 log CFU/g) from segmented core samples from chemically injected subprimals*

| | | ECOH | | | | STEC | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 0 | | Day 15 | | Day 0 | | Day 15 | |
| Brine formulation | Segment no. | Log CFU/g recovered | % transfer[a] | Log CFU/g recovered | % transfer | Log CFU/g recovered | % transfer | Log CFU/g recovered | % transfer |
| $Lac^-$ | Control[b] | 6.51 ± 0.37 A[c] | | 6.28 ± 2.12 A | | 6.31 ± 0.34 A | | 5.78 ± 0.41 A | |
| | 1 | 5.78 ± 0.41 A | 18.79 | 4.70 ± 1.04 B | 3.04 | 6.19 ± 0.38 A | 76.87 | 5.70 ± 0.47 A | 82.20 |
| | 2 | 4.42 ± 0.37 B | 0.81 | 4.01 ± 1.37 BC | 0.54 | 5.02 ± 0.60 B | 5.13 | 4.81 ± 0.80 B | 10.66 |
| | 3 | 3.81 ± 0.46 BC | 0.20 | 3.42 ± 1.03 CD | 0.14 | 4.09 ± 0.53 BC | 0.62 | 4.04 ± 0.59 BC | 1.79 |
| | 4 | 3.34 ± 0.53 C | 0.07 | 2.87 ± 0.37 D | 0.04 | 3.33 ± 0.65 C | 0.11 | 3.57 ± 0.61 C | 0.61 |
| | 5 | 4.84 ± 1.19 B | 2.16 | 2.97 ± 0.77 D | 0.05 | 4.64 ± 0.94 C | 2.14 | 3.59 ± 0.57 C | 0.64 |
| | 6 | 4.30 ± 0.94 B | 0.62 | 3.71 ± 1.28 CD | 0.27 | 4.11 ± 0.64 BC | 0.64 | 4.37 ± 0.68 BC | 3.70 |
| Total[d] | | 5.86 | 22.64 | 5.08 | 4.08 | 6.24 | 85.51 | 5.78 | 99.62 |
| $Lac^+$ | Control | 6.58 ± 0.31 A | | 5.98 ± 0.77 A | | 6.32 ± 0.33 A | | 6.11 ± 1.33 A | |
| | 1 | 6.32 ± 0.81 AB | 54.55 | 5.92 ± 0.38 A | 93.25 | 5.74 ± 0.41 A | 26.39 | 5.52 ± 0.77 A | 25.53 |
| | 2 | 5.53 ± 1.29 B | 8.85 | 4.89 ± 0.74 B | 8.10 | 4.55 ± 1.70 B | 1.72 | 4.65 ± 1.06 B | 3.47 |
| | 3 | 4.39 ± 0.97 CD | 0.64 | 4.37 ± 1.14 BC | 2.48 | 4.17 ± 1.39 BC | 0.72 | 3.76 ± 1.12 BC | 0.44 |
| | 4 | 3.77 ± 0.55 CD | 0.16 | 4.10 ± 0.92 CD | 1.33 | 3.59 ± 0.52 C | 0.19 | 3.08 ± 0.55 C | 0.09 |
| | 5 | 3.61 ± 0.75 D | 0.11 | 3.53 ± 1.12 D | 0.36 | 3.74 ± 0.64 C | 0.26 | 3.51 ± 0.64 C | 0.25 |
| | 6 | 4.42 ± 0.71 C | 0.69 | 4.38 ± 0.72 BC | 2.54 | 4.42 ± 0.88 D | 1.28 | 4.16 ± 0.81 B | 1.13 |
| Total | | 6.40 | 64.98 | 6.01 | 108.06[e] | 5.80 | 30.56 | 5.60 | 30.91 |

[a] Percent transfer was calculated as (CFU per gram of tenderized subprimal core segment divided by CFU per gram of segment 1 of nontenderized subprimal core) × 100.
[b] Control samples are segment 1 of nontenderized subprimal cores.
[c] For a given formulation and storage day, means with different letters within columns are significantly ($P \leq 0.05$) different by the LSD test.
[d] Total level of ECOH or STEC (log CFU per gram or percent) transferred into all six segments of a core sample.
[e] Total percent exceeded 100% because of sampling variability of control (nontenderized) treatment.

TABLE 2. *Levels of ECOH recovered from nonintact steaks inoculated with ca. 6.0 log CFU/g before and after cooking*

| Cooking temp (°C) | Storage (days) | ECOH level (log CFU/g ± SD) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Strips (S1 plus S2 plus S3) | | Quarters (Q1 plus Q2 plus Q3 plus Q4) | | Total steak (all strips plus all quarters)[a] | |
| | | Lac$^-$ | Lac$^+$ | Lac$^-$ | Lac$^+$ | Lac$^-$ | Lac$^+$ |
| Uncooked | 0 | 6.36 ± 0.24 A[b] | 6.25 ± 0.26 A | 5.24 ± 0.01 A | 5.25 ± 0.10 A | 6.40 ± 0.22 A | 6.30 ± 0.24 A |
| | 15 | 5.25 ± 0.14 A | 5.46 ± 0.41 A | 4.26 ± 0.02 A | 4.75 ±0.46 A | 5.30 ± 0.13 A | 5.60 ± 0.24 A |
| 37.8 | 0 | 5.11 ± 0.04 AB | 5.24 ± 0.20 AB | 4.37 ± 0.36 AB | 4.45 ± 0.71 AB | 5.19 ± 0.03 AB | 5.32 ± 0.28 AB |
| | 15 | 4.92 ± 0.38 A | 4.97 ± 0.03 A | 3.88 ± 0.22 AB | 4.31 ± 0.28 AB | 4.96 ± 0.36 A | 5.06 ± 0.03 A |
| 48.9 | 0 | 4.89 ± 0.23 B | 4.30 ± 0.56 BC | 3.85 ± 0.74 BC | 3.79 ± 0.16 B | 4.94 ± 0.28 B | 4.44 ± 0.46 BC |
| | 15 | 4.14 ± 1.81 AB | 4.29 ± 0.06 AB | 3.06 ± 1.72 ABC | 3.52 ± 0.13 AB | 4.17 ± 1.80 AB | 4.36 ± 0.07 AB |
| 60.0 | 0 | 4.24 ± 0.40 B | 4.19 ± 0.27 BC | 2.76 ± 1.03 CD | 3.69 ± 0.48 B | 4.26 ± 0.42 B | 4.32 ± 0.32 BC |
| | 15 | 2.91 ± 1.23 BC | 3.06 ± 1.61 BC | 2.84 ± 0.63 BC | 3.15 ± 0.11 B | 3.55 ± 0.35 BC | 3.67 ± 0.81 BC |
| 71.1 | 0 | 1.47 ± 0.07 C | 3.32 ± 0.29 C | 2.09 ± 0.78 D | 1.93 ± 0.48 B | 2.25 ± 0.59 C | 3.34 ± 0.30 C |
| | 15 | 2.66 ± 1.12 C | 2.48 ± 1.42 C | 2.07 ± 0.87 C | 1.64 ± 0.37 B | 2.77 ± 1.07 C | 2.61 ± 1.25 C |

[a] ECOH levels reported are the summation of total CFU from all strips plus all quarters and represents the results from two trials and 42 pieces of meat.

[b] For a given formulation and storage time, temperature means with different letters within a column are significantly ($P \leq 0.05$) different by the LSD test.

obtained by taking up to eight independent temperature readings from each steak immediately after it was removed from the grill (Table 6). The results revealed that, although on average the target endpoint temperatures were achieved or exceeded, the range in temperature for a given target endpoint temperature varied considerably. Of note, for 71.1°C (160°F), the recommended minimum internal instantaneous cooking temperature *(41, 43)*, the temperatures within steaks, that being for individual strips and/or quarters, ranged from 48.3 to 102.2°C (119 to 216°F).

## DISCUSSION

Historically, strains of O157:H7 are the most commonly recognized serotype of *E. coli* associated with foodborne illness. In recent years, however, non-O157 Shiga toxin–producing strains have also been linked to outbreaks and cases worldwide *(7, 27)*. Our group and other investigators validated that mechanical tenderization of beef forces cells of Shiga toxin–producing *E. coli* into the deeper tissue of the meat *(12, 15, 16, 25)*. Of particular note, colleagues at Kansas State University (Manhattan) reported that 3 to 4% of surface-inoculated ECOH were transferred into the approximate geometric center of beef subprimals by blade tenderization *(32, 39)*. Other investigators also confirmed that tenderization transfers cells into the interior of meat, but with decreasing levels correlated with the depth to which the blade penetrates the meat *(38)*. In addition, Gill and colleagues *(14)* subsequently reported that injection in combination with mechanical tenderization increased contamination of beef primal cuts with *Listeria innocua* by 1,000-fold. The results herein for chemical injection are in

TABLE 3. *Levels of STEC recovered from nonintact steaks inoculated with ca. 6.0 log CFU/g before and after cooking*

| Cooking temp (°C) | Storage (days) | STEC level (log CFU/g ± SD) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Strips (S1 plus S2 plus S3) | | Quarters (Q1 plus Q2 plus Q3 plus Q4) | | Total steak (all strips plus all quarters)[a] | |
| | | Lac$^-$ | Lac$^+$ | Lac$^-$ | Lac$^+$ | Lac$^-$ | Lac$^+$ |
| Uncooked | 0 | 5.71 ± 0.18 A[b] | 5.94 ± 0.19 A | 4.70 ± 0.34 A | 4.97 ± 0.22 A | 5.77 ± 0.19 A | 5.99 ± 0.15 A |
| | 15 | 6.02 ± 0.09 A | 6.04 ± 0.14 A | 4.86 ± 0.43 A | 5.01 ± 0.10 A | 6.06 ± 0.12 A | 6.09 ± 0.12 A |
| 37.8 | 0 | 4.95 ± 0.28 AB | 5.43 ± 0.14 AB | 3.83 ± 0.86 AB | 4.37 ± 0.27 AB | 4.99 ± 0.32 AB | 5.46 ± 0.15 AB |
| | 15 | 4.67 ± 0.25 AB | 4.60 ± 0.27 B | 4.21 ± 0.67 AB | 3.30 ± 0.11 B | 4.82 ± 0.36 AB | 4.61 ± 0.26 B |
| 48.9 | 0 | 4.42 ± 0.46 AB | 4.49 ± 0.89 B | 3.61 ± 0.25 AB | 4.22 ± 1.06 AB | 4.48 ± 0.43 AB | 4.68 ± 0.95 BC |
| | 15 | 4.21 ± 0.07 BC | 3.92 ± 0.16 BC | 4.09 ± 0.70 ABC | 3.42 ± 0.27 B | 4.51 ± 0.34 BC | 4.04 ± 0.19 BC |
| 60.0 | 0 | 4.05 ± 0.48 BC | 4.07 ± 1.55 B | 3.03 ± 0.65 BC | 3.38 ± 0.99 B | 4.09 ± 0.50 B | 4.18 ± 1.45 BC |
| | 15 | 3.55 ± 0.19 BC | 2.38 ± 0.06 D | 2.99 ± 0.54 BC | 1.68 ± 0.42 B | 3.66 ± 0.22 BC | 2.46 ± 0.53 D |
| 71.1 | 0 | 2.71 ± 1.41 C | 2.63 ± 0.44 C | 2.01 ± 0.82 C | 1.79 ± 0.43 B | 2.81 ± 1.26 C | 2.69 ± 0.43 C |
| | 15 | 2.83 ± 1.01 C | 2.81 ± 1.19 CD | 2.85 ± 0.22 C | 2.37 ± 1.31 BC | 3.31 ± 0.34 C | 2.94 ± 1.20 CD |

[a] STEC levels reported are the summation of total CFU from all strips plus all quarters and represents the results from two trials and 42 pieces of meat.

[b] For a given formulation and storage time, temperature means with different letters within a column are significantly ($P \leq 0.05$) different by the LSD test.

TABLE 4. *Postenrichment recovery rates for ECOH from cooked steak portions failing to yield the pathogen by direct plating*

| Brine formulation | Temp (°C) | Storage (days) | Strips (S1 plus S2 plus S3)[a] | Quarters (Q1 plus Q2 plus Q3 plus Q4)[b] |
|---|---|---|---|---|
| Lac$^-$ | 37.8 | 0 | 18/18 direct plating[c]<br>0/0 enrichment[e] | 24/24 direct plating[d]<br>0/0 enrichment[f] |
| | | 15 | 18/18 direct plating<br>0/0 enrichment | 23/24 direct plating<br>1/1 enrichment |
| | 48.9 | 0 | 18/18 direct plating<br>0/0 enrichment | 23/24 direct plating<br>1/1 enrichment |
| | | 15 | 12/18 direct plating<br>6/6 enrichment | 17/24 direct plating<br>6/7 enrichment |
| | 60.0 | 0 | 16/18 direct plating<br>1/2 enrichment | 17/24 direct plating<br>6/7 enrichment |
| | | 15 | 10/18 direct plating<br>6/8 enrichment | 14/24 direct plating<br>9/10 enrichment |
| | 71.1 | 0 | 8/18 direct plating<br>5/10 enrichment | 5/24 direct plating<br>6/19 enrichment |
| | | 15 | 6/18 direct plating<br>4/12 enrichment | 7/24 direct plating<br>6/17 enrichment |
| Lac$^+$ | 37.8 | 0 | 18/18 direct plating[c]<br>0/0 enrichment[e] | 24/24 direct plating[d]<br>0/0 enrichment[f] |
| | | 15 | 18/18 direct plating<br>0/0 enrichment | 24/24 direct plating<br>0/0 enrichment |
| | 48.9 | 0 | 17/18 direct plating<br>1/1 enrichment | 22/24 direct plating<br>2/2 enrichment |
| | | 15 | 16/18 direct plating<br>2/2 enrichment | 22/24 direct plating<br>1/2 enrichment |
| | 60.0 | 0 | 15/18 direct plating<br>2/3 enrichment | 20/24 direct plating<br>4/4 enrichment |
| | | 15 | 13/18 direct plating<br>1/5 enrichment | 18/24 direct plating<br>3/6 enrichment |
| | 71.1 | 0 | 11/18 direct plating<br>4/7 enrichment | 7/24 direct plating<br>14/17 enrichment |
| | | 15 | 9/18 direct plating<br>4/9 enrichment | 7/24 direct plating<br>2/17 enrichment |

[a] Enrichment and direct plating results for a composite of strips 1, 2, and/or 3 (summation of 3 steaks × 3 strips × 2 trials; 18 strips total per each temperature) obtained from cooked steaks.

[b] Enrichment and direct plating results for a composite of quarters 1, 2, 3, and/or 4 (summation of 3 steaks × 4 quarters × 2 trials; 24 quarters total per each temperature) obtained from cooked steaks.

[c] Number of strip composite samples from which ECOH were recovered by direct plating/total number of composite samples direct plated.

[d] Number of quarter composite samples from which ECOH were recovered by direct plating/total number of composite samples direct plated.

[e] Number of strip composite samples from which ECOH were recovered by enrichment/total number of composite samples enriched.

[f] Number of quarter composite samples from which ECOH were recovered by enrichment/total number of composite samples enriched.

agreement with the above-mentioned studies, in that most cells (3.0 to 93.3%) remained in the topmost 1 cm of beef subprimals after tenderization, and that both pathogens were transferred throughout the subprimal in decreasing order into the lower segments, that being segments 2 through 6. In general, we observed an increase in percent recovery in segment 6 compared with segments 3, 4, or 5. Although we have no data to support this contention, it is possible that in addition to the physical impingement or transfer of cells into the interior of the subprimals by the blades, any back pressure and/or vacuum created by the withdrawal of the blades from subprimals during tenderization could force additional cells into the deepest tissue of the meat, that being segment 6. Further studies are warranted to verify how and why more cells are recovered from segment 6 compared with segments 3, 4, and 5, and to confirm if this observation is reproducible and/or statistically relevant. Regardless, our data also revealed, for the first time, that in general, there were no discernible differences in the extent or levels of translocation between ECOH and STEC after chemical injection and/or in their viability during subsequent refrigerated storage of nonintact beef subprimals. The brine formulations used in the present study, which contained salt and phosphate, both with and without lactate and diacetate, were selected based on discussions with collaborators in the meat industry to be representative of what several commercial processors were using at the time this study was initiated, including a processor that supplied a major/global retail chain. It would be of value to evaluate other formulations and to test different salts, such as calcium, in

TABLE 5. *Postenrichment recovery rates for STEC from cooked steak portions failing to yield the pathogen by direct plating*

| Brine formulation | Temp (°C) | Storage (days) | Strips (S1 plus S2 plus S3)[a] | Quarters (Q1 plus Q2 plus Q3 plus Q4)[b] |
|---|---|---|---|---|
| $Lac^-$ | 37.8 | 0 | 17/18 direct plating[c] | 24/24 direct plating[d] |
| | | | 1/1 enrichment[e] | 0/0 enrichment[f] |
| | | 15 | 18/18 direct plating | 24/24 direct plating |
| | | | 0/0 enrichment | 0/0 enrichment |
| | 48.9 | 0 | 16/18 direct plating | 22/24 direct plating |
| | | | 1/2 enrichment | 2/2 enrichment |
| | | 15 | 17/18 direct plating | 20/24 direct plating |
| | | | 1/1 enrichment | 2/4 enrichment |
| | 60.0 | 0 | 14/18 direct plating | 14/24 direct plating |
| | | | 4/4 enrichment | 2/10 enrichment |
| | | 15 | 13/18 direct plating | 12/24 direct plating |
| | | | 1/5 enrichment | 2/12 enrichment |
| | 71.1 | 0 | 13/18 direct plating | 9/24 direct plating |
| | | | 1/5 enrichment | 7/15 enrichment |
| | | 15 | 9/18 direct plating | 7/24 direct plating |
| | | | 1/9 enrichment | 0/17 enrichment |
| $Lac^+$ | 37.8 | 0 | 18/18 direct plating[c] | 24/24 direct plating[d] |
| | | | 0/0 enrichment[e] | 0/0 enrichment[f] |
| | | 15 | 17/18 direct plating | 23/24 direct plating |
| | | | 1/1 enrichment | 1/1 enrichment |
| | 48.9 | 0 | 18/18 direct plating | 24/24 direct plating |
| | | | 0/0 enrichment | 0/0 enrichment |
| | | 15 | 16/18 direct plating | 21/24 direct plating |
| | | | 1/2 enrichment | 0/3 enrichment |
| | 60.0 | 0 | 18/18 direct plating | 18/24 direct plating |
| | | | 0/0 enrichment | 4/6 enrichment |
| | | 15 | 11/18 direct plating | 13/24 direct plating |
| | | | 1/7 enrichment | 5/11 enrichment |
| | 71.1 | 0 | 9/18 direct plating | 6/24 direct plating |
| | | | 3/9 enrichment | 8/18 enrichment |
| | | 15 | 12/18 direct plating | 8/24 direct plating |
| | | | 0/6 enrichment | 6/16 enrichment |

[a] Enrichment and direct plating results for a composite of strips 1, 2, and/or 3 (summation of 3 steaks × 3 strips × 2 trials; 18 strips total per each temperature) obtained from cooked steaks.
[b] Enrichment and direct plating results for a composite of quarters 1, 2, 3, and/or 4 (summation of 3 steaks × 4 quarters × 2 trials; 24 quarters total per each temperature) obtained from cooked steaks.
[c] Number of strip composite samples from which STEC were recovered by direct plating/total number of composite samples direct plated.
[d] Number of quarter composite samples from which STEC were recovered by direct plating/total number of composite samples direct plated.
[e] Number of strip composite samples from which STEC were recovered by enrichment/total number of composite samples enriched.
[f] Number of quarter composite samples from which STEC were recovered by enrichment/total number of composite samples enriched.

combination with other antimicrobials, including organic acids, in the brine used for injection to better tenderize and possibly protect nonintact products, with respect to spoilage and pathogenic microbes. To this end, Yoon et al. *(45)* reported that brines containing selected organic acids (e.g., acetic, citric) when used in combination with chemical tenderizers (e.g., calcium chloride) generated greater thermal destruction of ECOH during subsequent cooking of tenderized and enhanced nonintact raw beef. As noted by Shen et al. *(37)*, the choice of cooking appliance also affected thermal inactivation of ECOH in their model nonintact beef system.

Given the apparent rise in the United States in illnesses linked to verocytotoxigenic *E. coli* displaying serotypes other than ECOH *(35)*, considerable efforts have been directed to obtain information on the behavior of STEC in foods to facilitate the development of appropriate control strategies. The limited data collected thus far suggest that certain STEC might behave similarly to ECOH at the physiological level when challenged by food-relevant conditions of temperature, pH, salt, and water content *(27)*. As summarized by Mathusa et al. *(27)*, desiccation resistance on paper disks and in dry foods was not serotype dependent for comparisons among O157, O26, and O111 strains; there were no significant differences on beef tissue surfaces between ECOH and STEC in response to acidified sodium chlorite (1,000 ppm), octanoic acid (9,000 ppm), and peracetic acid (200 ppm), and in general, STEC displayed similar heat resistance (in apple juice) to ECOH. Our data are in general agreement with the above-mentioned studies with both ECOH and STEC showing similar reductions (0.3 to 4.1 log CFU/g) after cooking injected

TABLE 6. *Average temperature and range indentified for end target temperatures after cooking brine-injected beefsteaks on a gas grill*

| Brine formulation | Target cooking temp (°C)[a] | Storage (days) | Avg (range) temp achieved (°C)[b] | |
|---|---|---|---|---|
| | | | ECOH | STEC |
| $Lac^-$ | 37.8 | 0 | 47.2 (32.2–61.1) | 48.9 (31.7–70.0) |
| | | 15 | 47.2 (23.9–58.9) | 52.8 (40.0–77.2) |
| | 48.9 | 0 | 58.3 (27.2–81.1) | 58.3 (37.8–76.7) |
| | | 15 | 57.2 (33.3–72.2) | 57.2 (43.9–76.7) |
| | 60.0 | 0 | 66.1 (43.3–91.1) | 69.4 (49.4–97.2) |
| | | 15 | 68.3 (48.3–80.0) | 69.4 (55.6–82.2) |
| | 71.1 | 0 | 73.9 (63.9–88.9) | 77.2 (61.1–89.4) |
| | | 15 | 73.3 (48.3–91.6) | 76.1 (65.0–95.0) |
| $Lac^+$ | 37.8 | 0 | 45.5 (25.0–72.2) | 46.7 (28.9–67.2) |
| | | 15 | 49.6 (34.4–72.2) | 51.5 (37.8–71.1) |
| | 48.9 | 0 | 54.4 (27.2–70.0) | 58.3 (31.1–77.7) |
| | | 15 | 59.6 (35–73.3) | 56.7 (35.0–80.5) |
| | 60.0 | 0 | 62.4 (42.2–78.3) | 66.1 (43.9–83.9) |
| | | 15 | 69.3 (48.9–83.9) | 70.0 (52.2–82.2) |
| | 71.1 | 0 | 77.2 (64.4–87.8) | 80.5 (62.7–88.9) |
| | | 15 | 76.8 (59.4–89.4) | 80.0 (59.4–102.2) |

[a] The target cooking temperature was the temperature achieved by two independent, internal thermocouples within each steak.
[b] Values are the average of eight independent temperature readings within each steak after removing steaks from the grill (two trials, three steaks per trial, and 8 readings per steak for a total of 48 readings).

steaks on a gas grill. In related studies, we observed no discernible differences in thermal resistance between ECOH and STEC after cooking blade-tenderized steaks on a gas grill (data not shown). Moreover, in general, higher temperatures generated greater lethality (>2.5 log CFU/g), and there were no apparent differences in lethality based on thickness (1.0 versus 1.5 in. [2.5 to 3.8 cm]) of blade-tenderized steaks in our related studies (data not shown). Shen and colleagues *(37)* reported *E. coli* reductions of 1.1 to 4.2 log CFU/g after broiling or roasting of a simulated restructured beef product containing sodium chloride and sodium tripolyphosphate, whereas researchers at Kansas State University reported *E. coli* reductions of 3.0 to 6.0 log CFU/g *(39)* in blade-tenderized beefsteaks after cooking on a gas grill and an electric skillet. In related studies on ground beef, other investigators reported *E. coli* reductions of 1.5 to 5.5 log CFU/g after cooking to 60 or 68.3°C *(17, 18)*. Such differences among studies could be attributed, at least in part, to differences in strains, cooking methods–appliances, types of meat, and/or plating media. Regardless, federal agencies have specified cooking parameters deemed adequate for assuring the safety of red meat and poultry products *(41, 43)*. The existing literature and our findings suggest that interventions effective against ECOH (or even *Salmonella*) would be equally as effective toward STEC *(27)*. These findings will assist in the development of comparative risk assessments of intact and nonintact beef products.

In the present study, fortuitous survivors were recovered from chemically injected steaks after cooking. It must be stated, however, that non-ecologically relevant levels of ECOH and STEC were surface inoculated onto beef subprimals and, as such, cooking these highly contaminated steaks on a gas grill, even when the recommended temperature of 71.1°C (160°F) was achieved, was not sufficient to kill all cells of either of these pathogen cocktails. Fortuitous survivors were most likely observed because not all portions of the steak achieved the target end temperature, due to a reduction in heat penetration from the insulating effects of fat or connective tissue, or the added moisture from injection, and/or from the intrinsic variability in temperature at the cooking surface. As discussed, even when the target end temperature was achieved as recorded by two independent thermocouples inserted into the same steak, the observed range of temperatures, as subsequently measured postcooking by using a handheld temperature monitor, varied considerably despite the fact that the overall average temperatures substantially exceeded the intended target temperatures. This could be significant from the public health perspective, as it is likely that most people will take only a single measurement of temperature, if any, to determine doneness. Our findings are of immediate and appreciable relevance because we evaluated conditions likely practiced by consumers, and because we tenderized and cooked steaks by using commercial apparatuses rather than small-scale, laboratory-controlled conditions, and/or a model meat system to simulate tenderization and/or a water bath to simulate cooking. Given the nonhomogeneous nature of steaks and the related physics–kinetics associated with cooking, it is likely that not all portions of the meat achieved the target temperature; however, this would result in significant reductions in pathogen numbers (e.g., 2.5 to 5.0 log), albeit while allowing for the recovery of fortuitous survivors, as has been reported elsewhere *(13, 24, 37, 45)*. Thus, it may be necessary to evaluate slightly higher endpoint cooking temperatures, with or without a holding time, to ensure total elimination of ECOH and STEC. Alternatively, given that the risk might never be totally eliminated, and the extremely low prevalence or levels of ECOH and STEC likely to be encountered outside the

laboratory setting *(3, 19, 44)*, a 1.0- to 2.0-log reduction achieved by cooking could still have an appreciable and positive effect on public health. Future efforts should be directed to generate *D*-values in synthetic media or model meat systems for the individual strains composing these pathogen cocktails.

Although the National Advisory Committee on Microbiological Criteria for Foods *(30)* concluded that blade-tenderized, nonintact beefsteaks do not pose a greater risk to public health from ECOH than do intact beefsteaks, if the meat is oven broiled and cooked to an internal temperature of ≥60°C (140°F), the process of tenderization does indeed transfer pathogens that might be present on the surface of the meat, albeit at low occurrences and levels *(3, 19, 44)*, to the interior of the product. It should be noted that there are currently no requirements for such products to be labeled as "nonintact" and, moreover, based on the absence of an identifier on the label and/or due to difficulty with visually discerning differences between products that have been pierced and those that have not, there is growing concern that consumers and/or retail establishments would not know that such products are nonintact and, as such, might require longer cooking times and/or higher temperatures to prevent foodborne illness. As mentioned, this risk is compounded by the fact that consumers frequently order steaks cooked to less than a medium degree of doneness (<60°C [<140°F]) *(10, 21, 36)*, and that ca. 18% of beef sold at retail is mechanically tenderized and/or enhanced *(2)*. Regardless, our data validate that ECOH and STEC behave similarly with respect to translocation and thermal inactivation within chemically enhanced subprimals and steaks. Our findings also establish that proper cooking appreciably reduces the levels of Shiga toxin–producing *E. coli* in chemically tenderized meat, but does not eliminate the pathogen, due to nonuniform heating within steaks. Further research is warranted to develop interventions to treat subprimals prior to tenderization and/or to develop brines for injection that may lessen the prevalence and levels of ECOH and/or STEC during subsequent storage and cooking. Regardless, the data herein are useful to estimate the comparative risk between intact and nonintact meats and to assist in the validation of targeted interventions and the development of potential labeling requirements for such products.

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BEST PRACTICES

Tenderizing/Enhancing of Whole Muscle Cuts

# Pathogen Control During Tenderizing/ Enhancing of Whole Muscle Cuts

Full report at http://www.bifsco.org/uDocs/03_29_06%20Non-Intact%20Best%20Practices.pdf

**Prepared by: National Cattlemen's Beef Association, American Meat Institute, National Meat Association, Southwest Meat Association**

## Why Best Practices?

Best Practices for tenderizing or enhancing operation reduce the likelihood that contamination with potential pathogens (specifically *E. coli* O157:H7) will occur.

## What is the Issue?

Tenderized and enhanced products may pose a risk if potential pathogens are moved from the meat surface to the interior portions of the meat products and the product is not cooked adequately to destroy the pathogens inside the meat product. If equipment used in the operation is contaminated somehow, and not cleaned and sanitized, the tenderizing or enhancing equipment, and perhaps the solution to be injected, may become the vehicle of the contamination.

Although the likelihood that subprimals or other intact cuts of meat are contaminated with *E. coli* O157:H7 is very low, because tenderizing and enhancing operations are raw meat processing operations, consideration should be given to *E. coli* O157:H7 as a potential, sporadic contaminate that could find its way into the processing environment and specific tenderizing or enhancing processing systems. Additionally, FSIS gave notice that all processors must reassess their HACCP systems to consider *E. coli* O157:H7 in their hazard analysis.

Analysis of outbreaks has suggested that insufficient sanitation of equipment was the biggest issue in the three *E. coli* O157:H7 outbreaks possibly linked to enhanced/tenderized beef steaks. The agency believes proper sanitation to be the single most important control measure available to processors of mechanically tenderized and enhanced products to prevent foodborne outbreaks.

As the tenderizers/injectors pass through the product they may introduce biological hazards to the interior or the product. Inadequate injection needle sanitation poses the greatest risk to spread any microbial contaminants present on the incoming raw materials, thus needle sanitation is critical. All needles must be removed at least daily and soaked in a sanitation solution before inspection and reassembly of the needle injector. Ideally, two sets of needles could be rotated to allow for maximum soaking time and potentially greater sanitation efficacy.

Validation and verification of sanitation practices are always challenging, however the nature of small diameter hollow injection needles further compounds this issue. To validate the efficacy of the sanitation system needles can be sacrificed (broken) to determine if the cleaning and sanitizing procedures are adequate. Likewise, routine verification of sanitation practices for needles can be determined by sacrificing and sampling needles at some frequency.

For more information contact:

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BEEF

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- Reassess the HACCP plans for production of tenderized and enhanced products.
- Meet all Federal regulations (9 CFR 307, 310, 313, 314, 317, 318, 320, and 416).
- Optimize raw material (i.e., whole muscle cuts) quality and safety by purchasing from suppliers with E. coli O157:H7 control steps, and obtain letters of guarantee and certificates of analysis for raw materials and other ingredients.
- Criteria to select raw material suppliers should include that suppliers have process interventions in place to reduce or eliminate potential enteric pathogens.
- Achieve and maintain temperatures at 40°F or less for raw materials, water, brines and finished products.
- Do needle integrity checks for injecting operations.
- Needle product from the side opposite of the external surface to minimize any bacterial translocation.
- Rotate the use two sets of needles to allow for maximum soaking time and potentially greater sanitation efficacy.
- Injection systems should be cleaned in place using a validated sanitation process of cleaning followed by sanitizing. SOPs should include the chemical concentration, frequency of cleaning, responsible party and how it will be verified.
- Use chilled water to prepare brines and meat protein suspensions.
- Limit age for brine solutions (e.g., 24 hours) and sanitize after each break.
- Limit age for suspension solutions (e.g., 8 hours) and sanitize after each break.
- Remove and sanitize needles each day.
- Use an antimicrobial intervention (e.g., filtration, UV) for re-circulating pickle solution.
- Use a bacterostatic ingredient in the brine solution (e.g. lactate, diacetate).
- Use voluntary labeling of enhanced and mechanically tenderized products to identify them as non-intact and to include cooking instructions.
- Use microbiological testing to verify cleaning and sanitation and to ensure that E. coli O157: H7 is not being harbored in the processing equipment or environment.
- Use lotting and traceability systems to record raw material use, processing dates and times, and other in-process data such as temperatures of brines, rooms and products.
- Have a stock recovery program.
- Ensure all products are held if and when pathogen tests are conducted.

**For more information contact:**

**National Cattlemen's Beef Association**

BEEF

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# Best Practices: Pathogen Control During Tenderizing/Enhancing of Whole Muscle Cuts

Supported by:

**National Cattlemen's Beef Association**
**American Meat Institute**
**National Meat Association**
**Southwest Meat Association**

**Revised February 2006**

February 2006

The American Meat Institute (AMI), National Cattlemen's Beef Association (NCBA), National Meat Association (NMA), and Southwest Meat Association (SMA) are pleased to have developed these industry *Best Practices for Pathogen Control for Tenderizing Operations of Whole Muscle Cuts*. In September 2003 leading manufacturers of non-intact meat products collaborated under the guidance of the American Meat Institute, National Meat Association, Southwest Meat Association, National Cattlemen's Beef Association, and developed the Best Practices for review by the Beef Industry Food Safety Council (BIFSCo). The Best Practices for Beef Slaughter (NMA et al., 2003a) and Best Practices for Handling Vacuum Packed Subprimal Beef Cuts (AMI et al., 2003) were used as resources in developing recommendations for non-intact beef products. Substantial updating of this document was completed following the Non-intact Products Processing Workshop (December 2005) based on meeting participants' comments. A full summary of this meeting is documented in *Beef Industry Addresses the Safety of Non-Intact Beef Products* (NCBA, 2006).

While the operating practices at individual companies may vary, producers of non-intact whole-muscle cuts are urged to consider these Best Practices as guidelines for their own internal practices and documentation. These practices are the best conditions known at the date of publication.

The following individuals should be recognized for their contribution to the development of these Best Practices:

Dell Allen, Cargill (retired)
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Skip Seward, American Meat Institute

**Industry Best Practices for Pathogen Control During Tenderizing/Enhancing of Whole Muscle Cuts**

## Purpose

This document is designed to discuss Best Practices that can be implemented throughout the tenderizing or enhancing operation, as well as during cleaning and sanitizing operations, to reduce the likelihood that contamination with potential pathogens (specifically *E. coli* O157:H7) will occur. There are multiple ways to reach the optimal end-result, and each operator must be able to apply the practices and procedures that best fit an individual operation. This document is not designed to mandate the use of any specific system or technology, but rather, to stress the importance of validating that the tenderizing or enhancing system is optimized to reduce the risk of contamination.

## Introduction

FSIS defines non-intact beef products as ground beef; beef injected with solution, beef that has been mechanically tenderized by needling, cubing, frenching, or pounding devices, and beef that has been reconstructed into formed entrees. Whole muscle cuts (e.g., chucks, ribs, tenderloins, strip loins, top sirloin butts, rounds) may be treated to increase tenderness or to add ingredients for quality purposes, a practice that often occurs before subsequent fabrication at the same or external location. Treatments may include solid-needle tenderizing or hollow-needle tenderizing where a solution is pumped into the whole muscle. In the latter case, the solution typically is re-circulated, refrigerated and treated to ensure the quality of the pumping solution. It is important that the management of these operations be such that the equipment, refrigeration, solutions and product are optimized for quality and safety.

Producers of raw non-intact beef products recognize that these products may pose a risk if potential pathogens are moved to the interior portions of the meat products (Krizner, 1999; Phebus et al., 2000; Lambert et al., 2001; Hajmeer et al., 2002), and the product is not cooked adequately to destroy the pathogens inside the meat product. As is discussed below, the likelihood of potential pathogens being transferred to the inside from the outside of the product is extremely low because of a very low prevalence of pathogens on meat portions being tenderized or enhanced (Ransom et al., 2002; Warren et al., 2003). If equipment used in the operation is contaminated somehow, and not cleaned and sanitized, the tenderizing or enhancing equipment, and perhaps the solution to be injected, may become the vehicle of the contamination. To reduce the risk, it is extremely important that processors implement Best Practices by focusing on cleaning and sanitation practices for tenderizing and enhancing operations.

One of the primary considerations in assessing the likelihood of contamination of products that are tenderized or enhanced is whether or not contamination, especially with *E. coli* O157:H7, is a hazard reasonably likely to occur on the surface of intact meat portions before the tenderizing or enhancing operation. Several studies indicate that *E. coli* O157:H7 is not a hazard reasonably likely to occur on the surface of intact meat portions. A study was conducted by Warren et al. (2003) where sponge samples were taken of 1,014 subprimal cuts from six beef processing plants

over a five-week period. Only two samples (0.2%) tested positive for *E. coli* O157:H7. Enumeration indicated that each of the two positive samples had <3.0 CFU per 200 $cm^2$ sampled.

Two later studies were conducted by ABC Research Corporation (Gainesville, Fla.) throughout 2004 to determine the prevalence of *E. coli* O157:H7 and indicator organisms on the surface of beef subprimals that would be used as raw materials for tenderizing or enhancing operations. These studies used cuts of meat specifically used for tenderizing or enhancing operations, namely, briskets, rounds, chucks and middle meats. One study (I) focused on raw materials produced during the winter months (January and February); the second study (II) collected data during the late summer and fall (August into November).

In Study I, 600 samples comprising six subprimal cut types (100/type) were collected from five plants from the southern Midwest, Midwest, northern Midwest and the Southeast. Each sample was a sponge sample of the entire surface of a subprimal. None of the 600 samples had *E. coli* O157:H7. In study II, 599 samples (following the same scheme described above for study I) tested negative for *E. coli* O157:H7. Based on limits of methodologies and the results from Studies I and II, the authors concluded that the overall incidence of *E. coli* O157:H7 on beef subprimals was < 0.083% (Kennedy and Badnaruk, 2004, 2005).

This document provides Best Practices for tenderizing and enhancing operations and can be used by establishments to develop plant specific programs. Although these Best Practices are applicable to both production of raw and fully cooked tenderized and/or enhanced items, this document primarily focuses on the manufacture of raw non-intact products (excluding ground beef). These Best Practices are designed to provide a recommended set of practices and procedures that processors may want to adopt in their entirety, or in part to ensure optimal wholesomeness.

**Raw Material Control**

Best Practices begin with optimizing raw material (i.e. whole muscle cuts) quality and safety. Tenderizing and enhancing operations should identify requirements for raw material suppliers and have a system for verification that the requirements are being met and achieving the goals of the quality and safety program.

Criteria to select raw material suppliers should include that suppliers have process interventions in place to reduce or eliminate potential enteric pathogens. Raw material suppliers should have validated process interventions and/or validated critical control points (CCPs) in place to prevent, eliminate or reduce *E. coli* O157:H7 to a non-detectable level. As always, multiple interventions (hurdles) are preferable to single microbial interventions. Validation may include scientific literature and/or plant specific validation using indicator organisms, and it should be specific to the process being applied at the establishment. This validation can be incorporated into the processor's purchase specifications or other plant programs to ensure that all raw materials are produced using validated CCPs or process interventions. These purchase specifications should have a means to ensure that they are being met. Examples of such verification tools include, but are not limited to third party process reviews, customer audits and microbiological testing. This is true for both domestic and imported suppliers of raw materials to be used in production of non-intact product. Purchase specifications should be updated regularly

(at least annually). An example letter from a harvest/fabrication facility to meet the processor's prerequisite program requirements has been provided and is included in Best Practices: Appendix A.

Another important criterion for supplier selection is the ability and demonstrated maintenance of cold chain management. This includes rapid chilling of hot carcasses to control microbial growth and proper carcass rotation within the cooler to ensure timely fabrication.

Lastly, it is important for non-intact beef processors to have specific data on *E. coli* O157:H7 incidence to support the position taken during the hazard analysis as "not reasonably likely to occur." These data must relate to the raw materials and/or finished product(s). Routine microbiological testing may include sampling and testing for *E. coli* O157:H7. Other microbiological testing includes analyses for *Salmonella*, Aerobic Plate Count (APC), Total Plate Count (TPC), coliforms, and generic *E. coli.* For all microbiological testing, it is important that there be a written protocol for sample collection, lab analysis and proficiency testing, as well as the procedures for reporting the results. It is important to establish how the results will be used before the data are collected. Most of these microbiological tests are used for tracking supplier trends over time; however, each establishment must clearly define how they are going to use the information and the consequences of failing to meet internal microbiological guidelines.

**Supplier Evaluations**

Raw material suppliers are critical to both food safety and quality aspects of producing tenderized and enhanced products. In addition to well-defined requirements it is important that there are procedures established to evaluate the raw material supply whether from an internal or external vendor source. Guidelines developed for the Raw Ground Products Best Practices can be used to help design a system for evaluating supply sources for other non-intact raw materials. A more detailed discussion of supplier evaluations can be found in the *Best Practices for Raw Ground Products* document (NMA et al., 2003b; www.bifsco.org/BestPractices.htm).

**Temperature Control**

Cold chain management is a continuum from the time a carcass leaves the slaughter process and enters the chilling process through processing, packaging, storage and distribution. The goal is to achieve and maintain the temperature that will inhibit the growth of foodborne pathogens and slow the growth of spoilage microflora. The minimum growth temperatures for the pathogens of most concern are 44.6°F (7°C) for salmonellae and 44.6-46.4°F (7-8°C) for pathogenic *E. coli* (ICMSF, 1996). If cold chain control is violated at any point in the chain, product safety and quality may be compromised.

Cold chain management is especially important at the tenderizing or enhancing operation. Specific points where temperature should be controlled, other control points related to temperature control, and examples of operating limits in tenderizing or enhancing operations include:

- Receiving and storage of raw materials at 40°F or less
- Processing raw materials using a "First In First Out" (FIFO) rotation
- Monitoring raw materials and finished products using a process room/cooler control program

- Verifying the potability of process water
- Maintaining process water at 40°F or less
- Maintaining finished product temperatures at 40°F or less throughout their shelf life
- Controlling brine solutions to 40°F or less
- Pre-chilling shipping containers to 40°F or less before loading
- Maintaining temperatures at 40°F or less throughout transport

While temperatures are specified at 40°F or less in the above list based on the growth limitations for pathogenic *Salmonella* and *E. coli* O157:H7, it is generally recognized that the colder the temperature the better.

**Process Controls**

There are three general types of processing that are recognized within tenderizing and enhancing operations. These include needle tenderizing, brine-injecting (enhancing), and suspension injecting. Specific Best Practices will be presented for each of these categories due to unique differences between the processes. Example Standard Operating Procedures (SOP) are provided in the appendix as a reference for cleaning and sanitizing of injector assembly (Best Practices: Appendix B). Every process and enhancement system is unique and appropriate SOP's should be in place depending on the situation.

***Needle Tenderized Products***

- Documented GMPs (including needle integrity checks) exist for tenderizing operations
- If possible, needle the product from the side opposite of the external surface to minimize any bacterial translocation
- Traceability program is in place for all finished products
- Food Defense program exists to prevent tampering with operational equipment, and raw materials

***Enhanced/Brine-Injected Products***

- Letters of guarantee and certificates of analysis exist for ingredients used in pumping solution (brine or pickle solution)
- Documented General Manufacturing Practices (including needle integrity checks) exist for injecting operations
- Chilled water feeding system is preferable to complete chilling of brine following mixing
- Maximum age is established for reuse brine (pickle) solutions (e.g., 24 hours), with a mandatory break in the use cycle (e.g., every 24 hours)
- Use of an antimicrobial intervention (e.g., filtration, UV) for recirculating pickle solution is implemented if deemed necessary by the hazard analysis
- Use of bacterostatic ingredients in the brine solution (e.g. lactate, diacetate, sodium metasilicate) is implemented if deemed necessary by the hazard analysis
- If possible, inject the product from the side opposite of the external surface to minimize any bacterial translocation
- Daily needle removal and soaking in sanitation solution is conducted
- Established protocol exists for managing rework, including traceability and a time frame for incorporation into manufacturing

- Traceability program is in place for all finished products
- Food Defense program exists to prevent tampering with operational equipment, raw materials and pickle solutions

***Meat Protein Suspension Injection Products***[1]

- Letters of guarantee and certificates of analysis exist for ingredients used in the processing of the suspension solution (to include all meat and nonmeat ingredients in the brine or pickle solution, as well as documentation on "supplier evaluation" on the sources the trim raw material used)
- Documented GMPs (including needle integrity checks) exist for injecting operations
- Chilled water feeding system is preferable to complete chilling of brine following mixing and as the suspension is generated from it
- Maximum age is established for reuse brine (pickle) solutions (e.g., 24 hours), with a mandatory break in the use cycle (e.g., every 24 hours)
- Maximum age is established for reuse suspension solutions (e.g., 8 hours), with a mandatory break in the use cycle (e.g., every 16-20 hours)
- Use of an antimicrobial intervention (e.g., UV) for re-circulating pickle solution is implemented if needed as determined by the hazard analysis
- Use of bacterostatic ingredients in the brine solution (e.g. lactate, diacetate, sodium metasilicate) if needed as determined by the hazard analysis
- If possible, inject the product from the side opposite of the external surface to minimize any bacterial translocation
- Daily needle removal and soaking in sanitation solution is conducted
- Established protocol exists for managing rework, including traceability and a time frame for incorporation into manufacturing
- Traceability program is in place for all finished products
- Food Defense program exists to prevent tampering with operational equipment, raw materials and pickle solutions

**Lotting**

All non-intact processors should have a lotting mechanism for coding and recording all products to allow trace back and trace forward of products throughout the manufacturing and distribution system. FSIS recognizes that the establishment will define a lot and expects scientific or other supportive basis for defining the lot. Lotting systems can range from very simplistic, e.g., handwritten numbering, to very elaborate, e.g., computerized, automated bar coding. Lotting is often based on some unit of time (e.g., hour, shift, day); however lotting can be driven by other factors including raw material source, production line or processing room. Some processors may choose to further divide lots of product into sublots. By creating smaller lot units, process control can be demonstrated and documented more frequently; and there is a potential to minimize the

[1] Cozzini's SUSPENTEC ™ system is a patented method of reducing meat, poultry or fish trimmings to micron size and incorporating them into traditional brines to create a suspension; the suspensions can then be injected into whole-muscle products. The use of this equipment is governed by FSIS Policy Memo PM041B. At the time this document was put together, Cozzini's SUSPENTEC ™ system was the only such technology available for Beef, Pork and Poultry. These practices may or may not be applicable to other suspension technologies when they become available.

volume of product implicated in the event a recall is ever required. In tenderizing and enhanced operations, there is some precedence that FSIS will accept a single bag of subprimals as a lot, provided the processing facility can show adequate separation. If lots are intended to be broken at some frequency by needle rotation, accompanying sanitation of the feed-in area (debagging tables, conveyors) is also necessary. Additionally, establishments should maintain records associated with all production lots. Information to be recorded is dependent on the individual system; however the following data typically are recorded:

- Raw material vendor, vendor lot
- Process date, time of production
- Raw material, brine, room and product temperature
- Microbiological data
- Equipment evaluations

A more detailed discussion of lotting can be found in the *Best Practices for Raw Ground Products* document (NMA et al., 2003b; www.bifsco.org/BestPractices.htm ).

**HACCP System**

Non-intact products will be produced under FSIS or state inspection, thereby meeting all Federal or State (equal to) requirements pertaining to HACCP systems (9 CFR 417), Sanitation SOPs (9 CFR 416) and pre-requisite programs. All processors should be able to support the decisions that are made in the HACCP program and to use the documentation generated from the program to demonstrate product safety.

HACCP is a proactive, systematic approach to food safety designed to prevent, eliminate or reduce food safety hazards to an acceptable level. Processing establishments must consider biological, physical, and chemical food safety hazards. As far as the authors know, there are no data to suggest that through a hazard analysis, *E. coli* O157:H7 should be considered a hazard reasonably likely to occur in tenderizing or enhancing operations. In fact, as mentioned earlier, data (nearly 1200 data points collected in the winter, fall and summer of 2004) have established that *E. coli* O157:H7 is not a hazard reasonably likely to occur on whole muscle cuts destined for tenderizing or enhancing operations. Likewise, additional studies have documented the very low incidence of *E. coli* O157:H7 on the surface of subprimals destined to be enhanced or mechanically tenderized. Data show only three to four percent of surface bacterial populations are translocated to an average interior depth of ¼" of the cuts during processing (Sporing, 1999; Lambert et al., 2001). Thus, mechanically tenderized and enhanced products pose no greater risk than intact cuts when cooked to a rare degree of doneness (140°F) (Marsden et al., 1999). A review of current research results is presented by the NCBA white paper entitled *Beef Industry Addresses the Safety of Non-intact Beef Products* (NCBA, 2006).

However, because these are raw meat processing operations, consideration should be given to *E. coli* O157:H7 as a potential, sporadic contaminate that could find its way into the processing environment and specific tenderizing or enhancing processing systems. Additionally, FSIS gave notice that all processors must reassess their HACCP systems to consider three foodborne outbreaks of *E. coli* O157:H7 that may have been linked to enhanced/tenderized beef steaks in their hazard analysis (FSIS-USDA, 2005). Thus, processors must focus on what practical strategies can be applied during the tenderizing or enhancing process to minimize the potential

for growth of *E. coli* O157:H7 if present as a process contaminant or as a highly unlikely contaminant of subprimals. These strategies typically involve prevention of harborages and niches through cleaning and sanitation of equipment, maintaining cold temperatures and using antimicrobial interventions on the subprimals prior to processing and during recirculation of enhancement solutions. Occasional verification that *E. coli* O157:H7 is not being harbored in the plant environment by swabbing equipment is recommended.

**Sanitation and Facilities**

Production of tenderized and enhanced products must occur in facilities that meet all Federal regulations (9 CFR 307, 310, 313, 314, 317, 318, 320, and 416) and the equipment used must meet sanitary operating guidelines. Establishments should meet all regulatory requirements of the Sanitation Standard Operating Procedures and should consider the guidelines presented in the Sanitation Performance Standards.

For optimal operation, the entire system should be process engineered. The idea of process engineering encompasses facility design, equipment design, product movement, supply movement and employee movement to create an environment that minimizes microbial contamination. The American Meat Institute's *Sanitary Design of Equipment and Facilities* (AMI, 2003) serves as a good reference. A checklist and a fact sheet, can be accessed at the following Web sites:
(http://www.meatami.com/Content/ContentGroups/Food_Safety_Inspection/Inspection1/Sanitation1/AMIEquipmentdesignChecklist.xls

http://www.meatami.com/Content/NavigationMenu/PressCenter/FactSheets_InfoKits/FactSheetSanitaryDesign.pdf).

FSIS personnel (Engeljohn, 2005) have suggested that insufficient sanitation of equipment was the biggest issue in the three *E. coli* O157:H7 outbreaks possibly linked to enhanced/tenderized beef steaks. The agency believes proper sanitation to be the single most important control measure available to processors of mechanically tenderized and enhanced products to prevent foodborne outbreaks.

Specifically, enhanced and mechanically tenderized processors should follow sanitation practices much like those adhered to by ready to eat (RTE) operations. A comprehensive review of RTE sanitation and practices are found in the *Guidelines for Developing Good Manufacturing Practices (GMPs), Standard Operating Procedures (SOPs) and Environmental Sampling/Testing Recommendations (ESTRs) in Ready to Eat (RTE) Products* (NMA, 1999).

As the tenderizers/injectors pass through the product they may introduce biological hazards to the interior or the product. Inadequate injection needle sanitation poses the greatest risk to spread any microbial contaminants present on the incoming raw materials, thus needle sanitation is critical. All needles must be removed at least daily and soaked in a sanitation solution prior to inspection and reassembly of the needle injector. Ideally, two sets of needles could be rotated to allow for maximum soaking time and potentially greater sanitation efficacy. Injection systems should be cleaned in place (CIP) using a validated sanitation process of cleaning followed by

sanitizing. Standard operating procedures should include the chemical concentration, frequency of cleaning, responsible party and how it will be verified.

Validation and verification of sanitation practices are always challenging, however the nature of small diameter hollow injection needles further compounds this issue. To validate the efficacy of the sanitation system needles can be sacrificed (broken) to determine if the cleaning and sanitizing procedures are adequate. Likewise, routine verification of sanitation practices for needles can be determined by sacrificing and sampling needles at some frequency. One processor has reported sacrificing one needle per cleaning cycle to verify internal needle cleanliness.

**Interventions/Inhibitors**

When called for by the hazard analysis, a validated intervention may be appropriate. The most basic intervention is knife trimming; which can be utilized with primals, subprimals, roasts and steaks prior to penetration. Other current applied technologies include application of antimicrobial solutions to the raw materials before processing, treatment of the brine with an inhibitory process (e.g., ultraviolet and/or filtration), addition of inhibitory ingredient to the brine and the use of an intervention or inhibitor applied to the finished product or packaging materials. New antimicrobial intervention and inhibitors that may be applicable in tenderizing or enhancing operations continue to be developed. A list of potential interventions at the time this document was written is included in Best Practices: Appendix C. For illustrative purposes, an in-plant study on the antimicrobial properties of a tenderizing pickle solution has been provided in Best Practices: Appendix D.

**Microbiological Testing**

Some producers have elected to sample and test for *E. coli* O157:H7 on subprimals destined for non-intact processing operations. Therefore, their verification testing data would serve as a basis for the hazard analysis.

Finished product microbiological testing is a means to verify process control and evaluate that the Best Practices discussed throughout this document are being used effectively to reduce the likelihood of contamination by potential pathogens and the overall microbial load on the finished product. However, finished product sampling cannot be used to ascertain the safety of the product unless enough samples are taken to develop a statistically based rationale for acceptance (e.g., 95 percent confidence that the probability of contamination is no greater than five percent). Generally, the economics of testing finished products and the high numbers of samples required to have a relatively high degree of confidence that a low level of contamination will be detected, make finished product testing impractical. There may be instances where finished product testing has some value, e.g., for periodic verification using indicator organisms, or when a process is out-of-control and an assignable cause is being sought.

Processors can achieve verification of the efficacy of a harvest/fabrication facility's processes to minimize microbial contaminants without microbial testing of incoming raw materials (subprimals). One way is to obtain copies of the harvest/fabrication facility's latest (at least annually) third-party food safety/HACCP audit. Additionally, processors can request that the harvest/fabrication facilities share their own routine microbiological verification data with the non-intact processor.

**Packaging and Labeling**

Packaging of non-intact beef cuts must occur in a manner to minimize the likelihood of contamination from packaging equipment, the environment, or food contact surfaces. Routine microbiological audit sampling and testing may be used to verify the efficacy of cleaning and sanitation, both on a routine basis and following equipment maintenance or relocation (AMI et al., 2003).

It is the belief of FSIS that consumers do not understand or expect whole muscle steaks and roasts to have been needled. Thus, the agency has suggested that processors consider voluntary labeling of enhanced and mechanically tenderized products to identify them as non-intact and to include cooking instructions. At least one large processor currently includes cooking instructions (145°F for three minutes) on such products.

**Integrated Approach to Control**

One way to evaluate the overall safety of a product is by calculating the integrated control measures, which is an evaluation of the baseline incidence and the bateriostatic / bacteriocidal effects of all the variables which contribute to the safety of the end product. The integrated approach to control includes, but is not limited to the following factors:

- Organism incidence rates in live animals
- Interventions applied at harvest and fabrication
- Raw material incidence rates
- Application of industry recognized best practices
- Interventions (including knife trimming) applied prior to injection/mechanical tenderization
- Organism translocation rates due to injection/mechanical tenderization
- Antimicrobial effects of an enhancement brine
- Ingredients affecting the heat liability of the organism
- Temperature control to minimize microbial amplification
- Cooking practices applied to the products
- Integrated time-temperature processing (integrated lethality)—incorporates all heat treatments, i.e. the increase in temperature as the product heats and the temperature levels as the product cools. Microbial destruction takes place during the entire heating and cooling process, not just at the minimum internal temperature.
- Relationship between depth of possible translocation, cooking time and temperature to effectively destroy microorganisms

By considering all of these variables, the true safety of the product can be determined.

**Best Practices: References**

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**Best Practices: Appendix A**
**Example *E. coli* O157:H7 Purchase Specification Letter for Supplier Evaluations**

Attention: Customer Name

Edible beef products from the plants listed at the end of this letter meet all USDA requirements for the production, sale and distribution of meat products. Such requirements include, but are not restricted to the categories listed below. Updates will be issued annually or as significant changes are made.

**HACCP/Pathogen Reduction Regulation (Megareg) Compliance**

- Testing of carcasses for *E. coli* Biotype I (9 CFR Part 310, §310.25), effective June 1997. (all Beef Slaughter plants)
- Implementation of SSOP (Sanitation Standard Operating Procedures, 9 CFR, Part 416, §416.11 - §416.17), effective January 26, 1997 for all plants.
- Implementation of HACCP Systems (9 CFR, Part 417, §417.1 - §417.8), effective January 27, 1998 for plants with greater than 500 employees.
- Implementation of HACCP Systems (9 CFR, Part 417, §417.1 - §417.8), effective June 1, 1998 for smaller plants noted separately by "*"
- Testing of carcasses and/or ground beef for *Salmonella* as conducted by USDA in accordance with §310.25.

**Federal Register Docket 00-022N, dated 10/7/02 (*E. coli* O157:H7 Reassessment)**

- Reassessment of HACCP plans for *E. coli* O157:H7 in accordance with the Notice 22-04, dated 10/7/02 conducted in all Company Name beef plants effective 12/6/02.
- Completion of annual reassessment of HACCP plans in accordance with 9CFR 417.4 (a) (3) effective January each calendar year. This reassessment included review and verification of adequacy of the HACCP plans in addressing *E. coli* O157:H7.

**Directive 6420.2 – Issued 3/31/04**

- CCP's in place and effect for zero tolerance requirements for head meat, cheek meat and weasand meat for all plants effective 5/17/04. **Note:** Zero Tolerance on carcasses has been in place as a CCP since the implementation of HACCP in 1998.

**Directive 10,010.1 – revised 3/31/04**

Labeling

- USDA approval for the following label disclaimer/instructional statements are available on site at the producing est.:
    - *For Cooking Only*
    - *Lot Tested and Found Negative for ECH7"*

Disposition CCP's

- All materials that are tested for *E. coli* O157:H7 that are not negative are addressed within the HACCP plans under a product disposition CCP.
- These materials are controlled, relabeled (when applicable) with the statement, "For Cooking Only" and are cooked or otherwise disposed of to inedible or rendering.
- Records reflect appropriate disposition of affected material.

Testing for *E. coli* O157:H7

Carcasses – Daily validation testing for *E. coli* O157:H7 is conducted at each beef slaughter plant. This has been in place and effect since 2000. Carcasses are sampled at the same sites as listed in 9CFR 310.25 for *E. coli* Biotype I and are retained pending results.

Beef Materials Destined For Non-Company Name Grinding

In accordance with the intended use described in the plants' Raw Not Ground HACCP plans (including trim and some variety meats harvested in slaughter), all materials destined for raw ground use are subjected to a statistically based sampling plan[1] for *E. coli* O157:H7. All boxed materials that are *"Lot tested and found to be negative for E. coli O157:H7"* are labeled with that statement. Combo'd trim does not carry this on the label as combo'd trim materials are tested per customer order and a Certificate of Analysis, (COA), specific to those combos is provided to the contracted end user. Since boxes may be broken down into smaller ship units by a primary (or secondary or tertiary, etc.) distributor, we deemed it necessary to label the individual box so the ultimate end user is aware that the materials were part of sampling lot that tested negative for *E. coli* O157:H7.

These labeling components are addressed in our HACCP plan as they are an integral part of the intended use.

Ground Beef

- All raw materials destined for grinding in the plants listed in this document are pre-tested[1] and negative for *E. coli* O157:H7 prior to grinding.
- External sources of trim raw material must have a validated carcass intervention for *E. coli* O157:H7 in place and a copy of that compliance is maintained on file at the receiving establishment.
- External sources of raw material must meet Company Name requirements for outside vendors including but not limited to: validated HACCP systems, 3rd party food safety/GMP audits, *E. coli* O157:H7 testing programs that meet or exceed 95% confidence for detection capability.
- Certificate of Analysis (COA's) received for all outside materials sent to grind.

Laboratory Verification Testing

- Verification of *E. coli* O157:H7 lab methods is routinely performed at each Company Name Laboratory in conjunction with the American Proficiency Institute Microbiological Performance Evaluation Program.

HACCP

Critical Control Points in place and in effect at present include:

| HACCP Category | Critical Control Points |
|---|---|
| **Slaughter** | Steam Cabinet operational and functional with regard to ambient temperature and transit time to deliver a minimum of 160°F to the carcass surface to address *E. coli* O157:H7. |
| | Zero Tolerance for feces, ingesta and milk on carcasses. |
| | Carcass Chilling to reduce the surface down to 45°F or less within 24 hours to control microbial growth. |
| | Disposition CCP to assure proper disposition of any carcasses that do not test negative for *E. coli* O157:H7. |
| **Raw Not Ground – Trim** | Pre-cut Carcass Surface Temperature below 45°F to control microbial growth. |
| | Disposition CCP to assure proper disposition of any products that do not test negative for *E. coli* O157:H7. |
| **Raw Not Ground – Variety Meats** | Zero Tolerance for feces, ingesta and milk on head, cheek and weasand meat. |
| | Chilling to reduce the surface down to 45°F or less within 24 hours to preclude microbial growth. |
| | Disposition CCP to assure proper disposition of any products that do not test negative for *E. coli* O157:H7 |
| **Raw Ground** | Inbound Raw Material Temperature $\leq$ 45°F to preclude microbial growth |
| | Functioning metal detector, verified for timing and sensitivity at the start of operations. |
| | Disposition CCP to assure proper disposition of any products that do not test negative for *E. coli* O157:H7 |

A CCP is *"A point, step, or procedure in a food process at which control can be applied and, as a result, a food safety hazard can be prevented, eliminated, or reduced to acceptable levels"*[2] It should be clearly understood that these CCP's are in place to accomplish just that for *E. coli* O157:H7; control, eliminate or reduce to an acceptable level. The acceptable level for *E. coli* O157:H7 is undetectable.

Best Practices/Good Manufacturing Practices

In addition to the CCP's, the following practices are utilized in our beef slaughter operations.

- **Steam Vacuums** – are located strategically throughout the slaughter floor and are used on pattern mark areas.
- **Pre-Evisceration Cabinet System (PECS)** – eligible beef carcasses are treated with up to 2.5% organic acid pre-evisceration.
- **Anti microbial spray** – carcasses are treated with an anti microbial spray of organic acid or acidified sodium chlorite after the Steam Cabinet. Heads are treated with an organic acid application immediately after the head wash, prior to USDA Inspection.

Verification

- In accordance with the facilities' HACCP plans, all CCP's have been validated and are verified at the specified frequencies in the HACCP plan in accordance with 9CFR 417.4.
- Company Name is audited on an annual basis by an independent third party auditor. That audit encompasses both regulatory compliance (HACCP, SSOP, 10,010.1, etc.) and good manufacturing practices. A summary matrix of audit scores is available upon request.

Customer Notification

- Company Name plants have a recall plan on file that includes notification to affected customers of any product that may be adulterated or misbranded.

Last, the Company Name plants listed below are federal establishments and operate under the regulatory requirements promulgated in Title 9 of the Code of Federal Regulations. By dint of the Mark of Inspection, we are obligated to adhere to all applicable requirements contained therein.

**COMPANY NAME BEEF PLANTS**

| **EST.** | **Location** | **Comments** |
|---|---|---|
| Est. ### | City, ST | |
| Est. ### | City, ST | |
| Est. ### | City, ST | |
| Est. ### | City, ST | |
| Est. ### | City, ST | |

**Best Practices: Appendix B**
**Standard Operating Procedures for Cleaning and Sanitizing Injector Assembly: Example I**

Purpose: To effectively clean and sanitize the injector assembly

Program: At the end of each production day, production personnel will perform the following tasks:

**Injector Needles**

1. Open the needle assembly and inspect for cleanliness. If any residual brine residue remains, rinse the housing and needles completely.
2. Remove all needles and carefully place the needles in a clean meat lug that has not been used during that day's production.
3. Rinse housing after needles are removed to ensure that all areas of the head are free of visible residue.
4. Add clean & soak chemicals to the meat lug to a level that completely submerges all needles in the container. Needles must soak for a minimum of 6 hours or as recommended by the sanitation chemical manufacture. If necessary, use a second set of cleaned and sanitized needles to ensure adequate cleaning while meeting production requirements.
5. After the needles have soaked for a minimum of 6 hours, each needle must be "blown out" with clean air before being replaced in the injector assembly.
6. Once clean needles have been placed in the injector assembly, they must be sanitized and rinsed before being used in production.

**Cleaning and Sanitizing Solutions**

1. The composition of the cleaning solution used for nightly cleaning can be used for cleaning the needles and assembly parts unless other solutions have been validated for efficacy.
2. The cleaning and sanitizing chemicals should be rotated periodically.
3. The amount of chemical solution used and the soak time for cleaning should be documented, and verified periodically, e.g., quarterly.

**Monitoring & Verification:** QA and Production Management will monitor the cleaning and sanitizing process during cleanup hours to ensure proper compliance. QA will verify sanitation daily during pre-operational inspections. An authorized person verifies solution composition and chemical strength nightly. Microbial sampling of cleaned and sanitized surfaces will be conducted as per the documented microbiological sampling schedule.

## Standard Operating Procedure Clean In Place System Cleaning: Example II

**PURPOSE:** To minimize bacterial growth.

**PROGRAM:** A CIP cleaning solution will be ran through the injection process to ensure proper cleaning of the injection process.

**PROCEDURE:**

1. Drain all brine material from lines, pumps, and tanks. During the draining process production personnel will continue to rinse all six tanks with potable water until all visible brine residue has disappeared.
2. Fill the two mixing tanks (# 3 & # 6) with 200 Gal. of cold potable water each.
3. Flush 100 Gal. from the line 1 mixing tank (#3) to each of the rear holding tanks (#2 & #1).
4. Flush 100 Gal. from the line 2 mixing tank (#6) to each of the rear holding tanks (#5 & #4).
5. Flush all water from all holding tanks through the CIP system and a minimum of 50 Gal. through each of the injectors (line 1 and line 2).
6. Fill mixing tanks( #3) and (#6) again with 200 Gal. of cold potable water and add appropriate amount of the approved CIP cleaning solution.
7. Mix thoroughly.
8. Flush 100 Gal. of the mixed cleaning solution from the line 1 mixing tank (#3) to each of the rear holding tanks (#2 & #1).
9. Flush 100 Gal. of the mixed cleaning solution from the line 2 mixing tank (#6) to each of the rear holding tanks (#5 & #4).
10. Flush all cleaning solution from all holding tanks through the CIP system pumping from each tank a minimum of 5 minutes.
11. A minimum of 50 Gal. will be pumped from one of the holding tanks of each line through its designated injector (line 1 and line 2).
12. Fill the two mixing tanks (# 3 & # 6) with 200 Gal. of cold potable water each.
13. Flush 100 Gal. from the line 1 mixing tank (#3) to each of the rear holding tanks (#1 & #2).
14. Flush 100 Gal. from the line 2 mixing tank (#6) to each of the rear holding tanks (#5 & #4).
15. Flush all water from all holding tanks through the CIP system and a minimum of 50 Gal. through each of the injectors (line 1 and line 2).

The currently used cleaning solution is STERIS brand Process Klenz alkaline cleaner used at 2.5% by volume. (5 gallons Process Klenz mixed with 200 gallons potable water.)

**CORRECTIVE ACTION:** Production will not be allowed to start until CIP cleaning has taken place.
**RELATED FORMS:** CIP System Cleaning Verification Process Check

**MATERIALS NEEDED:** Steris brand process klenz alkaline cleaner.

**FREQUENCY:** Daily

**MONITORED BY:** QA and Production Management will routinely monitor to ensure proper compliance.

General Manager _____ Date _____

QA Manager _____ Date _____

## Standard Operating Procedure Clean In Place System Sanitizing: Example III

**PURPOSE:** To minimize bacterial growth.

**PROGRAM:** A CIP Sanitizing solution will be ran through the injection process to ensure proper cleaning of the injection process.

**PROCEDURE:**

1. Fill the two mixing tanks (# 3 & # 6) with 200 Gal. of cold potable water each.
2. Flush 100 Gal. from the line 1 mixing tank (#3) to each of the rear holding tanks (#2 & #1).
3. Flush 100 Gal. from the line 2 mixing tank (#6) to each of the rear holding tanks (#6 & #4).
4. Flush all water from all holding tanks through the CIP system and a minimum of 50 Gal. through each of the injectors (line 1 and line 2).
5. Fill mixing tanks #3 and #6 again with 200 Gal. of cold potable water and add appropriate amount of the approved CIP sanitizing solution.
6. Mix thoroughly.
7. Flush 100 Gal. of the mixed sanitizing solution from the line 1 mixing tank (#3) to each of the rear holding tanks (#2 & #1).
8. Flush 100 Gal. of the mixed sanitizing solution from the line 2 mixing tank (#6) to each of the rear holding tanks (#5 & #4).
9. Flush all sanitizing solution from all holding tanks through the CIP system pumping from each tank a minimum of 5 minutes.
10. A minimum of 50 Gal. will be pumped from one of the holding tanks of each line through its designated injector (line 1 and line 2).
11. Fill the two mixing tanks (# 3& # 6) with 200 Gal. of cold potable water each.
12. Flush 100 Gal. from the line 1 mixing tank (#3) to each of the rear holding tanks (#2 & #1).
13. Flush 100 Gal. from the line 2 mixing tank (#6) to each of the rear holding tanks (#5 & #4).
14. Flush all water from all holding tanks through the CIP system and a minimum of 50 Gal. through each of the injectors (line 1 and line 2).

The currently used cleaning solution is STERIS brand Process LCS liquid chlorinating sanitizer used at .25 ounce per gallon. (50 ounces mixed with 200 gallons potable water.) Chlorine Days Monday, Wednesday, Friday, Saturday, Sunday. Quat Days: Tuesday, Thursday.

**CORRECTIVE ACTION**: Production will not be allowed to start until sanitizing has taken place.

**RELATED FORMS**: NA

**MATERIALS NEEDED:** Quat or Chlorine

**FREQUENCY:** Daily

**MONITORED BY:** QA and Production Management will routinely monitor to ensure proper compliance.

General Manager _____ Date _____

QA Manager _____ Date _____

## Standard Operating Procedure Operational Cleaning of Injector Reservoir In-Line Filters: Example IV

**PURPOSE:** To minimize bacterial growth.

**PROGRAM:** Injection filters will be cleaned on a regular basis to ensure the injectors operate at an optimal level.

**PROCEDURE:**

1. Remove the machine side in-line final filter by rotating its holding cylinder to the vertical position where it will latch against the wall of the reservoir.
2. From this position the end cap can be threaded back and spun out of the way so the filter may be removed for cleaning.
3. Remove filter and clean with tempered water of sufficient pressure to remove any built up residue.
4. Replace filter into its holding cylinder and thread back its end cap to secure filter in the cylinder.
5. Return filter assembly to the horizontal position inside the reservoir tank.
6. Remove the off side in-line final filter by rotating its holding cylinder to the vertical position where it will latch against the wall of the reservoir.
7. From this position the end cap can be threaded back and spun out of the way so the filter may be removed for cleaning.
8. Remove filter and clean with tempered water of sufficient pressure to remove any built up residue.
9. Replace filter into its holding cylinder and thread back its end cap to secure filter in the cylinder.
10. Return filter assembly to the horizontal position inside the reservoir tank.

**CORRECTIVE ACTON:** NA

**RELATED FORMS:** NA

**MATERIALS NEEDED:** Tempered Water

**FREQUENCY:** Operational cleaning of injector reservoir filters should be conducted on the hourly basis in order to maintain consistent pump settings.

**NOTE:** Each employee who handles injector equipment must change gloves before and after as well as clean any additional utensils needed for the tasks. This ten-step process will be used for the reservoir tanks of both line one and line two injectors. If filters are cleaned one at a time than the injector does not need to be shut down for this SOP.

**MONITORED BY:** QA and Production Management will routinely monitor to ensure proper compliance.

General Manager: _____ Date: _____

QA Manager: _____ Date: _____

**Best Practices: Appendix C**
**Decontamination Interventions for Primals, Subprimals, Trim and Ground Meat**

**Decontamination Interventions**

| | Intervention | Effectiveness in Lab setting | Effectiveness in Field / Plant | Regulatory Status |
|---|---|---|---|---|
| MECHANICAL TREATMENT | | | | |
| | **Irradiation** | Widely studied. Effective in reducing pathogens at varying levels depending on dose. | Effective, but control of dose is critical to minimize effects on organoleptic factors. | Approved, labeling required |
| | **Trimming** | CSU study indicates surface trimming is as effective as certain chemical treatments. 1.1 log CFU/$cm^2$ reduction (inoculated with 3.7 log CFU/$cm^2$). | Effective and implemented widely | Not a limitation |
| | **Steam** | Initial results are limited, but may have an effect. | Unknown | Unknown |
| | **Hot water wash** | CSU study indicates a significant log reduction. 1.0 log CFU/$cm^2$ reduction (inoculated with 3.6 log CFU/$cm^2$). | Unknown | Unknown |

| | Intervention | Effectiveness in Lab setting | Effectiveness in Field / Plant | Regulatory Status |
|---|---|---|---|---|
| CHEMICAL TREATMENT | | | | |
| | **Acidified Sodium Chlorite** | Company data 2.9 log reduction of *E. coli* O157. 2.0 log reduction of *E. coli* (generic). KSU 2-3 log CFU/cm$^2$ reduction of APC. ABC Research found up to a 0.63 log reduction of *E. coli* O157 on inoculated subprimals | Initial trials show approximately a 2 log reduction of APC. | Approved, however weight gain over 0.5% must be labeled. |
| | **Lactic Acid** | CSU data supports 2.5% LA @ 55°C resulted in 1.0 log CFU/cm$^2$, while 5.0% LA @ 55°C resulted in a 1.1 log CFU/cm$^2$ (inoculated at 3.6 and 3.5 log CFU/cm$^2$, respectively). | Unknown. 0.4% by weight, of a 2.5% solution was not effective. | Pending approval at 2.5% and 5.0% levels. |
| | **Acidified Calcium Sulfate** | Company trials are encouraging. | Unknown | Not approved in Beef trim |
| | **CPC** | Company trials show significant log reductions. | Unknown | Not approved in Beef trim, residual levels cited as concern. |
| | **Peroxyacetic acid** | ABC Research data found .63 - .71 log reduction of *E. coli* O157:H7 on inoculated subprimals. | Unknown | Approved |
| | **Citric Acid** | Laboratory trials show promise. | Unknown | Approved |

| | Intervention | Effectiveness in Lab setting | Effectiveness in Field / Plant | Regulatory Status |
|---|---|---|---|---|
| BIOLOGICAL | | | | |
| | **Lactoferrin** | CSU study indicates that Lactoferrin applied to inoculated subprimals allowed 4.6 log less growth of *E. coli* O157:H7. Additionlly 5.0% lactic acid used in combination with activated Lactoferrin at 55°C resulted in 0.9 log CFU/cm$^2$ reduction (inoculated at 3.5 log CFU/cm$^2$). | Unknown | Approved for Carcasses and parts Directive 7120.1 |
| | **Lactobacillus acidophilus** | TTU study demonstrated a 90% reduction in *E. coli* O157:H7 and a 99.9% reduction in *Salmonella* | Unknown | Working on petition |

**Best Practices: Appendix D**
**Studies on the Antimicrobial Properties of Tenderizing Pickle Solution**

Preliminary Report

*September 10, 2003*

Study I

Objective: To determine antimicrobial properties of a pickle solution used in tenderizing whole muscle cuts

Composition of pickle solution: A typical pickle solution will contain phosphate, salt and flavorings. The solution used in this study contained a proprietary formula based on in finished products, e.g., 0.5%.

Measurement of the antimicrobial effect: The antimicrobial effect of the pickle solution was measured using a micro-titer assay (i.e., providing minimum inhibitory concentrations) and traditional laboratory plating procedures.

Results: Using micro-titer assays, initial experiments determined that the pickle solution reduced the concentrations of *E. coli* O157:H7 and *Salmonella* by at least 2 logs (100-fold). In follow-up experiments, direct inoculation of pickle solution with a cocktail of 3 *E. coli* O157:H7 strains and 3 *Salmonella* strains at levels near $10^6$ per mL resulted in complete lethality for all pathogens after 30 minutes of exposure (the first measurement time interval after the zero time measurement).

In a laboratory setting using traditional microbiological techniques, the antimicrobial properties of the pickle solution were determined. Pickle solution was inoculated to 1.73 logs per mL with *E. coli* O157:H7 and stored at room temperature (~73°F) or under refrigeration (37°F). No *E. coli* O157:H7 were recovered from the pickle solution after 2 hours at room temperature and after 24 hours under refrigerated conditions.

| | **Storage temp** | |
|---|---|---|
| ***Time*** | **Room** | **Refrigerator** |
| 0 min | Positive | Positive |
| 30 min | Positive | Positive |
| 1 hour | Positive | Positive |
| 2 hour | Negative | Positive |
| 4 hour | Negative | Positive |
| 24 hour | Negative | Negative |

These data represent the results of a single study using inoculated organisms, and should not be extrapolated to all situations. The storage temperature and times, while different for room temperature versus refrigerated, simply indicate that the brine solution may exhibit inhibitory properties against *E. coli* O157:H7. However, further research would be needed to confirm that this is the case, and multiple variables may be contributing to this effect.

Next steps: Additional validation work will be repeated with meat extract added to evaluate effects of meat components on bactericidal activity and with inoculated meat exposed to the pickle solution.

Study II

Objective: To determine the prevalence of *E. coli* O157:H7 in injection solutions used to enhance various beef products.

Sampling Procedures: One-quart samples of injection solutions were taken from the brine return, before the brine entered the reservoir for recycling with fresh solution, before filtration. Samples were collected at least 20 minutes into production, with each sample set of three samples spaced throughout the scheduled production run. Samples were then sealed and sent to the laboratory for testing.

Results: In total, 19 sample sets (57 samples) were collected through July and August 2003. All samples (Table 1) tested negative for the presence of *E. coli* O157:H7. Preliminary investigation into the recovery of *E. coli* O157:H7 that were inoculated into brine samples indicated that the organism could be recovered from the brine solution, if present.

Table 1. Injection Solution Results for Study II

| Date | Meat Cut | *E. coli* O157:H7 Result 1 | *E. coli* O157:H7 Result 2 | *E. coli* O157:H7 Result 3 |
|---|---|---|---|---|
| 29-Jul-03 | Flat | NEG | NEG | NEG |
| 29-Jul-03 | Flat | NEG | NEG | NEG |
| 29-Jul-03 | Ribeye | NEG | NEG | NEG |
| 30-Jul-03 | Capoff Inside | NEG | NEG | NEG |
| 30-Jul-03 | Flat | NEG | NEG | NEG |
| 30-Jul-03 | Ribeye | NEG | NEG | NEG |
| 31-Jul-03 | Ribeye | NEG | NEG | NEG |
| 05-Aug-03 | Capoff Inside | NEG | NEG | NEG |
| 05-Aug-03 | Ribeye | NEG | NEG | NEG |
| 05-Aug-03 | Capoff Inside | NEG | NEG | NEG |
| 06-Aug-03 | Ribeye | NEG | NEG | NEG |
| 06-Aug-03 | Capoff Inside | NEG | NEG | NEG |
| 06-Aug-03 | Inside | NEG | NEG | NEG |
| 11-Aug-03 | Ribeye | NEG | NEG | NEG |
| 13-Aug-03 | Ribeye | NEG | NEG | NEG |
| 20-Aug-03 | Inside | NEG | NEG | NEG |
| 20-Aug-03 | Capoff Inside | NEG | NEG | NEG |
| 20-Aug-03 | Capoff Inside | NEG | NEG | NEG |
| 20-Aug-03 | Inside | NEG | NEG | NEG |

AMERICAN MEAT INSTITUTE

February 1, 2010

David Goldman, M.D.
Assistant Administrator, Office of Public Health Science
Food Safety and Inspection Service
United States Department of Agriculture
Jamie Whitten Building
Room 341-E
Washington, DC 20250

***RE: Risk assessment for non-intact beef steaks***

Dear Dr. Goldman:

On December 24, 2009, the Food Safety and Inspection Service (FSIS or the agency) initiated a recall (FSIS-RC-067-2009) of *ca.* 248,000 pounds of mechanically tenderized beef steak associated with illnesses caused by *Escherichia coli* O157:H7. The American Meat Institute (AMI) treats each recall very seriously because of the adverse health risks they present for consumers. Recalls also can serve as a vehicle for the industry to learn more about a foodborne illness outbreak and the cause or causes of that outbreak. That information, in turn, can assist the meat and poultry industry and other stakeholders in implementing better processes or procedures that can help eliminate further outbreaks.

In the above-referenced recall, as in some other recalls, the underlying cause of the illness is unclear. Were the products that caused the illnesses mechanically tenderized only, mechanically tenderized and then marinated by a solution in a tumbler or by needle injection? Gleaning the correct information from this, and other, recalls can be very helpful in developing strategies and procedure to prevent future outbreaks.

AMI has conducted a review of available information regarding illness-related recalls linked to mechanically tenderized beef products. From this review AMI has determined that all of the recalls due to outbreaks were related to the consumption of marinated or enhanced steak products. (See Table 1 below.) Because of potential cross contamination issues, the recalled product may include more than just the implicated product.

Thank you again for your letter. We appreciate your input and your commitment to food safety.

Sincerely,

David P. Goldman, M.D., M.P.H.
Assistant Administrator
Office of Public Health Science

USDA

FSIS

# Risk Assessment Update for *E. coli* O157:H7 in non-intact beef

Risk Assessment Division
Office of Public Health Science
Food Safety and Inspection Service

*Better information. Better decisions. Risk assessment.*

# Objective

- To estimate comparative risks between intact and tenderized steaks that are either mechanically tenderized or chemically injected.
- To be used to guide FSIS labeling requirements that would mitigate the risk of *E. coli* O157:H7 illness from the consumption of tenderized steaks.

# Background

- 2002 FSIS Comparative Risk Assessment of E. coli O157:H7 in intact and mechanically tenderized steaks
- Six outbreaks since 2003
- New data from ARS study (2010)
- Update of the 2002 FSIS RA

# 2002 vs. 2010 Model

| | 2002 Model | 2010 Model |
|---|---|---|
| Product Type | Intact vs. MTB | Intact vs. MTB vs. CIB |
| Cooking Data Source | Sporing et al. | Sporing et al. and ARS |
| Cooking Method | Grilling, Broiling, and Frying | Grilling |
| Thickness of steaks | Differentiated | Combined |
| Cooking Lethality Model | Exponential | Log-linear |
| Model Outputs | Risk per serving | Number of illness |

# Model Inputs (1)

- 3 Scenarios based on product type
  - Intact
  - Mechanically Tenderized Beef (MTB)
  - Chemically Injected Beef (CIB)
- Initial Contamination
- Cooking temperature:
  - 120, 130, 140, 150, and 160 °F
- Cooking Effects

# Model Inputs (2)

- Growth Effects
- Health Effect
  - Dose-response model: beta-Poisson model
  - Population:
    - General population
    - Susceptible population
- Consumption data
  - FSIS product volume data, ERS, and NHANES

# Total # of illness in the US

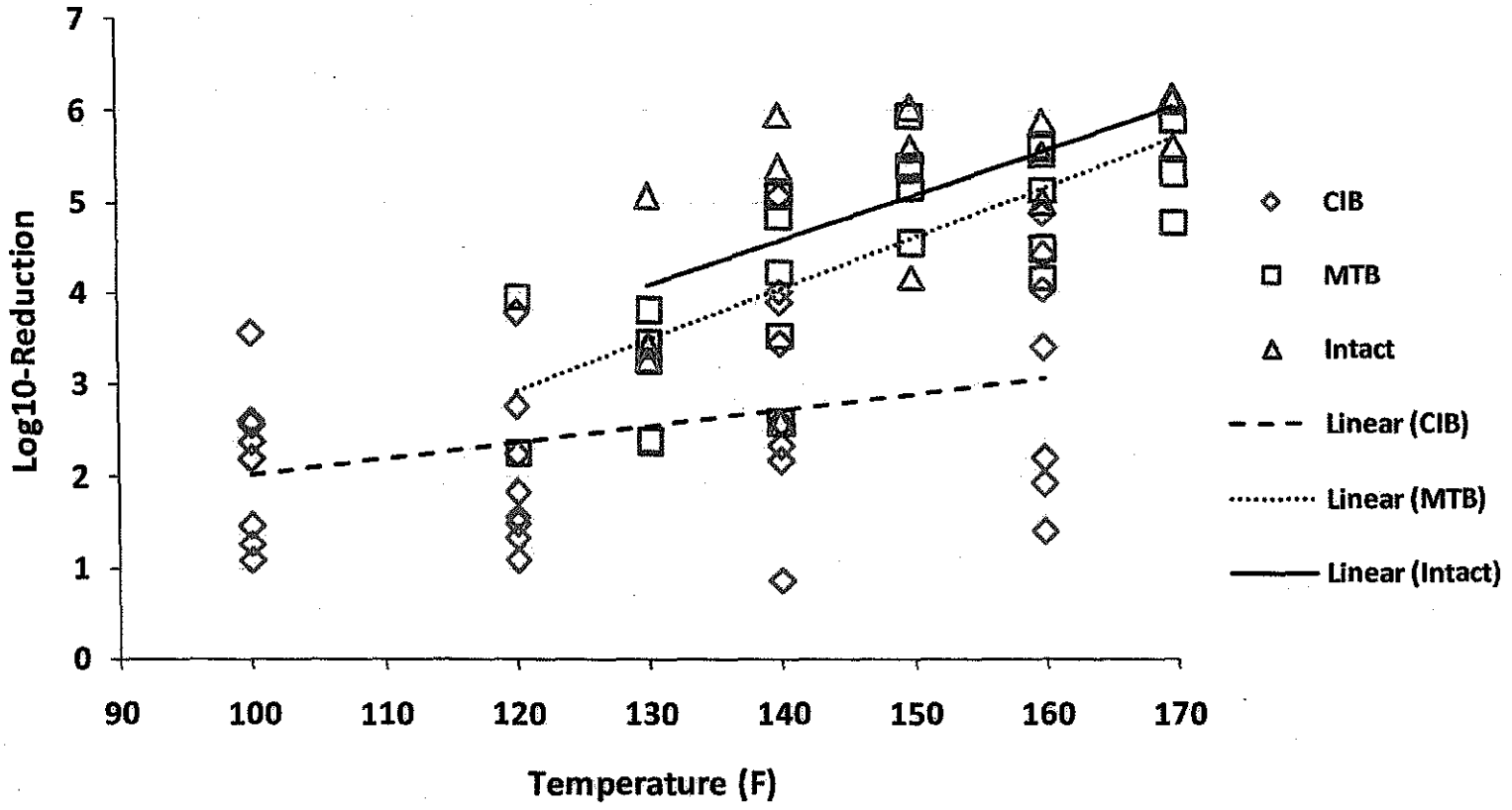
- # of Illness = (Risk / serving) x
  total # of serving in the US

  e.g.

  $5 \times 10^{-4}$ (Illness/serving) x
  1,000,000 servings = 500 illness

# Preliminary Results (1):

## $Log_{10}$-Reductions for 3 Scenarios with Trend Lines



# Preliminary Results (2):

## Predicted Outcomes
## (assuming 10 billion servings steaks)

| | Intact | MTB | CIB |
|---|---|---|---|
| P (I/S) | $4.39 \times 10^{-7}$ | $8.04 \times 10^{-7}$ | $3.04 \times 10^{-6}$ |
| Illness | 4,390 | 8,044 | 30,402 |
| Hosp. (21.6%) | 949 | 1,739 | 6,572 |
| HUS (5.1%) | 223 | 410 | 1,548 |
| Death (0.6%) | 27 | 50 | 190 |
| Relative Risk | 1 | 1.8 | 6.9 |

**Assumes all serving are Intact, MTB, or CIB**

# Conclusion

- Risks from MTB and CIB are higher than that from Intact Beef by ~2 and ~7 fold, respectively.
- This preliminary estimation only provides information on cooking effect and the relative risks for 3 different scenarios.

# Data Needs

- Cooking effect for tumbling and/or marinated steaks vs. intact steaks
- Comparative study on thermal resistance cooking lethality of *E. coli* O157:H7 in ground vs. whole muscle (Data available for *Salmonella*)

# Next Steps

| | |
|---|---|
| February – March, 2010 | -Consumption Analysis<br>-The 1st version of risk assessment model<br>-Work with FSIS/OPPD on Preamble for labeling |
| April – May, 2010 | -Populate the model with RA data<br>-Run and Test the model<br>-Scenario Analysis |
| June – July, 2010 | -Risk assessment Report to OPPD<br>-Presentations and Briefing<br>-Development of Statement of Work for Independent Peer Review |

# Next Steps

| | |
|---|---|
| August – September, 2010 | -Peer Review<br>-Response to peer review comments<br>-Make report 508–compliant<br>-Publish on FSIS website |
| September – November, 2010 | -Submit manuscript for publication |

# Tenderized steaks a health hazard?

Viewpoint

with RICHARD RAYMOND*

ARE tenderized steaks a culinary delight or a health hazard? The Safe Food Coalition thinks the latter and sent a letter to Agriculture Secretary Tom Vilsack Aug. 24 asking him to approve a draft from the U.S. Department of Agriculture's Food Safety & Inspection Service (FSIS) that would require special labeling of blade- and/or needle-tenderized steaks and move the draft to the Office of Management & Budget, which is basically the President.

The American Meat Institute (AMI) thinks it is the former and that the only documented risk is when the steaks are further processed, such as being injected with marinade for flavoring.

I tend to be on AMI's side on this one, and as some FSIS insiders know, there was an internal meeting on labeling tenderized steaks in 2007 after a small outbreak in Pennsylvania that resulted in "several illnesses" forced a sizeable recall of tenderized steaks. The source of the illnesses was a local restaurant.

The historical grand total of illnesses caused by consuming blade/needle-tenderized steaks at that time was very small, and I felt that the labeling was overkill for a very small problem and would probably discourage the consumption of some cuts of meat, which would drive up the price of others.

A historical review of the numbers of illnesses is no easy task, but besides the Pennsylvania outbreak and recall, I also uncovered these facts:

- In August 2000, two illnesses resulted from eating blade/needle-tenderized steaks at a restaurant in Michigan. The meat was also marinated.
- In June 2003, 11 people in five states fell ill after they ate tenderized, marinated steaks at restaurants belonging to a national chain, and 739,000 lb. were recalled.
- In August 2004, four people in Colorado were sickened, again from eating tenderized, marinated steaks in national chain restaurants.
- On Christmas Eve in 2009, 21 people in 16 states who ate blade-tenderized, marinated steaks at Applebee's fell ill, and 248,000 lb. were recalled.

Since that 2009 outbreak, the Safe Food Coalition has been pushing for change, and FSIS responded by having undersecretary Dr. Elisabeth Hagen testify at her appropriations hearing that she and the agency intended to proceed with requiring this product to be properly labeled and identified as a risk to health if not properly cooked.

In the five instances listed, all steaks were consumed in restaurants, and all were injected with marinade. My conclusion is the same as AMI, which is that it must be related to the marinade, not simply the blade or needle tenderizing.

In fact, an independent study by Weigand et al. published in the January 2012 *Journal of Food Protection* (75:48-61) showed that pathogens in tenderized meat were rendered more heat resistant when marinade or spices were added.

The proposed labeling recommendations, I am told, would state that intact steak can be safely consumed if the core temperature reaches 145°F; for tenderized steaks, the recommended core temperature would be 160°F, the same as ground beef.

That makes sense until you read another article in the January 2012 *Journal of Food Protection* (75:62-70) from lead author John Luchansky, a scientist who has written many scholarly articles on tenderized meats.

In the article, Luchansky and his fellow researchers reached the conclusion that the same 2-4 log reduction in *Escherichia coli* was achieved at core temperatures ranging from 122°F to 160°F. Some viable pathogens remained, presumably from inconsistent internal cooking temperatures.

The researchers further concluded that regardless of meat temperature or thickness, the results were consistently the same.

So, I do not understand the proposed cooking instructions. The temperature does not seem to affect the safety of the meat.

I do not understand why some think labeling will make for a better-informed consumer unless restaurants also are required to label the product on the menu as being non-intact and state that improper cooking may make you ill. This product, I am advised, is not sold in meat cases in grocery stores; it is sold almost exclusively to restaurants to fill their requests.

Menus already have generic messaging because of the Food Code that says improperly cooked meat, poultry, fish and seafood can make you sick. Why specifically pick on this small segment that has made so very few ill?

I must digress and explain the "small segment" statement.

According to FSIS's own beef checklist report in 2007, 18% of all beef steaks and roasts were blade or needle tenderized, for a total of 50 tons per month. That is a lot of meat being fabricated using this process, which breaks down muscle fiber and connective tissue to make a more tender piece of meat for those who can't or won't buy USDA Prime cuts.

So, approximately 40 people are known to have become ill from eating blade/needle-tenderized and marinated steaks in restaurants over 12 years.

In that same time frame, 14.4 million lb. of tenderized steaks and roasts have been fabricated, sold and, I presume, consumed.

If the government really wants to significantly reduce *E. coli*–related illnesses, it should require restaurants to cook hamburgers to 160°F if they are to be served to children and not ruin the steaks that account for only two to three illnesses per year.

By the way, no deaths have been linked to the consumption of tenderized steaks — probably because the very young and very old can't or don't want to chew a tougher piece of meat.

Let's also consider this: The Centers for Disease Control & Prevention estimates that 2,000 Americans choke to death annually, most due to improperly masticated beef steak.

Furthermore, the Food & Drug Administration requires no labeling on cantaloupe, the source of one of the deadliest foodborne illness outbreaks in U.S. history.

I do not criticize Hagen for her actions; she and I worked for different secretaries and presidents who eventually call all the shots.

I eat my steaks medium rare and my burgers well done and will continue to do so, label or no label. ■

*Dr. Richard Raymond is a medical doctor by training and a former undersecretary of agriculture for food safety at the U.S. Department of Agriculture.

Viewpoint

# Don't let ag become dirty topic of conversation

Viewpoint

with MIKE HALEY*

**MAKING the decision about what candidate is best to lead this country has always been a tough decision for me.**

**Oftentimes, I find myself evaluating each candidate right up until it's time to cast my ballot.**

**This year, however, while I believe both candidates truly want what is best for this country, it was much easier for me to decide which candidate I think can lead us along the path that I feel is best; thus, I easily made my decision months ago.**

**For the most part, politics is something I enjoy discussing with others who both agree and disagree with my viewpoints. However, those discussions have been few and far between this year as most individuals seem to be deeply entrenched in their camps defending the candidate of their choice and attacking the opposition with every misconstrued fact that can be found.**

**While this attack mentality does not make a whole lot of sense to me, I do understand that people are trying to convince others that their candidate of choice should win the election.**

**Instead, it seems like the extreme mentality nearly makes it almost impossible to have any productive conversation, and when attempted, it only works to further divide individuals into their respective camps.**

**In the end, it leads to a situation where politics are only discussed when in like company; discussing politics openly is often shunned because it's a topic that only leads to a heated debate that cannot easily be resolved as opinions vastly differ.**

**I learned a long time ago at a family dinner that it's best to try to avoid bringing up the topics I am passionate about like politics and religion because I think my relationships matter more than stating my personal beliefs.**

**However, this is often not the case when I engage in conversations revolving around another topic I am passionate about: raising crops and animals on my farm.**

**Just like with politics, farming is a topic that often starts a fierce debate about what is right and wrong; we can easily find ourselves deeply engrained in one camp and arguing relentlessly against another.**

**It's easy to convince ourselves that our beliefs on a topic are superior to others' view and that by yelling more loudly and more frequently, we will somehow convince them that they are wrong and to join our crusade in solving the world's problems.**

**This leads to my fear that our inability to listen to each other's thoughts about agriculture is only turning agriculture into a dirty topic like politics — one that cannot be discussed without increasing tensions.**

**Despite this fear, conversations about farming must continue if the tensions created from differences of opinion are ever to be resolved.**

**In doing so, we need to strive to keep the conversations civil and positive in tone while considering more than just our point of view. Without such an approach, our opinions become moot and are only heard within our own choir.** ■

*Mike Haley farms alongside his father Steve and wife Pam in Ohio, where they raise corn, soybeans, wheat and registered Simmental cattle. He is passionate about sharing information about agriculture with others. He is active in online conversations and can be found at http://haley-farms.com, http://justfarmers.biz and on Twitter @farmerhaley.