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Fate of Shiga Toxin–Producing O157:H7 and Non-O157:H7 *Escherichia coli* Cells within Blade-Tenderized Beef Steaks after Cooking on a Commercial Open-Flame Gas Grill†

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ABSTRACT

We compared the fate of cells of both Shiga toxin–producing *Escherichia coli* O157:H7 (ECOH) and Shiga toxin–producing non-O157:H7 *E. coli* (STEC) in blade-tenderized steaks after tenderization and cooking on a gas grill. In phase I, beef subprimal cuts were inoculated on the lean side with about 5.5 log CFU/g of a five-strain mixture of ECOH or STEC and then passed once through a mechanical blade tenderizer with the lean side facing up. In each of two trials, 10 core samples were removed from each of two tenderized subprimals and cut into six consecutive segments starting from the inoculated side. Ten total cores also were obtained from two nontenderized (control) subprimals, but only segment 1 (the topmost segment) was sampled. The levels of ECOH and STEC recovered from segment 1 were about 6.0 and 5.3 log CFU/g, respectively, for the control subprimals and about 5.7 and 5.0 log CFU/g, respectively, for the tenderized subprimals. However, both ECOH and STEC behaved similarly in terms of translocation, and cells of both pathogen cocktails were recovered from all six segments of the cores obtained from tenderized subprimals, albeit at lower levels in segments 2 to 6 than those found in segment 1. In phase II, steaks (2.54 and 3.81 cm thick) cut from tenderized subprimals were subsequently cooked (three steaks per treatment) on a commercial open-flame gas grill to internal temperatures of 48.9, 54.4, 60.0, 65.6, and 71.1°C. Regardless of temperature or thickness, we observed 2.0- to 4.1-log and 1.5- to 4.5-log reductions in ECOH and STEC levels, respectively. Both ECOH and STEC behaved similarly in response to heat, in that cooking eliminated significant numbers of both pathogen types; however, some survivors were recovered due, presumably, to uneven heating of the blade-tenderized steaks.

Recent data from the Centers for Disease Control and Prevention (CDC) *(26)* for the period 2000 through 2008 revealed that overall estimates for illnesses (9.4 million), hospitalizations (55,961), and deaths (1,351) attributed to foods have declined considerably since the publication of a similar report by the CDC for the 1990s *(21)*. However, Scallan et al. *(26)* also reported that the number of foodborne illnesses caused by Shiga toxin–producing *Escherichia coli* O157:H7 (ECOH) (ca. 73,000 to 97,000) and by non-O157:H7 serotypes of Shiga toxin–producing *E. coli* (STEC) (ca. 37,000 to 169,000) increased dramatically since the report by Mead et al. *(21)* a decade earlier. Historically, ECOH has been linked to several recalls and outbreaks of foodborne illness involving meat products, whereas with the exception of a relatively small recall of approximately 8,500 lb (3,859 kg) of ground beef due to contamination with STEC serotype O26 that caused a cluster of illnesses in Maine and New York *(34),* STEC has only rarely been associated with illness when meat was a vehicle *(8, 20)*.

Among meat-related outbreaks and recalls since ECOH was first identified as a foodborne pathogen approximately 30 years ago, ground beef has been the most frequently incriminated vehicle *(12, 38)*. However, within the past 10 years, tenderized and/or enhanced beef products have been also associated with several recalls and/or illnesses *(1, 2, 5, 6, 15, 25, 33)*. For products that are chemically enhanced or that contain added substances, as detailed elsewhere *(31),* the product label must declare all added ingredients and include an appropriate qualifying statement such as ''Injected with up to 10% of a flavoring solution.'' In contrast, regardless of how products are mechanically tenderized, at present such products are not required by the U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS) to be labeled as ''blade tenderized.'' Given the demonstrated potential for transfer of pathogens from the surface to the deeper tissues of the meat via mechanical

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or chemical tenderization *(9, 17, 29, 30, 39),* in addition to safe handling instructions consideration should be given to labeling such products as tenderized and/or enhanced and to educating consumers, restaurant, and/or food service personnel about proper cooking and handling of nonintact meats. Before regulatory agencies make any policy changes to possibly require labeling of nonintact products or make modifications to existing cooking instructions, additional research is needed to determine whether mechanically tenderized beef poses a greater risk than do otherwise similar but intact and/or chemically injected products. Further research is also needed to establish whether STEC is more persistent within deeper tissues or has greater heat resistance than ECOH within nonintact steaks and other types of beef.

At present, the FSIS considers ECOH an adulterant in raw nonintact beef, i.e., both raw ground beef and raw whole muscle cuts that are tenderized via blades, injection, restructuring, application of proteolytic enzymes, and/or vacuum tumbling *(32, 33).* In September 2011, the FSIS determined that a subset of STEC serotypes (i.e., O26, O45, O103, O111, O121, and O145) would also be considered adulterants in raw nonintact beef *(36).* Although surveys have revealed that the prevalence and levels of ECOH and STEC in meats are quite low *(2, 14, 37),* these serotypes continue to cause serious illness through ingestion of contaminated foods, including meats. Thus, considerable research has been conducted and/or initiated to gain insight into strategies to better characterize and control ECOH and STEC across the beef chain continuum. To this end, several studies have been conducted to quantify the translocation and thermal destruction of ECOH in nonintact beef *(9–11, 17, 19, 28, 39).* With the exception of our recent publication *(18),* no published studies have quantified translocation or fate of STEC in direct comparison with ECOH after tenderization, enhancement, storage, and/or cooking of nonintact beef. There is also an immediate need for further discussion, awareness, and research related to whether ECOH and/or STEC are a greater threat to public health in nonintact beef than in otherwise similar meat that is not enhanced or tenderized. Thus, our primary objectives were to compare the fate of both ECOH and STEC after translocation into beef subprimal cuts during blade tenderization (phase I) and their thermal destruction in steaks derived from tenderized subprimals following cooking on a gas grill (phase II).

MATERIALS AND METHODS

Bacterial strains. The five rifampin-resistant (Rif^r) strains of ECOH were (i) USDA-FSIS 011-82 (meat isolate), (ii) ATCC 43888 (human isolate, CDC B6914-MS-1), (iii) ATCC 43889 (human isolate, CDC B1409-C1), (iv) ATCC 43890 (human isolate, CDC C984), and (v) USDA-FSIS 45756 (meat isolate). The five kanamycin-resistant (Kan^r) strains of non-O157:H7 STEC were (i) JB1-95 (clinical isolate, serotype O111:H^-), (ii) CDC 96-3285 (human stool, serotype O45:H2), (iii) CDC 90-3128 (human stool, serotype O103:H2), (iv) CDC 97-3068 (human stool, serotype O121:H19), and (v) 83-75 (human stool, serotype O145:NM). All strains used in this study were confirmed, cultured, and maintained as described previously *(17–19).* Note, strain JB1-95 was listed as serotype O111:H7, strain B395, in our previous article *(18).* The Kan^r STEC strains originated in our companion study on brine-injected steaks *(18),* whereas the Rif^r ECOH strains originated in our previous study on blade-tenderized steaks *(17).* All genetically marked strains were generated by sequential transfer on agar plates containing incrementally higher levels of either rifampin or kanamycin as described previously *(30).* As detailed elsewhere *(17),* the cocktails were prepared by taking a loopful of an isolated colony of each of the ECOH or STEC strains and transferring it to separate test tubes containing 10 ml of tryptic soy broth (BD, Franklin Lakes, NJ) that were subsequently incubated for ca. 20 h at 37°C. The entire contents (10 ml) of each tube of the freshly grown five strains of ECOH or five strains of STEC were separately combined (50 ml total for each cocktail) and then separately washed and separately resuspended in 0.1% peptone water (PW; BD). Each cocktail was serially diluted in PW as appropriate to achieve the target inoculation level and then held at 4°C for about 30 min.

Inoculation and tenderization of subprimals and cooking of tenderized steaks. Top butt beef subprimals (ca. 15 to 20 lb [6.8 to 9.1 kg] each; USDA Institutional Meat Purchase Specifications no. 184) were purchased from a local wholesale distributor and stored at 4°C for up to 12 days. Subprimals were inoculated by pipetting 10 ml of either the ECOH or the STEC cocktail onto the lean side surface to a target level of about 5.5 log CFU/g for tenderization experiments (phase I) and about 3.5 or 5.5 log CFU/g for cooking experiments (phase II). Inoculated subprimals were then tenderized in a single pass with the lean side facing up as previously described *(17).* In phase I, for each of two trials, two subprimals were not tenderized (positive control), whereas an otherwise similar set of two subprimals were single-pass tenderized. A total of 10 core samples were obtained from each tenderized subprimal with a sterile stainless steel coring device (4 in. [10.2 cm] long and 2 in. [5.1 cm] in diameter) *(18)* and cut into six consecutive segments to quantify pathogen translocation (Fig. 1). For control subprimals, 10 core samples also were obtained from each nontenderized subprimal; however, only segment 1 samples were tested. The translocation matrix for experimental treatments consisted of one inoculation level by 10 core samples per each subprimal by 6 segments per core sample by two trials by two subprimals per trial by two pathogen types per trial per treatment, for a total of 480 segments tested. In contrast, the translocation matrix for the control treatments consisted of one inoculation level by 10 core samples per inoculation level by 1 segment per core sample by two trials by two subprimals per trial by two pathogen types per trial per treatment, for a total of 80 segments tested.

Inoculated and tenderized subprimals also were cut into steaks 2.54 or 3.81 cm thick, and a total of three steaks per treatment were cooked on an open-flame commercial gas grill (model XXE-4, Baker's Pride, New Rochelle, NY) with all four burners fully utilized to achieve target internal steak temperatures of 48.9°C (120°F), 54.4°C (130°F), 60°C (140°F), 65.5°C (150°F), or 71.1°C (160°F), as previously described *(19).* Each of the two cooking trials consisted of 2 inoculation levels × 5 cooking temperatures × 3 steaks per temperature × 2 thicknesses of steaks × 2 trials × 2 pathogen types, for a total of 240 steaks.

The come-up times, the temperature of the grill surface, and the temperature of the ambient air about 30 cm above the cast iron grill grates were monitored and recorded at 5-s intervals as described previously with calibrated stainless steel type J thermocouples (model HQTQIN-116-18, Omega Engineering,

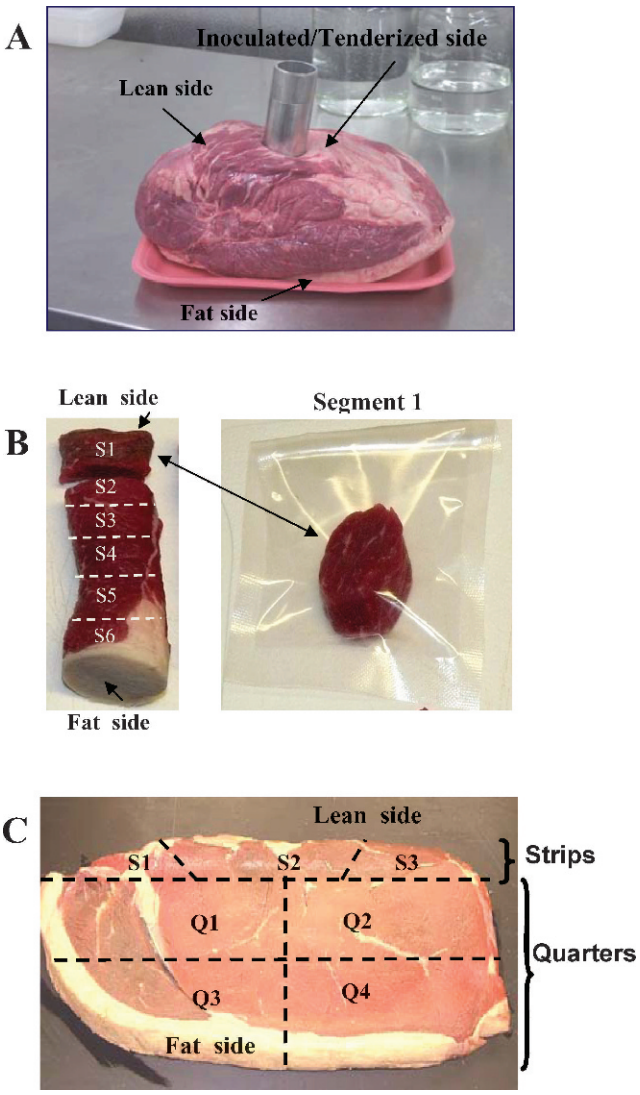

FIGURE 1. *(A) Coring of a beef subprimal. (B) Segmentation of a core sample into six consecutive segments and segment 1 vacuum packaged. (C) Segmentation of a blade-tenderized steak into strips and quarters.*

Inc., Stamford, CT) *(18).* The target internal temperature of each steak also was monitored using two type J thermocouples that were inserted into the approximate geometric center from opposing sides of each steak; readings were taken at 5-s intervals. Steaks were removed from the grill when both thermocouples within the steak reached the target internal temperatures of 48.9, 54.4, 60, 65.5, or 71.1°C. About 3 to 5 min elapsed before strips and quarters were cut from steaks (Fig. 1) after the meat was removed from the grill.

Viability of ECOH and STEC in blade-tenderized beef during storage. In related experiments to separately monitor the fate of each pathogen cocktail during simulated storage of nonintact beef steaks, multiple core samples were taken randomly from across the entire inoculated lean side surface of each tenderized subprimal with the sterile coring device. The topmost 1 cm (segment 1) of each tenderized core sample was separately placed into a sterile polyethylene bag (Fig. 1), vacuum packaged, and stored at 4 or 10°C for up to 28 days and at 25°C for up to 6 days. The matrix for the shelf life study consisted of 2 trials × 3 storage temperatures × 3 replicates for each of 6 sampling intervals for each pathogen type for a total of 108 samples.

Microbiological analyses. Cells for ECOH and STEC were enumerated from cores and segments and from strips and quarters as previously described *(18, 19).* Macerated meat samples were plated, with and without prior dilution in sterile 0.1% PW, onto sorbitol MacConkey agar (BD) plus rifampin (100 μg/ml; Sigma Chemical Company, St. Louis, MO) (SMACR) or sorbitol MacConkey agar plus kanamycin (100 μg/ml; Sigma) (SMACK) for enumeration of ECOH and STEC, respectively. After incubation at 37°C for 24 h, sorbitol-negative and sorbitol-positive colonies were enumerated as ECOH and STEC, respectively. When pathogen levels decreased to below the detection limit (≤1.4 log CFU/g for cores and/or segments, ≤0.80 log CFU/g for strips, and ≤0.70 log CFU/g for quarters) by direct plating, these cores, segments, strips, or quarters were enriched as previously described *(17)* by transferring 1 ml of each macerated sample into 9 ml of modified EC broth (BD) containing novobiocin (10 mg/liter; Sigma). Each sample was incubated without shaking at 37°C for 18 h and then streaked onto SMACR or SMACK plates and incubated at 37°C for 24 h for determining the presence or absence of ECOH and STEC, respectively.

Statistical analyses. For phase I of this study, as described previously *(17),* transfer of ECOH or STEC into the deeper tissues of subprimals by mechanical tenderization was expressed as a percentage: the average of the number of cells recovered separately from each of the six segments obtained from tenderized subprimal cores divided separately by the average of the number of cells recovered from segment 1 of the cores obtained from the nontenderized positive control subprimals and multiplied by 100. The standard deviations for the levels of the pathogen recovered from each of the six segments and the cumulative totals recovered from core samples were calculated using the statistical function option provided with Excel 2003 software (Microsoft, Redmond, WA). For phase II of this study, the SAS system (version 9.2, SAS Institute, Cary, NC) was used to determine significant differences ($P \leq 0.05$) in the pathogen levels among steak thicknesses, cooking temperatures, pathogen types, and sample types (i.e., strips versus quarters). Means and standard deviations of these levels in the cooking experiments were calculated from individual sets of data for each of the two separate trials at each of the five temperatures tested using triplicate samples and/or steaks at each time interval. An analysis of variance was used to determine differences in the log reduction obtained for each temperature, thickness, pathogen, and/or combinations thereof, and significance was determined using the least significant difference (LSD) technique at $P \leq 0.05$.

RESULTS

Translocation of ECOH and STEC into beef subprimals via blade tenderization. In general, there were no significant differences ($P \geq 0.05$) between the 5.5 log CFU/g ECOH and STEC cocktails in the extent of penetration and/or transfer of the cells into the deeper tissues of the meat after blade tenderization (Table 1). Similar results were observed when comparing translocation of these two pathogen cocktails using an initial inoculation level of ca. 3.5 log CFU/g (data not shown). Data from our previous work *(17)* also confirmed that, in general, the total levels recovered from all six segments of a core sample were essentially the same regardless of the initial inoculum level (ca. 0.5 to 3.5 log CFU/g) applied to the surface of subprimals, that is, transfer of cells into deeper tissues by

TABLE 1. *Levels of Shiga toxin–producing O157:H7 and non-O157:H7* E. coli *recovered from segmented core samples obtained from subprimals inoculated on the lean side and single-pass tenderized with the lean side facing up*

Segment no.	*E. coli* O157:H7 (ECOH)		Non-O157:H7 *E. coli* (STEC)	
	Mean ± SD (log CFU/g)[a]	% transfer[b]	Mean ± SD (log CFU/g)	% transfer
Nontenderized (control)[c]				
1	5.96 ± 0.03 A		5.26 ± 0.06 A	
Tenderized[d]				
1	5.74 ± 0.02 A	61.3	4.99 ± 0.10 A	53.8
2	3.49 ± 0.46 B	0.34	3.32 ± 0.05 B	1.14
3	3.04 ± 0.81 BC	0.12	2.70 ± 0.14 BC	0.27
4	2.84 ± 0.71 C	0.07	2.79 ± 0.41 BC	0.33
5	2.99 ± 0.30 BC	0.11	2.30 ± 0.55 CD	0.11
6	3.36 ± 0.02 B	0.25	2.22 ± 0.95 D	0.09
Total[e]	5.75 ± 0.01	62.18	5.01 ± 0.03	55.71

[a] For each pathogen, means within a column with different letters are significantly different ($P \leq 0.05$).
[b] Percent transfer was calculated as (average CFU per gram of tenderized subprimal core segment/average CFU per gram of segment 1 of nontenderized control subprimal core) × 100.
[c] Values are the mean ± SD of 10 cores from each of two nontenderized subprimals from each of two trials (40 total cores).
[d] Values for pathogen levels are the mean ± SD of 40 samples for segments obtained from 10 cores from each of two tenderized subprimals from each of two trials (40 total cores).
[e] Total pathogen level or percentage transferred into all six segments of a core sample.

tenderization was approximately the same for higher and lower initial levels of ECOH.

The use of lower initial levels (i.e., 3.5 log CFU/g) resulted in transfer to all six segments, but most cells (32.1 to 65.1%) of both ECOH and STEC remained within the topmost 1 cm (segment 1) of a subprimal (data not shown). Likewise, use of higher initial levels of ECOH or STEC (i.e., 5.5 log CFU/g) did not result ($P \geq 0.05$) in greater transfer of cells into the deeper tissues (Table 1) compared with the use of the lower initial levels, with the majority (53.8 to 61.3%) of the cells of both pathogen types remaining within the topmost 1 cm (segment 1). In contrast, the total percentage of ECOH and STEC cells recovered from segments 2 through 6 were about 112.4- and 51.5-fold lower, respectively, than levels recovered from segment 1 of nontenderized subprimals (control). There also was a significant linear decrease ($P \leq 0.05$) in pathogen levels from the top surface (the lean side that was inoculated and/or tenderized) through to the fat side, with lower levels of ECOH and STEC being internalized into the deeper tissues of the meat (segments 2 through 6). From among the about 62.2% (ECOH) and 55.7% (STEC) of cells that were transferred into all six segments of tenderized subprimals, the vast majority of the cells of each pathogen type resided within segment 1 (61.3% for ECOH and 53.8% for STEC), whereas the remaining 1.0% (ECOH) and 1.9% (STEC) of the cells were distributed in segments 2 through 6. About 5.8 and 5.0 log CFU/g ECOH and STEC, respectively, were recovered from all six segments of all cores tested. Some cells probably remained associated with the meat, purge, tenderizer blades, and/or other parts of the tenderizer machine, including the conveyer belt.

Viability of ECOH and STEC in blade-tenderized subprimals during storage. ECOH and STEC levels in segment 1 of extracted core samples decreased by 0.69 and 0.13 log CFU/g, respectively, over 28 days of storage at 4°C. However, when otherwise similar samples were stored at 10°C for 28 days or at 25°C for 6 days, ECOH increased by about 0.7 and 3.3 log CFU/g, respectively. When tenderized core samples were stored at 10°C for 28 days or at 25°C for 6 days, STEC increased by about 1.7 and 3.6 log CFU/g, respectively.

Come-up time, target internal meat temperature, air temperature, and surface temperature of the grill during cooking of blade-tenderized steaks. The average come-up times to achieve target internal temperatures of 48.9, 54.4, 60.0, 65.6, and 71.1°C were 5.1 ± 0.6, 6.0 ± 0.01, 6.5 ± 0.06, 6.9 ± 0.1, and 8.9 ± 0.7 min, respectively, for 2.54-cm-thick steaks and 10.2 ± 0.9, 10.8 ± 1.2, 12.0 ± 1.2, 14.2 ± 1.7, and 15.1 ± 1.3 min, respectively, for 3.81-cm-thick steaks. The internal temperatures of steaks cooked to a target internal temperature of 48.9, 54.4, 60.0, 65.6, and 71.1°C ranged from 48.9 to 117.4, 54.4 to 111.9, 60.0 to 109.8, 65.6 to 115.1, and 71.1 to 134.9°C, respectively, with average internal temperatures of 55.5 ± 10.7, 61.5 ± 11.2, 68.3 ± 12.0, 72.4 ± 10.4, and 78.6° ± 12.0°C, respectively, at the time when both thermocouples achieved and recorded the target end point temperature. For both trials, measurements for the ambient air and grill temperatures were taken at 5-s intervals, and the data were averaged. During cooking, the average temperature of the air above the grill grates was 100.8 ± 21.0°C, and the average temperature of the grill surface was 376.8 ± 19.95°C. These data are the average of 27,285 total temperature measurements, representing the summation of all grill and air temperatures, both inoculation levels (3.5 and 5.5 log CFU/g), both steak thickness (2.54 and 3.81 cm), and three steaks cooked in each of two trials for each of the five cooking temperatures tested.

TABLE 2. E. coli *O157:H7 recovered from nonintact steaks before and after cooking*

Temp (°C)	Thickness (cm)	Mean ± SD *E. coli* O157:H7 recovered (log CFU/g)[a]					
		Strips (S1 + S2 + S3)		Quarters (Q1 + Q2 + Q3 + Q4)		Total steak (all strips + all quarters)[b]	
		3.5 log CFU/g	5.5 log CFU/g	3.5 log CFU/g	5.5 log CFU/g	3.5 log CFU/g	5.5 log CFU/g
Uncooked	2.54	4.02 ± 0.07 A	5.98 ± 0.34 A	2.65 ± 0.03 A	4.54 ± 0.22 A	3.68 ± 0.06 A	5.64 ± 0.34 A
	3.81	3.70 ± 0.01 A	5.63 ± 0.45 A	2.58 ± 0.16 A	4.50 ± 0.45 A	3.38 ± 0.02 A	5.30 ± 0.45 A
48.9	2.54	1.51 ± 0.60 B	2.09 ± 0.58 B	1.33 ± 0.11 B	1.99 ± 0.86 B	1.49 ± 0.27 B	2.05 ± 0.72 B
	3.81	1.11 ± 0.03 B	1.26 ± 0.18 B	1.25 ± 0.05 B	1.36 ± 0.02 B	1.19 ± 0.05 B	1.32 ± 0.08 B
54.4	2.54	1.15 ± 0.01 B	2.73 ± 0.99 A	1.23 ± 0.08 B	1.83 ± 0.39 B	1.20 ± 0.05 B	2.46 ± 0.89 B
	3.81	1.05 ± 0.01 B	1.63 ± 0.34 B	1.16 ± 0.05 B	1.28 ± 0.08 B	1.11 ± 0.03 B	1.48 ± 0.24 B
60	2.54	1.15 ± 0.01 B	1.86 ± 0.48 B	1.22 ± 0.10 B	1.58 ± 0.36 B	1.19 ± 0.05 B	1.72 ± 0.43 B
	3.81	1.13 ± 0.02 B	2.09 ± 0.55 B	1.48 ± 0.45 B	1.45 ± 0.51 B	1.39 ± 0.33 B	1.83 ± 0.54 B
65.6	2.54	1.26 ± 0.17 B	2.39 ± 0.35 B	1.23 ± 0.08 B	2.27 ± 1.19 A	1.25 ± 0.03 B	2.42 ± 0.77 B
	3.81	1.03 ± 0.36 B	1.70 ± 0.28 B	1.17 ± 0.03 B	1.21 ± 0.32 B	1.11 ± 0.04 B	1.48 ± 0.29 B
71.1	2.54	1.15 ± 0.04 B	1.92 ± 0.96 B	1.26 ± 0.09 B	1.94 ± 0.54 B	1.22 ± 0.07 B	1.95 ± 0.74 B
	3.81	1.22 ± 0.28 B	1.20 ± 0.10 B	1.14 ± 0.01 B	1.28 ± 0.01 B	1.19 ± 0.14 B	1.25 ± 0.03 B

[a] For an inoculation level and steak thickness, means followed by different letters are significantly different ($P \leq 0.05$) by the LSD test.
[b] Levels of *E. coli* O157:H7 reported for total steak are the summation of total CFU from all strips plus all quarters and represent the results from two trials and 42 pieces of meat.

Thermal inactivation of ECOH and STEC in blade-tenderized steaks cooked on a gas grill. With the exception of 3.81-cm-thick steaks inoculated with 3.5 or 5.5 log CFU/g that were cooked to 54.4 or 48.9°C, respectively, there were no significant differences ($P \geq 0.05$) in the extent of thermal inactivation between ECOH and STEC in blade-tenderized beef regardless of steak thickness, inoculation level, or target cooking temperature (data not shown). In general, the higher the internal temperature and the thicker the steak, the greater the lethality for ECOH and STEC compared with the lower temperatures and thinner steaks tested. These differences could be due, in part, to the additional time needed for the thicker steaks to achieve the target temperature and/or to the fact that the majority of the pathogen cells were in the outermost 1 cm and, therefore, received more heat during cooking to a higher internal temperature. In general, when subprimals were inoculated with 3.5 or 5.5 log CFU/g ECOH or STEC and the control steaks cut from these subprimals were portioned (before cooking) into strips (S1 through S3) and quarters (Q1 through Q4), the sum of all three strips had appreciably more pathogen cells than did the sum of all four quarters (Tables 2 and 3). Also, more cells were recovered from Q1 and Q2 than from Q3 and Q4 (data not shown).

With the exception of strips and quarters cut from 3.81-cm-thick steaks inoculated with 5.5 log CFU/g ECOH and subsequently cooked to 54.4 or 65.6°C, respectively, for a given inoculation level and given cooking temperature, the thickness of the steak did not have a significant effect ($P \geq 0.05$) on lethality for ECOH (Table 2) or STEC (Table 3) in strips or quarters or on the total recovery of ECOH or STEC from strips plus quarters. Regardless of steak thickness or whether more cells were distributed on the surface (i.e., strips) or into the deeper tissues (i.e., quarters) of steaks cut from tenderized subprimals that were inoculated with 3.5 or 5.5 log CFU/g, there was no significant difference ($P \geq 0.05$) in lethality for ECOH among the various cooking temperatures tested. Likewise, when subprimals were inoculated with ca. 3.5 log CFU/g STEC and then cut into steaks that were about 2.54 cm thick, there were no significant differences ($P \geq 0.05$) in the extent of thermal inactivation of the pathogen among cooking temperatures for strips, quarters, or total steaks. However, when subprimals were inoculated with about 3.5 log CFU/g STEC and then cut into steaks that were about 3.81 cm thick, with the exception of quarters cut from these steaks, significant differences ($P \leq 0.05$) in the extent of thermal inactivation of STEC were observed for strips and total steaks that were cooked to a target internal temperature of 54.4°C when compared with otherwise similar strips and total steaks cooked to a target internal temperature of 48.9, 65.6, or 71.1°C, but not those cooked to 60.0°C. Similarly, significant differences ($P \leq 0.05$) were found in the extent of thermal inactivation of STEC (5.5 log CFU/g inoculum) transferred via blade tenderization into strips or on total steaks cut to a thickness of 2.54 cm and then cooked to 54.4°C compared with strips and total steaks that were cooked to a target internal temperature of 60.0, 65.6, or 71.1°C but not those cooked to 48.9°C. However, no significant differences ($P \geq 0.05$) in lethality for STEC were observed for quarters that were cut from 2.54-cm-thick steaks cooked to target internal temperatures of 48.9 and 54.4°C compared with those quarters cooked to a target internal temperature of 60.0, 65.6, or 71.1°C. When subprimals were inoculated with ca. 5.5 log CFU/g, inactivation of STEC was greater ($P \leq 0.05$) for strips and quarters that were cut from 3.81-cm-thick steaks and for total steaks that were cooked to a target internal temperature of 60.0, 65.6, and 71.1°C compared with otherwise similar strips, quarters, and total steaks cooked to a target internal temperature of 48.9 and 54.4°C. Our findings confirmed that cooking mechanically tenderized steaks inoculated with ca. 3.5 or 5.5 log CFU/g ECOH to target internal instantaneous

TABLE 3. *Non-O157:H7 Shiga toxin–producing* E. coli *recovered from nonintact steaks before and after cooking*

Temp (°C)	Thickness (cm)	Mean ± SD non-O157:H7 *E. coli* recovered (log CFU/g)[a]					
		Strips (S1 + S2 + S3)		Quarters (Q1 + Q2 + Q3 + Q4)		Total steak (all strips + all quarters)[b]	
		3.5 log CFU/g	5.5 log CFU/g	3.5 log CFU/g	5.5 log CFU/g	3.5 log CFU/g	5.5 log CFU/g
Uncooked	2.54	3.44 ± 0.312 A	5.96 ± 0.09 A	2.54 ± 0.01 A	4.59 ± 0.26 A	3.15 ± 0.26 A	5.61 ± 0.10 A
	3.81	3.68 ± 0.29 A	5.94 ± 0.05 A	2.51 ± 0.17 A	4.97 ± 0.21 A	3.35 ± 0.29 A	5.63 ± 0.02 A
48.9	2.54	1.61 ± 0.29 B	2.59 ± 0.93 BC	1.15 ± 0.01 B	2.33 ± 0.35 B	1.42 ± 0.19 B	2.51 ± 0.69 BC
	3.81	1.23 ± 0.21 C	3.22 ± 0.25 B	1.15 ± 0.05 B	2.39 ± 0.16 B	1.19 ± 0.13 C	2.93 ± 0.24 B
54.4	2.54	1.49 ± 0.00 B	3.40 ± 0.00 B	1.13 ± 0.00 B	2.58 ± 0.00 B	1.33 ± 0.00 B	3.11 ± 0.00 B
	3.81	2.54 ± 0.25 B	2.37 ± 1.11 B	1.36 ± 0.10 B	2.01 ± 0.36 B	2.21 ± 0.23 B	2.27 ± 0.83 BC
60	2.54	1.89 ± 0.33 B	1.92 ± 0.05 CD	1.30 ± 0.14 B	1.43 ± 0.25 C	1.65 ± 0.28 B	1.72 ± 0.04 CD
	3.81	1.75 ± 0.77 BC	2.31 ± 0.04 BC	1.08 ± 0.06 B	1.20 ± 0.01 C	1.56 ± 0.55 BC	1.99 ± 0.03 C
65.6	2.54	1.17 ± 0.19 B	1.98 ± 1.42 CD	1.15 ± 0.03 B	1.58 ± 0.52 C	1.16 ± 0.10 B	1.90 ± 1.09 CD
	3.81	1.02 ± 0.01 C	1.41 ± 0.60 CD	1.16 ± 0.04 B	1.71 ± 0.74 BC	1.10 ± 0.02 C	1.61 ± 0.70 CD
71.1	2.54	1.10 ± 0.02 B	1.43 ± 0.45 D	1.64 ± 0.77 B	1.31 ± 0.09 C	1.53 ± 0.62 B	1.38 ± 0.27 D
	3.81	1.00 ± 0.02 C	1.13 ± 0.05 D	1.43 ± 0.45 B	1.17 ± 0.04 C	1.32 ± 0.34 C	1.16 ± 0.05 D

[a] For inoculation level and steak thickness, means followed by different letters are significantly different ($P \leq 0.05$) by the LSD test.
[b] Levels of non-O157:H7 Shiga toxin–producing *E. coli* reported for total steak are the summation of total CFU from all strips plus all quarters and represent the results from two trials and 42 pieces of meat.

temperatures of 48.9, 54.4, 60.0, 65.6, or 71.1°C reduced pathogen levels by about 2.0 to 2.5 and 3.2 to 4.1 log CFU/g, respectively. Similarly, levels of STEC were reduced by about 1.5 to 2.3 and 2.5 to 4.5 log CFU/g when mechanically tenderized steaks were inoculated with ca. 3.5 or 5.5 log CFU/g, respectively, of this pathogen and then cooked to target internal instantaneous temperatures 48.9, 54.4, 60.0, 65.6, or 71.1°C.

After cooking to the recommended internal instantaneous temperature of 71.1°C *(32, 36),* depending on the initial inoculation level there was a total reduction of ECOH and STEC of ca. 2.2 to 4.1 and 1.7 to 4.5 log CFU per steak, respectively; however, it was still possible to recover viable cells of both pathogen types from all strips and quarters by direct plating or enrichment after cooking to any of the internal temperatures tested (Tables 4 and 5).

DISCUSSION

Although O157:H7 strains are the most common serotypes of *E. coli* associated with foodborne illness,

TABLE 4. *Pathogen recovery and enrichment of cooked steak portions testing negative for Shiga toxin–producing* E. coli *O157:H7 by direct plating*

Temp (°C)	Thickness (cm)	3.5 log CFU/g intial level				5.5 log CFU/g initial level			
		Strips (S1 + S2 + S3)[a]		Quarters (Q1 + Q2 + Q3 + Q4)[b]		Strips (S1 + S2 + S3)		Quarters (Q1 + Q2 + Q3 + Q4)	
		Direct plating[c]	Enrichment[d]	Direct plating	Enrichment	Direct plating	Enrichment	Direct plating	Enrichment
48.9	2.54	4/18	2/14	4/24	1/20	6/18	2/12	12/24	1/12
	3.81	4/18	3/14	1/24	2/23	6/18	4/12	11/24	4/13
54.4	2.54	1/18	0/17	0/24	0/24	11/18	0/7	10/24	2/14
	3.81	2/18	0/16	2/24	2/22	8/18	3/10	11/24	11/13
60	2.54	0/18	0/18	0/24	0/24	10/18	0/8	4/24	2/20
	3.81	6/18	2/12	4/24	1/20	6/18	4/12	7/24	6/17
65.6	2.54	3/18	0/15	0/24	1/24	10/18	1/8	11/24	4/13
	3.81	2/18	0/16	0/24	0/24	6/18	3/12	6/24	6/18
71.1	2.54	2/18	1/16	0/24	0/24	11/18	1/7	8/24	1/16
	3.81	1/18	3/17	0/24	1/24	5/18	3/13	4/24	3/20

[a] Strips, 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] Quarters, 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] Direct plating, number of strip or quarter composite samples from which *E. coli* O157:H7 was recovered by direct plating /total number of composite samples direct plated.
[d] Enrichment, number of strip or quarter composite samples from which *E. coli* O157:H7 was recovered by enrichment/total number of composite samples enriched.

TABLE 5. *Pathogen recovery by direct plating and enrichment of cooked steak portions testing negative for non-O157:H7 Shiga toxin–producing* E. coli

		3.5 log CFU/g intial level				5.5 log CFU/g initial level			
		Strips (S1 + S2 + S3)[a]		Quarters (Q1 + Q2 + Q3 + Q4)[b]		Strips (S1 + S2 + S3)		Quarters (Q1 + Q2 + Q3 + Q4)	
Temp (°C)	Thickness (cm)	Direct plating[c]	Enrichment[d]	Direct plating	Enrichment	Direct plating	Enrichment	Direct plating	Enrichment[6]
48.9	2.54	12/18	2/6	6/24	4/18	13/18	1/5	14/24	2/10
	3.81	5/18	4/13	7/24	4/17	15/18	2/3	18/24	2/6
54.4	2.54	4/18	1/14	1/24	2/23	14/18	0/4	16/24	1/8
	3.81	7/18	2/11	3/24	5/21	12/18	4/6	15/24	4/9
60	2.54	4/18	0/14	1/24	5/23	5/18	3/13	7/24	5/17
	3.81	3/18	4/15	1/24	8/23	9/18	3/11	6/24	2/18
65.6	2.54	2/18	1/16	1/24	1/23	6/18	2/12	4/24	0/20
	3.81	0/18	4/18	2/24	4/22	5/18	1/13	5/24	2/19
71.1	2.54	2/18	1/16	2/24	2/22	4/18	1/14	3/24	0/21
	3.81	0/18	3/18	1/24	1/23	1/18	2/17	2/24	0/22

[a] Strips, 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] Quarters, 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] Direct plating, number of strip or quarter composite samples from which non-O157:H7 Shiga toxin–producing *E. coli* was recovered by direct plating/total number of composite samples direct plated.

[d] Enrichment, number of strip or quarter composite samples from which non-O157:H7 Shiga toxin–producing *E. coli* was recovered by enrichment/total number of composite samples enriched.

non-O157:H7 Shiga toxin–producing strains also have recently been linked to outbreaks and individual cases worldwide *(4, 20)*. In a recent report, the CDC estimated that the number of illnesses in the U.S. population caused by both STEC and ECOH were appreciably higher for 2000 through 2008 compared with the 1990s *(21, 26)*. Several recent outbreaks and recalls associated with ECOH and STEC contamination of meat products have contributed to the observed increases in illnesses attributable to Shiga toxin–producing strains of *E. coli*. To date, there have only been a handful of studies detailing the fate of STEC in nonintact beef processed via blade tenderization or chemical injection *(10, 17–19, 22, 25, 30, 37)*. The potential for illness may be exacerbated in nonintact products, such as steaks cut from blade-tenderized subprimals, because ECOH and/or STEC cells may reside within the deeper tissues. Thus, nonintact steaks and related cuts of meat may require higher cooking temperatures and/or longer cooking times to eliminate pathogens than would be required for otherwise similar pieces of meat on which pathogens may only reside on the surface and probably would be killed by direct contact with the heat source used for cooking. Some concern also exists that STEC may differ from ECOH in tolerance to stresses such as tenderization and cooking; if so, existing conditions and practices for processing, storing, handling, and heating of beef steaks should be reevaluated. Therefore, we compared the thermal stability of ECOH and STEC in steaks cut from mechanically tenderized subprimals for risk assessment and product labeling purposes.

Our data establish for the first time that mechanical tenderization transfers ECOH and STEC throughout beef subprimals to the same extent; however, more cells of both pathogen types were transferred into the topmost 1 cm than into the deeper tissues. These data are similar to our previous findings *(17–19)* and those reported by other investigators *(9, 11, 25, 30)*. Although blade tenderization, chemical injection, application of proteolytic enzymes, vacuum tumbling, cubing, pounding, and/or frenching can force ECOH and STEC cells into the interior of a whole-muscle piece of meat, the number of cells transferred within nonintact beef is likely to be quite low because of the very low prevalence (<0.083 to 2.0%) *(2, 14)* and levels (<0.375 CFU/cm^2) *(36)* of pathogenic *E. coli* typically found on the surface of beef subprimals. From a public health standpoint, because at least 18% of beef sold at retail is tenderized *(1)* and cells of Shiga toxin–producing *E. coli* may reside within the interior of tenderized subprimals (including the geometric center), further evaluation of the adequacy of existing parameters and procedures for cooking nonintact products is needed.

As concluded by the National Advisory Committee on Microbiological Criteria for Foods *(23)*, the presence of pathogenic *E. coli* within nonintact steaks is a potential public health threat when such products are not properly cooked. Regardless of steak thickness or initial (inoculation) pathogen levels, cooking nonintact steaks cut from tenderized subprimals to internal temperatures of 48.9 to 71.1°C resulted in average total reductions of 2.0 to 4.1 log of ECOH. Likewise, average total reductions of 1.5 to 4.5 log of STEC were achieved after cooking to 48.9 to 71.1°C. These data are similar to prior reports of 1.1- to 4.2-, 2.4- to 5.3-, and 0.5- to 4.1-log reductions of ECOH in restructured beef *(28)*, blade-tenderized steaks *(30)*, and ground beef patties *(13)*, respectively. However, surviving cells of both ECOH and STEC were recovered by direct plating or enrichment at all

cooking temperatures tested herein. Our laboratory *(18)* and other investigators have also reported survival of ECOH and/or STEC in nonintact beef after cooking *(10, 24, 30, 39)*. Survivors were presumably recovered, at least in part, due to the (i) inability to achieve the target end point temperature throughout the steak (i.e., existence of cold spots), (ii) reduction in heat penetration due to insulating effects of fat and/or connective tissue (i.e., uneven heating), (iii) variability in temperature at the cooking surface, and/or (iv) use of unrealistically high inoculation levels (e.g., 5.5 log CFU/g) of ECOH and STEC that probably would not be encountered in the ''real world.'' Thermal resistance of ECOH and STEC in nonintact beef can be influenced by the (i) prior history of the cells, (ii) age of the culture and whether cells were repeatedly passaged in the laboratory, (iii) growth medium and incubation temperatures, and (iv) initial inoculation levels. Muscle type and species of meat, especially the moisture and fat content, also may have an effect on thermal resistance. The cooking appliance used, e.g., grill, oven, or skillet, also can have an appreciable effect on the extent and rate that microbes are inactivated in foods *(28)*.

The ultimate goal of our ongoing research is to significantly reduce the prevalence and/or levels of ECOH and STEC in nonintact beef and, thus, to make a significant and positive impact on public health. Our findings have immediate and practical relevance based on our use of (i) pathogenic (i.e., Shiga toxin–producing) strains of *E. coli* rather than surrogates, (ii) pilot scale commercial food processing equipment rather than bench top or laboratory apparatuses, and (iii) entire beef subprimals and whole steaks rather than simulated (e.g., 3-g balls) or restructured beef products. Preliminary models generated from these data revealed estimated two- and fourfold greater risks for mechanically tenderized and chemically injected steaks, respectively, compared with otherwise similar but intact steaks *(3)*. These data call into question whether tenderized and/or enhanced products should be labeled as such and, if so, whether these products also should have additional labeling and cooking instructions tailored to the nature of the product, i.e., intact, ground, blade tenderized, vacuum tumbled, treated with proteolytic enzymes, and/or brine injected.

Our results add to the growing pool of knowledge establishing that STEC and ECOH behave similarly under food-relevant conditions. These data and related findings previously published by both our laboratory *(18)* and other investigators *(20)* provide a sufficient and scientifically sound basis for rendering a decision regarding the relative risk of ECOH and STEC associated with intact compared with nonintact beef and, in turn, for fostering debate on the associated public health risk(s), labeling and packaging information, modified cooking instructions, modified hazard analysis and critical control point procedures, and/or revised sampling regimens that may be required for such products. At present, the FSIS does not require mechanically tenderized nonintact meat to be identified or labeled as such. Often, however, it is difficult to discern whether products have been blade tenderized, and it has not yet been established whether such products require special handling and cooking instructions. Thus, consumers and retail establishments may not have the ability or sufficient information to assure that they properly cook such products. The problem may be exacerbated by consumer preference for steaks cooked to a medium degree of doneness ($<60^{\circ}$C [140°F]) *(7, 16, 27)* and the fact that at least 18% of retail beef is tenderized and/or enhanced *(1)*.

If regulations make it mandatory to label nonintact beef products as tenderized or needle injected, this requirement should be applicable throughout fabrication and further processing of a given product until it reaches the end user. In the present study, no discernible differences in translocation or thermal stability were noted between the STEC and ECOH cocktails inoculated onto beef that was then blade tenderized and then cooked. Ultimately, the potential for illness can be appreciably lessened by ensuring that all portions of each steak or piece of meat achieve the recommended end point temperature of 160°F (71.1°C). Existing cooking regimens and associated interventions already validated for ECOH will likely be equally effective against STEC. Additional studies to address key parameters such as strain-to-strain variation, as well as the effects of fat, temperature of the meat when placed on the cooking appliance, and/or differences among meat species are currently being evaluated as part of our continuing effort to address the comparative fates of ECOH and STEC in intact versus nonintact meat during processing, storage, and/or cooking.

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