Journal of Food Protection, Vol. 74, No. 7, 2011, Pages 1054–1064
doi:10.4315/0362-028X.JFP-10-579

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

JOHN B. LUCHANSKY,[1]* ANNA C. S. PORTO-FETT,[2] BRADLEY A. SHOYER,[1] JEFFREY E. CALL,[1] WAYNE SCHLOSSER,[3] WILLIAM SHAW,[3] NATHAN BAUER,[3] AND HEEJEONG LATIMER[3]

[1]*U.S. Department of Agriculture, Agricultural Research Service, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038;* [2]*Food Safety Connect, P.O. Box 62, Blacksville, West Virginia 26521; and* [3]*U.S. Department of Agriculture, Food Safety and Inspection Service, 1400 Independence Avenue S.W., Washington, D.C. 20250, USA*

MS 10-579: Received 31 December 2010/Accepted 20 February 2011

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

* Author for correspondence. Tel: 215-233-6676; Fax: 215-233-6581; E-mail: John.Luchansky@ars.usda.gov.

† Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

‡ Portions of this research were presented at the Annual Meeting of the International Association for Food Protection, Anaheim, CA, 1 to 4 August 2010 *(23, 24)*.

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

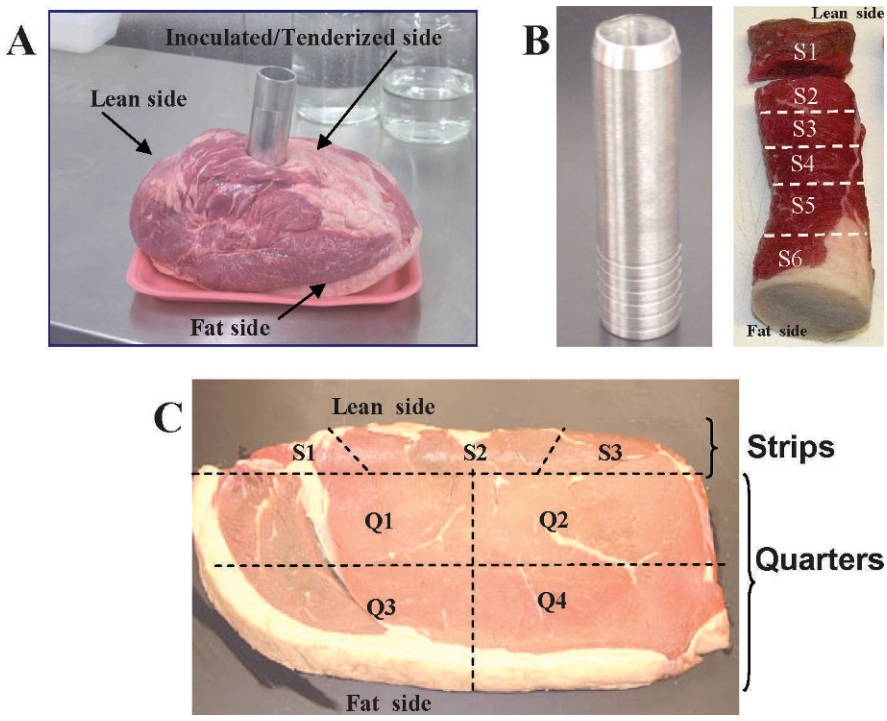

FIGURE 1. *(A) Coring of a beef subprimal. (B) Core apparatus and segmentation of a core sample into six consecutive segments. (C) Segmentation of a brine-injected steak into strips and quarters.*

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] 0/0 enrichment[e]	24/24 direct plating[d] 0/0 enrichment[f]
		15	18/18 direct plating 0/0 enrichment	23/24 direct plating 1/1 enrichment
	48.9	0	18/18 direct plating 0/0 enrichment	23/24 direct plating 1/1 enrichment
		15	12/18 direct plating 6/6 enrichment	17/24 direct plating 6/7 enrichment
	60.0	0	16/18 direct plating 1/2 enrichment	17/24 direct plating 6/7 enrichment
		15	10/18 direct plating 6/8 enrichment	14/24 direct plating 9/10 enrichment
	71.1	0	8/18 direct plating 5/10 enrichment	5/24 direct plating 6/19 enrichment
		15	6/18 direct plating 4/12 enrichment	7/24 direct plating 6/17 enrichment
Lac$^+$	37.8	0	18/18 direct plating[c] 0/0 enrichment[e]	24/24 direct plating[d] 0/0 enrichment[f]
		15	18/18 direct plating 0/0 enrichment	24/24 direct plating 0/0 enrichment
	48.9	0	17/18 direct plating 1/1 enrichment	22/24 direct plating 2/2 enrichment
		15	16/18 direct plating 2/2 enrichment	22/24 direct plating 1/2 enrichment
	60.0	0	15/18 direct plating 2/3 enrichment	20/24 direct plating 4/4 enrichment
		15	13/18 direct plating 1/5 enrichment	18/24 direct plating 3/6 enrichment
	71.1	0	11/18 direct plating 4/7 enrichment	7/24 direct plating 14/17 enrichment
		15	9/18 direct plating 4/9 enrichment	7/24 direct plating 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.

ACKNOWLEDGMENTS

We thank John Phillips (U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center [USDA-ARS-ERRC], Wyndmoor, PA) for statistically analyzing these data. We extend our sincere appreciation to Rosemary Martinjuk, Peggy Tomasula, Chris Sommers, Lihan Huang, and Nelly Osoria of the USDA-ARS-ERRC (Wyndmoor, PA) for their feedback or technical assistance on this project. In addition, we are grateful to Jim Lindsay and Mary Torrence (USDA-ARS, Beltsville, MD); Denise Eblen, Janell Kause, David Goldman, and Paul Uhler (USDA Food Safety and Inspection Service [USDA-FSIS], Washington, DC); Steve Campano (Hawkins, Inc., Minneapolis, MN); Tim Freier, Ted Brown, Dan Schaefer, Nancy Rathe, Francois Bere, and Scott Eilert (Cargill, Inc., Minneapolis, MN); Betsy Booren and Jim Hodges (American Meat Institute, Washington, DC); Randy Phebus (Kansas State University, Manhattan); Harshavardhan Thippareddi (University of Nebraska–Lincoln); John Sofos (Colorado State University, Fort Collins); Ernie Illg (Illg's Meats, Chalfont, PA); and Ron Tew (Deli Brands of America, Baltimore) for contributing their time, talents, and/or resources. This project was funded, in part, through an interagency agreement between USDA-ARS (J.B.L.) and USDA-FSIS.

REFERENCES

1. American Meat Institute. 2010. Letter to the U.S. Department of Agriculture, Food Safety and Inspection Service regarding risk assessment for nonintact beefsteaks. Personal communication.
2. Anonymous. 2005. Beef industry addresses the safety of nonintact beef products. Summary report of the Nonintact Products Processing Workshop. Coordinated by the National Cattlemen's Beef Association and sponsored by the Cattlemen's Beef Board, Denver.
3. Anonymous. 2006. Best practices for pathogen control during tenderizing/enhancing of whole-muscle cuts. Beef Industry Food Safety Council, Denver.
4. Beauchamp, C. S., and J. N. Sofos. 2010. Diarrheagenic *Escherichia coli,* chap. 5, p. 71–94. *In* V. K. Juneja and J. N. Sofos (ed.), Pathogens and toxins in foods: challenges and interventions. ASM Press, Washington, DC.
5. Bettelheim, K. A. 2001. Enterohaemorrhagic *Escherichia coli* O157:H7: a red herring? *J. Med. Microbiol.* 50:201–202.
6. Bettelheim, K. A. 2007. The non-O157 Shiga-toxigenic (verocytotoxigenic) *Escherichia coli*; under-rated pathogens. *Crit. Rev. Microbiol.* 33:67–87.
7. Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra, and N. A. Strockbine. 2005. Non-O157 Shiga toxin–producing *Escherichia coli* infections in the United States, 1983–2002. *J. Infect. Dis.* 192:1422–1429.
8. Centers for Disease Control and Prevention. 2010. From the Centers for Disease Control and Prevention: multistate outbreak of *E. coli* O157:H7 infections associated with beef from national steak and poultry. Available at: http://www.cdc.gov/ecoli/2010/index.html. Accessed 27 December 2010.
9. Centers for Disease Control and Prevention. 2010. Two multistate outbreaks of Shiga toxin–producing *Escherichia coli* infections linked to beef from a single slaughter facility—United States, 2008. *Morb. Mortal. Wkly. Rep.* 59:557–560.
10. Cox, R. J., J. M. Thompson, C. M. Cunial, S. Winter, and A. J. Gordon. 1997. The effect of degree of doneness of beefsteaks on consumer acceptability of meals in restaurants. *Meat Sci.* 45:75–85.
11. Duffy, G., E. Cummins, P. Nally, S. O'Brien, and F. Butler. 2006. A review of quantitative microbial risk assessment in the management of *Escherichia coli* O157:H7 on beef. *Meat Sci.* 74:76–88.
12. Gill, C. O., and J. C. McGinnis. 2004. Microbiological conditions of mechanically tenderized beef cuts prepared at four retail stores. *Int. J. Food Microbiol.* 95:95–102.
13. Gill, C. O., L. F. Moza, and S. Barbut. 2009. Survival of bacteria in less than thorough cooked, brine-injected steaks. *Food Control* 20: 501–507.
14. Gill, C. O., B. Uttaro, M. Badoni, and S. Zawadski. 2008. Distributions of brine and bacteria in beef prime cuts injected with brine without, or before or after mechanical tenderizing. *Meat Sci.* 79: 181–187.
15. Hajmeer, M. N., E. Ceylan, J. L. Marsden, and R. K. Phebus. 2000. Translocation of natural microflora from muscle surface to interior by blade tenderization. Available at: http://www.oznet.ksu.edu/library/lvstk2. Accessed 20 October 2010.
16. Heller, C. E., J. A. Scanga, J. N. Sofos, K. E. Belk, W. Warren-Serna, G. R. Bellinger, R. T. Bacon, M. L. Rossman, and G. C. Smith. 2007. Decontamination of beef subprimal cuts intended for blade tenderization or moisture enhancement. *J. Food Prot.* 70:1174–1180.
17. Jackson, C. J., M. D. Hardin, and G. R. Acuff. 1996. Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures. *J. Food Prot.* 59:230–237.
18. Juneja, V. K., O. P. Snyder, A. C. Williams, and B. S. Marmer. 1997. Thermal destruction of *Escherichia coli* O157:H7 in hamburger. *J. Food Prot.* 60:1163–1166.
19. Kennedy, J. E., S. K. Williams, T. Brown, and P. Minerich. 2006. Prevalence of *Escherichia coli* O157:H7 and indicator organisms on

the surface of intact subprimal beef cuts prior to further processing. *J. Food Prot.* 69:1514–1517.
20. Laine, E. S., J. M. Scheftel, D. J. Boxrud, K. J. Vought, R. N. Danila, K. M. Elfering, and K. E. Smith. 2005. Outbreak of *Escherichia coli* O157:H7 infections associated with nonintact blade-tenderized frozen steaks sold by door-to-door vendors. *J. Food Prot.* 68:1198–1202.
21. Lorenzen, C. L., T. R. Neely, R. K. Miller, J. D. Tatum, J. W. Wise, J. F. Taylor, M. J. Buyck, J. O. Reagan, and J. W. Savell. 1999. Beef customer satisfaction: cooking method and degree of doneness effects on the top sirloin steak. *J. Anim. Sci.* 77:637–644.
22. Luchansky, J. B., J. E. Call, R. K. Phebus, and T. Harshavardan. 2008. Translocation of surface inoculated *Escherichia coli* O157:H7 into beef subprimals following blade tenderization. *J. Food Prot.* 71: 2190–2197.
23. Luchansky, J. B., A. C. S. Porto-Fett, B. Shoyer, J. E. Call, C. Sommers, W. Schlosser, W. Shaw, N. Bauer, and H. Latimer. 2010. Thermal inactivation of Shiga toxin–producing cells of *Escherichia coli* in chemically injected beefsteaks cooked on a commercial open-flame gas grill, abstr. P3-75, p. 179. Abstr. Annu. Meet. IAFP 2010, Anaheim, CA, 1 to 4 August 2010.
24. Luchansky, J. B., A. C. S. Porto-Fett, B. Shoyer, J. E. Call, C. Sommers, W. Schlosser, W. Shaw, N. Bauer, and H. Latimer. 2010. Translocation of Shiga toxin–producing cells of *Escherichia coli* in chemically injected beef subprimals, abstr. P3-76, p. 179. Abstr. Annu. Meet. IAFP 2010, Anaheim, CA, 1 to 4 August 2010.
25. Luchansky, J. B., A. C. S. Porto-Fett, B. Shoyer, R. K. Phebus, T. Harshavardan, and J. E. Call. 2009. Thermal inactivation of *Escherichia coli* O157:H7 in blade tenderized beefsteaks cooked on a commercial open-flame gas grill. *J. Food Prot.* 72:1404–1411.
26. Marler, B. 2010. Cargill meat solutions recalls *E. coli* O26–tainted ground beef. Available at: http://www.foodpoisionjournal.com/admin/trackback/219273. Accessed 27 December 2010.
27. Mathusa, E., Y. Chen, E. Enache, and L. Hontz. 2010. Non-O157 Shiga toxin–producing *Escherichia coli* in foods. *J. Food Prot.* 73: 1721–1736.
28. Mukherjee, A., Y. Yoon, K. E. Belk, J. A. Scanga, G. C. Smith, and J. N. Sofos. 2008. Thermal inactivation of *Escherichia coli* O157:H7 in beef treated with marination and tenderization ingredients. *J. Food Prot.* 71:1349–1356.
29. Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.* 11:142–201.
30. National Advisory Committee for Microbiological Criteria for Foods. 2002. Final report: *E. coli* O157:H7 in blade tenderized beef. Available at: http://www.fsis.usda.gov/OPHS/NACMCF/2002/rep_blade1.htm. Accessed 17 December 2010.
31. Pfeiffer, K., R. Nickelson II, and K. D. Pfeiffer. 2008. *Escherichia coli* O157:H7 risk mitigation for blade-tenderized and enhanced nonintact beef muscle, p. 1–4. Proceedings of the 61st American Meat Science Association Reciprocal Meat Conference, Muscle Biology/Molecular Biology Section, Gainesville, FL, 22 to 25 June 2008.
32. Phebus, R. K., H. Thippareddi, S. Sporing, J. L. Marsden, and C. L. Kastner. 2000. *Escherichia coli* O157:H7 risk assessment for blade-tenderized beefsteaks. Available at: http://krex.k-state.edu/dspace/bitstream/2097/4685/1/cattle00pg117-118.pdf. Accessed 20 November 2010.
33. Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Swerdlow. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerg. Infect. Dis.* 11:603–609.
34. Rhee, M. S., S. Y. Lee, V. N. Hillers, S. M. McCurdy, and D. H. Kang. 2003. Evaluation of consumer-style cooking methods for reduction of *Escherichia coli* O157:H7 in ground beef. *J. Food Prot.* 66:1030–1034.
35. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17:7–15.
36. Schmidt, T. B., M. P. Keene, and C. L. Lorenzen. 2002. Improving consumer satisfaction of beef through the use of thermometers and consumer education by wait staff. *J. Food Sci.* 67:3190–3193.
37. Shen, C., J. M. Adler, I. Geornaras, K. E. Belk, G. C. Smith, and J. N. Sofos. 2010. Inactivation of *Escherichia coli* O157:H7 in nonintact beefsteaks of different thickness cooked by pan broiling, double pan broiling, or roasting by using five types of cooking appliances. *J. Food Prot.* 73:461–469.
38. Sofos, J. N., I. Geornaras, K. E. Belk, and G. C. Smith. 2008. Nonintact whole muscle food safety: the problem and research needs, p. 1–7. Proceedings of the 61st American Meat Science Association Reciprocal Meat Conference, Muscle Biology/Molecular Biology Section, Gainesville, FL, 22 to 25 June 2008.
39. Sporing, S. B. 1999. *Escherichia coli* O157:H7 risk assessment for production and cooking of blade-tenderized beefsteaks. Available at: http://www.fsis.usda.gov/OPPDE/rdad/ FRPubs/01-013N/Blade3.pdf. Accessed 27 December 2010.
40. Swanson, L. E., J. M. Scheftel, D. J. Boxrud, K. J. Vought, R. N. Danila, K. M. Elfering, and K. E. Smith. 2005. Outbreak of *Escherichia coli* O157:H7 infections associated with nonintact blade-tenderized frozen steaks sold by door-to-door vendors. *J. Food Prot.* 68:1198–1202.
41. U.S. Department of Agriculture, Food Safety and Inspection Service. 1999. Performance standards for the production of certain meat and poultry products. *Fed. Regist.* 64:732–749.
42. U.S. Department of Agriculture, Food Safety and Inspection Service. 2005. HACCP plan reassessment for mechanically tenderized beef products. *Fed. Regist.* 70:30331–30334.
43. U. S. Food and Drug Administration. 2001. Destruction of organisms of public health concern. Food Code, sect. 3-401. Available at: http://cfsan.fda.gov/~dms/fc01-3.html. Accessed 15 November 2010.
44. Warren, W. 2002. Characterization of *E. coli* O157:H7 subprimal beef cuts prior to mechanical tenderization. Project summary, National Cattlemen's Beef Association. Available at: http://www.beef.org/uDocs/E.%20coli%20Mech%20Tenderization_Warren_6_6_03.pdf.Accessed 16 November 2010.
45. Yoon, Y., A. Mukherjee, K. E. Belk, J. A. Scanga, G. C. Smith, and J. N. Sofos. 2009. Effect of tenderizers combined with organic acids on *Escherichia coli* O157:H7 thermal resistance in nonintact beef. *Int. J. Food Microbiol.* 133:78–85.