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When Is A Steak Not A Steak?

By Barbara Kowalcyk

October 4, 2012

Have you ever noticed that the USDA recommends that steaks and roasts be cooked to 145° while ground beef should be cooked to 160°? Have you ever wondered why there is a difference? After all, beef is beef -- right?

Actually there is a very good reason for the difference. While meat starts out sterile, it can become contaminated with bacteria – like *E. coli* O157:H7 – when it isn't handled properly during slaughtering or processing, and once contaminated, the only thing that will kill the bacteria is heat. With intact cuts of meat – like steaks and roasts – that contamination will be on the surface, not on the inside. Pathogens on the surface are much easier to kill, after all, the outside of the meat heats up much faster than the inside does, so the recommended temperature can be lower. However, with non-intact meat – like ground beef – surface bacteria can be moved moved or "translocated" to the inside of the meat where it is harder to kill, so a higher temperature is required. Sounds pretty straightforward, right? Intact meat gets cooked to a lower temperature of 145° and non-intact meat gets cooked to 160°.

Unfortunately, steaks and roasts are often not as they seem.

So, when is a steak not a steak and a roast not a roast? As soon as its surface has been pierced, a steak or roast becomes a non-intact cut of meat and should be cooked to a higher temperature. Many of us grew up learning to make our steaks and roasts juicer and more flavorful by piercing them with a fork and letting them sit in a marinade for a while. Now, you may not be fully aware of it, but stabbing the meat created a "hide-out" for bacteria like *E. coli* O157:H7. And it means that the pierced steak or roast should be cooked differently. Now, assuming that you know about the increased risk of illness, you can make an informed choice about how well you want to cook that steak or roast. But what happens when you don't know that the steak or roast you have just bought or been served was already tenderized? How do you make an informed choice then?

According to the USDA estimates, 18% of all beef steaks and roasts manufactured in the United States are mechanically tenderized. This mechanically tenderized process pushes hundreds of needles or sharp blades into steaks and roasts to make tougher cuts of meat more tender and therefore more palatable to consumers. It also takes any pathogens on the outside of the meat and pushes them to the inside - as shown by multiple scientific studies, including ones conducted by USDA. So, for food safety reasons, the "steak" or "roast" needs to be treated like ground beef and must be cooked thoroughly to kill any bacteria that are still alive inside the meat. Eating an

undercooked steak or roast that has been mechanically tenderized has a higher risk of causing foodborne illness. Children, pregnant and post-partum women, senior citizens and anyone with a compromised immune system are particularly vulnerable and could develop serious, life-threatening complications.

Currently, mechanically tenderized cuts of meat are not labeled, even though they look the same as non-tenderized products. Basically, they are non-intact cuts of meat that look intact. Consumers cannot tell the difference and, therefore, have no way of knowing that they need to be prepared differently. To be safe, these steaks and roasts cannot be grilled and eaten medium rare – like intact steaks and roasts can be. Consumers need to know that there really is a difference in risk, which means "a steak is not always a steak and a roast is not always a roast." This is especially true when a major *E. coli* outbreak is in process, like today.

XL Foods Inc., a Canadian firm, has recalled all the beef produced in one of their slaughtering plants for unsanitary conditions, after a United States border checkpoint found *E. coli* O157:H7 in its testing. Over the past two weeks, Canada has expanded its original recall of beef trim (used in making ground beef) thirteen times. The most recent update, on October 1st, expanded the growing list of retailers and included all steaks, roasts and other cuts destined for retail sale. Mechanical tenderization was applied to some of the recalled steaks and roasts, which means that consumers eating mechanically tenderized Canadian XL Food products are at a higher risk for acquiring an *E. coli* O157:H7 infection. Four of Canada's reported eight illnesses are linked to steaks, and U.S. officials know that a large amount of product, including beef steaks and roasts, have been shipped to stores throughout the United States. Consumers should view all of the Canadian XL Foods' beef products as being potentially contaminated with deadly *E. coli* O157:H7 and should return or discard the product. More information about the recall is available here.

The recall of Canadian beef that began last month highlights mechanically tenderized meat as a critical food safety issue. For the past three years, consumer groups have worked to put in place labeling requirements for mechanically tenderized meats. Those efforts have paid off – a rule that would require mechanically tenderized products to be labeled has recently been approved by the Secretary of Agriculture and sent to the White House for final approval. Consumers can write to the White House's Office of Management and Budget and tell the President's staff that USDA's label proposal for mechanically tenderized meat must be approved immediately. Labeling of mechanically tenderized meat products is a consumer protection that is fairly inexpensive to implement, but the benefits for labeling these products could be great.

Ultimately, consumers have a right to know what they are buying and eating. They must be given the information they need to make informed decisions about their food choices. After all, what you - the consumer - put into your mouth can directly impact your health. You have a right to know.

Barbara Kowalcyk Ph.D. is a Co-Founder and the CEO of the Center for Foodborne Illness Research & Prevention. Her story was featured in Food, Inc. and in 2010 was voted an Ultimate Game Changer by the Huffington Post.

HEALTH CANADA

Information for Canadians on cooking mechanically tenderized beef

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For immediate release

OTTAWA - Health Canada has started a review of the science around the safe handling and cooking of beef products that are mechanically tenderized, to identify what advice should be communicated to consumers and the food industry.

Some meat handlers and even some Canadians at home tenderize cuts of beef, including steaks and roasts, using machines or tools made for this process. Mechanically tenderizing meat is a very common practice and has been used by suppliers, restaurants and retailers for many years to improve the tenderness and flavour of cooked beef.

While this review is ongoing, and to make sure that any bacteria that may be present in the meat are killed, Health Canada and the Public Health Agency of Canada are encouraging Canadians to cook mechanically tenderized steak and beef cuts to an internal temperature of at least 71 degrees Celsius (160 degrees Fahrenheit). Reaching 71 degrees Celsius (160 Fahrenheit) would cook a steak or roast to approximately "medium" doneness, although a digital food thermometer should be used to be sure that the safe internal temperature is reached.

In addition to this interim advice, Health Canada and the Public Health Agency continue to recommend that Canadians take steps to protect against the risks of food-borne illness, including *E. coli*. These steps include:

- Wash your hands before and after cooking;
- Keep knives, counters and cutting board clean;
- Keep raw meats separate from other foods when you store them; and
- Refrigerate or freeze left-overs promptly.

Canadians who are at greater risk of complications from foodborne illness, and their caregivers, should be particularly cautious about making sure any mechanically tenderized beef products are thoroughly cooked and handled safely. These groups include seniors, pregnant women, young children and those with weakened immune systems.

In general, the internal temperature of a steak or other solid cut of meat is not a significant health concern given that any harmful bacteria that may be present would normally only be on the surface of the meat and would be eliminated even if cooked "rare". However, when steaks and beef cuts are mechanically tenderized, there is a potential for bacteria to spread from the surface into the centre of the meat. As a result, there may be an increased chance that bacteria like *E. coli* O157:H7 are not fully eliminated when these beef products are cooked "rare".

Health Canada's scientific review will look at the likelihood that the tenderizing process can spread bacteria, along with additional steps and best practices that can be applied by industry to prevent the spread of bacteria before a product reaches Canadian consumers. The review will also evaluate the effectiveness of measures a consumer can take, including whether an internal temperature lower than 71 degrees Celsius (160 degrees Fahrenheit) would be as effective at reducing the risk from these products.

Health Canada is also actively working with the retail and food industry to support its efforts to identify mechanically tenderized beef for consumers through labels, signage or other means. The industry expects to start putting these measures in place over the next two to three weeks. In the meantime, should consumers be uncertain if a product has been mechanically tenderized, they are encouraged to ask the food seller or food service provider.

Once the scientific review has been completed, Health Canada will update Canadians on any changes to these recommendations.

For more information on safe food handling, please visit the Government of Canada's food safety portal.

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Media Enquiries:
Public Health Agency of Canada
(613) 941-8189

Health Canada
(613) 957-2983

Public Enquiries:
(613) 957-2991
1-866 225-0709

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Ottawa Citizen

Timeline: E. coli crisis, from slaughterhouse to fridge

Edmonton Journal October 8, 2012
http://www.ottawacitizen.com/health/Timeline+coli+outbreak+meat+recall/7318030/story.html

Aug. 23: Cattle are slaughtered at a plant in Brooks run by Edmonton-based XL Foods Inc. Beef slaughtered that day is later recalled.

Aug. 24, 27, 28, 29: Beef produced at the Brooks plant is later recalled.

Sept. 3: U.S. officials detect E. coli 0157:H7 bacteria in beef trimmings from the Brooks plant and alert the Canadian Food Inspection Agency (CFIA). Both agencies start investigating.

Sept. 4: The CFIA detects E. coli in beef trimmings processed at the Brooks plant. Consumers buy Kirkland Signature brand strip loin grilling steaks from a northeast Edmonton Costco at 13650 50th St. Four people later become ill.

Sept. 5: Beef produced at the Brooks plant is later recalled. The CFIA investigates how two batches of beef trimmings, which never made it to market, were contaminated. The agency tells the Brooks plant to take preventive actions and CFIA inspectors supervise plant operations.

Sept. 6: The CFIA asks XL Foods for information about distribution and test results for all products produced Aug. 24 and 28, the dates the contaminated products were made.

Sept. 7: The CFIA tells XL Foods to strengthen sampling and testing controls in the plant.

Sept. 8-9: CFIA officials continue to oversee plant operations and speak with management.

Sept. 10-11: CFIA analyses information from XL Foods and decides to take a closer look at products produced Aug. 24, Aug. 28 and Sept. 5.

Sept. 11: A four-year-old Calgary girl is hospitalized with symptoms caused by E. coli bacteria.

Sept. 11-12: Four people who ate Costco Kirkland strip loin steaks seek medical treatment in Edmonton for symptoms of E. coli poisoning. Two were hospitalized but all four are recovering.

Sept. 12: Food safety officials in the United States notify the CFIA two more meat samples from XL Foods have tested positive for E. coli. Both batches are held at the border and destroyed. The CFIA sends technical experts to the Brooks plant to investigate how contamination occurred.

Sept. 13-16: The CFIA removes XL Foods Inc. from the list of companies eligible to export to the United States. The CFIA's technical review team determines no single critical factor would have caused E. coli contamination. XL Foods starts telling its customers beef trimmings produced Aug. 24, Aug. 28 and Sept. 5 are being recalled.

Sept. 16: The CFI sends out the first alert warning people not to eat, sell or serve 26 ground beef and ground-beef products sold at several major stores because they "may be contaminated with E. coli." The alert says XL Foods Inc. voluntarily issued the recall though no reported illnesses have been linked to the recalled products.

Sept. 17: The CFIA expands the voluntary recall to add 55 more ground beef and ground-beef products to the list of those recalled across Canada. All the products were manufactured at the Brooks plant.

Sept. 18: The CFIA expands the recall to add 14 more products and issues five more corrective-action requests to XL Foods to fix plant deficiencies.

Sept. 19: The CFIA adds 75 more products to the recalled list. XL Foods and its parent company, Edmonton-based Nilsson Bros., release a recorded statement saying XL Foods prides itself on providing safe and high-quality beef products.

Sept. 20: The CFIA adds 37 products to the recall. The U.S. Food Safety and Inspection Service issues a public-health alert.

Sept. 21: The CFIA adds 47 products to the recall and discovers two more processing dates, Aug. 27 and 29, have a higher risk of E. coli contamination. XL Foods starts notifying customers. The U.S. Food Safety and Inspection Service updates its public-health alert.

Sept. 22: The CFIA adds 10 products to the recall and warns the public about the two new suspect production dates.

Sept. 24: The CFIA issues a summary that says an in-depth review uncovered "several deficiencies" during an investigation into the slaughterhouse. Alberta Health Services tells the CFIA there is no link between an illness it's investigating and an XL Foods product. U.S. officials notify CFIA that beef trimmings produced by XL Foods Aug. 27 has tested positive for E. coli.

Sept. 25: The CFIA adds 60 products to the recall. The U.S. recalls products distributed to California, Texas, Washington, Oregon, Michigan, Nebraska, Utah and Wisconsin. Alberta Health Services officials say they are investigating a total of eight E. coli cases, four in Edmonton, three in Calgary and one in central Alberta. Lab test results come in to Alberta Health Services that confirm the four Edmonton patients were infected by tainted strip loin grilling steaks purchased at a northeast Edmonton Costco. Alberta Health Services notifies the CFIA about the test results.

Sept. 26: The CFIA recalls Kirkland brand steaks packaged and sold Sept. 4-7 from the Costco at 13650 50th St. A CFIA spokesman confirms the steaks were processed at the plant in Brooks. Top public health doctors in Alberta say they have asked Costco stores to stop using a meat-tenderizing machine that could push E. coli bacteria from the surface of meat inside, where it is protected from high cooking temperatures that kill the bacteria. The U.S. Food Safety and Inspection Service expands its recall to cover 10 states.

Sept. 27: The CFIA suspends operations at the Brooks plant, takes control of all the plant's products and tells customers to expect further recalls. Alberta Health Services confirms it is investigating a fourth case in Calgary of E. coli poisoning, bringing the total number of recent cases in Alberta to nine. The health authority is still investigating what caused E. coli poisoning in four Calgary patients and one central Alberta patient.

Sept. 28: The CFIA warns the public, restaurants, retailers, distributors and manufacturers not to eat, serve or sell beef produced at the Brooks plant on Aug. 24, between Aug. 27-29 or on Sept. 5. The recall now includes whole-muscle meats such as steaks and roasts. The U.S. Food Safety and Inspection Service expands its recall to cover 30 states for all beef products produced in Brooks on the same dates as well as mechanically tenderized steaks.

Compiled by Andrea Sands

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

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*

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ABSTRACT

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

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

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

* 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

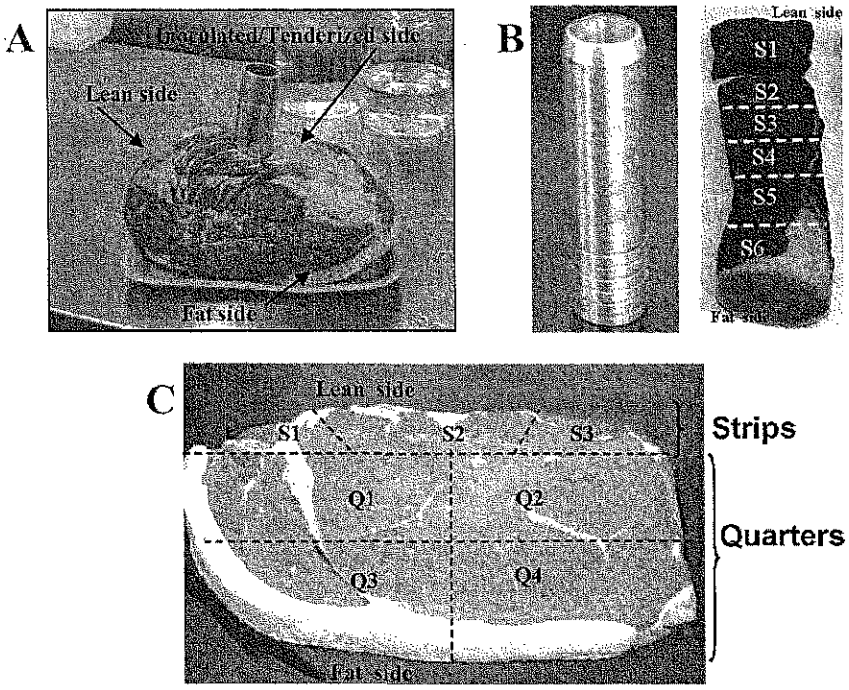

FIGURE 1. *(A) Coring of a beef subprimal. (B) Core apparatus and segmentation of a core sample into six consecutive segments. (C) Segmentation of a brine-injected steak into strips and quarters.*

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] 1/1 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	16/18 direct plating 1/2 enrichment	22/24 direct plating 2/2 enrichment
		15	17/18 direct plating 1/1 enrichment	20/24 direct plating 2/4 enrichment
	60.0	0	14/18 direct plating 4/4 enrichment	14/24 direct plating 2/10 enrichment
		15	13/18 direct plating 1/5 enrichment	12/24 direct plating 2/12 enrichment
	71.1	0	13/18 direct plating 1/5 enrichment	9/24 direct plating 7/15 enrichment
		15	9/18 direct plating 1/9 enrichment	7/24 direct plating 0/17 enrichment
Lac$^+$	37.8	0	18/18 direct plating[c] 0/0 enrichment[e]	24/24 direct plating[d] 0/0 enrichment[f]
		15	17/18 direct plating 1/1 enrichment	23/24 direct plating 1/1 enrichment
	48.9	0	18/18 direct plating 0/0 enrichment	24/24 direct plating 0/0 enrichment
		15	16/18 direct plating 1/2 enrichment	21/24 direct plating 0/3 enrichment
	60.0	0	18/18 direct plating 0/0 enrichment	18/24 direct plating 4/6 enrichment
		15	11/18 direct plating 1/7 enrichment	13/24 direct plating 5/11 enrichment
	71.1	0	9/18 direct plating 3/9 enrichment	6/24 direct plating 8/18 enrichment
		15	12/18 direct plating 0/6 enrichment	8/24 direct plating 6/16 enrichment

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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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*

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ABSTRACT

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

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

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

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

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

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

MATERIALS AND METHODS

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

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

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

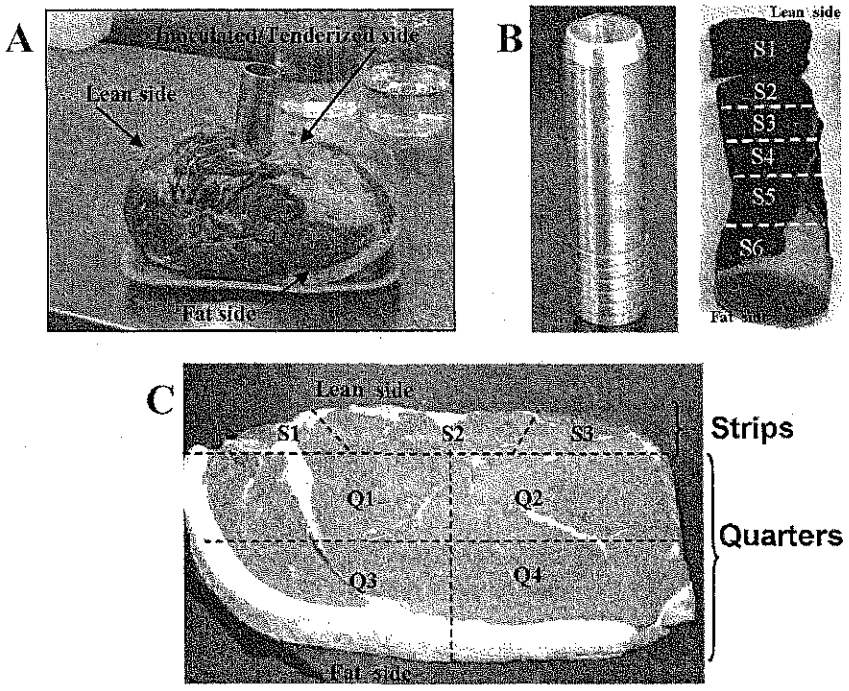

FIGURE 1. *(A) Coring of a beef subprimal. (B) Core apparatus and segmentation of a core sample into six consecutive segments. (C) Segmentation of a brine-injected steak into strips and quarters.*

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

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

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

RESULTS

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

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

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

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

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

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

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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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†

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, DC 20250, USA*

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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

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

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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,

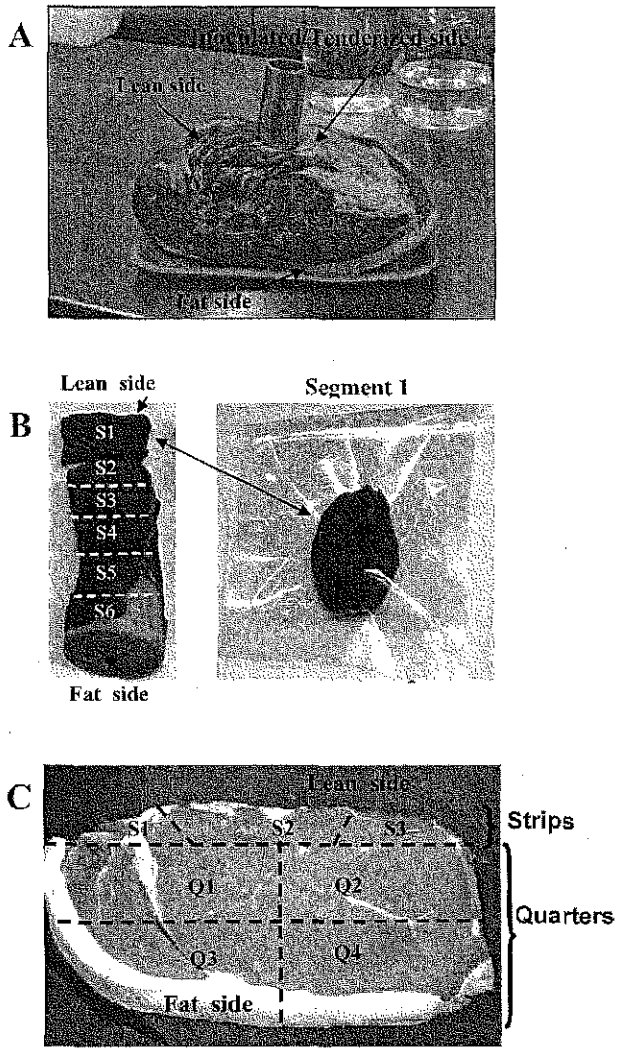

FIGURE 1. *(A) Coring of a beef subprimal. (B) Segmentation of a core sample into six consecutive segments and segment 1 vacuum packaged. (C) Segmentation of a blade-tenderized steak into strips and quarters.*

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°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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Consumer Groups Call on USDA to Take Action on Mechanically Tenderized Meat Products

Following a December 24, 2009 recall of 248,000 pounds of mechanically tenderized steaks that has sickened twenty-one people in 16 states, nine of whom were hospitalized, consumer groups are calling on the U.S. Department of Agriculture to require labeling identifying all mechanically tenderized meat products; to include these products in its sampling program; and to inform the public and restaurants about the need for adequate cooking of these products. USDA's Food Safety and Inspection Service, in conjunction with the Centers for Disease Control and Prevention, has linked the illnesses to mechanically tenderized steaks produced by National Steak and Poultry and distributed to restaurant chains.

Often used on less expensive cuts of meat to increase tenderness, mechanical tenderization is a process that inserts small needles or blades into a meat product, such as a steak or roast. These needles or blades can transfer any pathogens located on the surface of the product to the interior, increasing the risk to consumers if the product is not cooked to a high enough temperature to kill the pathogens. FSIS estimates that over 50 million pounds of mechanically tenderized products are produced each month. Currently this product is unidentifiable to consumers or institutions.

Assuring adequate cooking temperatures for mechanically tenderized products is particularly important. USDA currently recommends that consumers cook beef steaks and roasts to 145°F while it recommends that consumers cook ground beef products to 160°F in order to kill any pathogens that may have been distributed throughout the product. The higher cooking temperature for ground beef products is warranted, given that ground products may have pathogens distributed throughout the product, not just on the surface.

Mechanically tenderized steaks and roasts present a similar risk to consumers because pathogens may not be just on the surface of the product. These products require higher cooking temperatures to ensure that all internal pathogens have been killed. This is especially important since many consumers prefer steaks cooked to rare or medium, which means the products are cooked to a temperature lower than 160°F. Since mechanically tenderized products are not labeled, food preparers may be cooking these products to unsafe temperatures and putting themselves, their families and customers at risk of deadly foodborne illness.

In a June 2009 letter to USDA, consumer groups outlined concerns that mechanically tenderized products presented an unnecessary risk to consumers. The letter, signed by the Center for Foodborne Illness Research & Prevention, Center for Science in the Public Interest, Consumer Federation of America and Food & Water Watch urged USDA to issue labeling requirements for mechanically tenderized products and to develop educational materials for the restaurant industry and the public. To date, USDA has not responded to those requests.

These groups, along with Consumers Union, National Consumers League and S.T.O.P., Safe Tables Our Priority, urge USDA to take steps immediately to address this risk to the public. The groups specifically ask USDA to:

- Require labeling that will allow all meat purchasers to clearly identify mechanically tenderized, non-intact meat products;
- Develop an educational outreach campaign to inform the public and retail meat purchasers about the proper cooking and handling procedures necessary to reduce the risk of foodborne illness from mechanically tenderized meat products; and
- Develop and implement a sampling program for the detection of *E. coli* O157:H7 in mechanically tenderized meat products.

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Additional information is available in the attached June 2009 letter and backgrounder. For further information, contact:

- Center for Foodborne Illness Research & Prevention, Patricia Buck, 724-458-0767
- Center for Science in the Public Interest, Sarah Klein, 202-777-8339
- Consumer Federation of America, Chris Waldrop, 202-797-8551
- Food & Water Watch, Tony Corbo, 202-683-2449
- Consumers Union, Jean Halloran, 914-378-2457
- National Consumers League, Courtney Brein, 202-835-3323
- S.T.O.P., Safe Tables Our Priority, Nancy Donley, 773-419-0128

SAFE FOOD COALITION

1620 I Street, NW, Suite 200, Washington, DC 20006 202-797-8551

Date: June 12, 2009

The Honorable Tom Vilsack
Secretary
U.S. Department of Agriculture
1400 Independence Avenue, SW
Washington, DC 20520

Dear Secretary Vilsack,

In recent years, several outbreaks and illnesses have been associated with mechanically tenderized (MT) meat products. These products, such as steaks and roasts, have been tenderized through a process that repeatedly inserts small needles or blades into the product. These needles or blades pierce the surface of the product increasing the risk that any pathogens located on the surface of the product can be transferred to the interior of the product.

FSIS classifies a product to be non-intact if the product has been injected or if its surface has been pierced. Therefore, MT beef and pork products are non-intact products, even though many MT non-intact meat products look like intact products.

Currently, FSIS does not require MT non-intact meat products to be identified. Therefore, consumers and retail outlets, such as restaurants, do not know whether the products they have purchased are intact or MT non-intact meat products. In addition, FSIS' current advice to consumers and retail outlets about cooking temperatures for products, such as steaks and roasts, does not differentiate between intact products and MT non-intact products. As a result, consumers and retail outlets do not have sufficient information to assure that these products are cooked to an appropriate and safe temperature.

We have now entered the grilling season. Grilling is a safe method for killing pathogens on the surface of meat products since grilling sears the surface with high, intense heat. However, searing the surface does not provide uniform, high heat to the interior of meat products. Therefore, when grilling ground meat or MT non-intact meat products, consumers need to know that these products require longer cooking times, accompanied by higher instant-read temperatures, to prevent foodborne illness.

We urge you to act immediately to address this important public health issue by beginning consumer and retail education, and initiating regulatory action to require labeling of these products. These recommendations are enumerated in detail in the following background memorandum. It is critical for consumers and retail outlets to have the information necessary to

safely prepare these products.

Sincerely,

Patricia Buck
Center for Foodborne Illness Research & Prevention

Michael Jacobson
Center for Science in the Public Interest

Chris Waldrop
Consumer Federation of America

Wenonah Hauter
Food & Water Watch

SAFE FOOD COALITION

1620 I Street, NW, Suite 200, Washington, DC 20006 202-797-8551

Background information for letter to Secretary Vilsack

On Mechanically Tenderized (MT) beef products.

In recent years, several outbreaks and illnesses have been associated with mechanically tenderized (MT) beef products,[1] making them a public health concern. The Safe Food Coalition is very concerned about USDA's:

- Lack of testing of MT beef source materials, including bench trim, and final products;
- Lack of labeling requirements for MT beef products;
- Inappropriate MT beef cooking guidelines listed in the bulk of the agency's publications.

According to the 2008 FSIS *Checklist Report*, over 50 million pounds of mechanically tenderized beef products are produced each month.[2] Most of these products have been mechanically tenderized through a process that repeatedly inserts small needles or blades into the product, generally with product being exposed to 2-3 passes.[3] A 2008 *Journal of Food Protection* article by Luchansky et al.[4] reports that a 2003 National Cattlemen's Beef Association survey found that 188 of 200 processors (94%) use mechanical tenderization to improve product quality. Taken together, this information indicates that a preponderance of beef plants are processing, distributing and selling MT beef products.

FSIS classifies a product to be non-intact if the product has been injected or if its surface has been pierced, even though the product may look intact. Therefore, MT beef and pork products are nonintact products.[5] USDA currently recommends that consumers cook intact beef products to 145°F while it recommends that consumers cook all pork and ground and non-intact beef products to 160°F.[6] The higher cooking temperature for ground and non-intact beef products is warranted,

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[1] U.S. Department of Agriculture. 2005. HACCP plan reassessment for mechanically tenderized beef products. *Fed. Register* 70:30331-30334.

[2] U.S. Department of Agriculture. 2008. Results of Checklist and Reassessment of Control for *Escherichia coli* O157:H7 in Beef Operations, p. 35. www.fsis.usda.gov/PDF/Ecoli_Reassement_&_Checklist.pdf.

[3] *Ibid.*, p. 93.

[4] Luchansky JB, Phebus RK, Thippareddi H, Call JE. Translocation of surface-inoculated Escherichia coli O157:H7 into beef subprimals following blade tenderization. *J Food Prot.* 2008 Nov; 71(11):2190-7.

[5] U.S. Department of Agriculture. 2008. Results of Checklist and Reassessment of Control for *Escherichia coli* O157:H7 in Beef Operations, p. 110. www.fsis.usda.gov/PDF/Ecoli_Reassement_&_Checklist.pdf.

[6] A 2006 USDA/FSIS Fact Sheet titled *Foodborne Illness Peaks in Summer – Why?* recommends that tenderized steaks and roasts be cooked to an internal temperature of 160°F; however in most other places on their website, FSIS makes no distinction in cooking temperature recommendations between intact beef products and non-intact beef products that have been mechanically tenderized.
http://www.fsis.usda.gov/Fact_Sheets/Foodborne_Illness_Peaks_in_Summer/index.asp

given the low infectious dose and high toxicity of *E. coli* O157:H7 and given that ground and nonintact beef products may have pathogens distributed throughout the product, not just on the surface.

Several studies have been undertaken to determine if the mechanical tenderization process transfers pathogens from the surface to the interior of beef products. A study by Luchansky et al.[7] found that, depending on the level of surface contamination, mechanical tenderization of beef products transferred *E. coli* O157:H7 into the topmost 1 cm of product in 90% to 100% of samples and into the topmost 2 cm of product in 55% to 98% of samples. The authors conclude:

Assuming that the prevalence and levels of E.coli O157:H7 on the surface of nonintact subprimals remain low and that best practices are followed for operating and monitoring tenderization equipment, then our data and the reports cited herein support the conclusion of others that nonintact, blade-tenderized beef steaks do not present a greater risk to consumers than otherwise similar meat that is intact, provided that the meat is properly cooked (underlining added).

In other words, according to this article, consumers and restaurant cooks are expected to use cooking as a kill step when preparing MT beef. However, without labeling by the beef manufacturer, these food providers would not know that they should cook the product to a "proper" temperature. Luchansky et al. do not attempt to establish what the correct cooking temperature should be for MT beef products, but the authors do report that studies to validate cooking guidelines to effectively kill *E. coli* O157:H7 in MT beef products are on-going.

With the onset of the grilling season, this non-labeling of MT beef is a serious public health threat. Again, nonintact MT beef products look like intact products. Without labeling, consumers cannot differentiate MT beef products from intact products and would not know that MT beef products need to be cooked to a higher temperature to ensure killing internal pathogens. Contrary to FSIS's stated public health goals, there is no policy/regulation that requires labeling of MT beef products, even though it is known that these products are capable of causing disease.

In the FSIS 2008 *Checklist Report*, Table 5.4.95 shows that 74% of establishments performing mechanical tenderization operations do not label the product.[8] While this statistic is disturbing, in recent conversations with FSIS, the Safe Food Coalition has learned that the agency has no knowledge of any processing plant that labels its product as being mechanically tenderized.

In addition, in the summary for the mechanical tenderization section, the *Checklist Report* highlights the following:

- Fifty-three percent (452) of establishments did not have purchase specifications for suppliers requiring intervention methods (see Table 5.4.82).

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[7] Luchansky JB, Phebus RK, Thippareddi H, Call JE. Translocation of surface-inoculated Escherichia coli O157:H7 into beef subprimals following blade tenderization. *J Food Prot.* 2008 Nov; 71(11):2190-7.

[8] U.S. Department of Agriculture. 2008. Results of Checklist and Reassessment of Control for *Escherichia coli* O157:H7 in Beef Operations, p. 93. www.fsis.usda.gov/PDF/Ecoli_Reassement_&_Checklist.pdf

- Less than 15 percent of establishments conducted validated interventions on mechanically tenderized product (see Table 5.4.83).

- More than 80 percent of establishments did not conduct ongoing verification testing of source materials, and only 3 percent used FSIS "best practices" as outlined in Attachment 5 of Notice 65-07 (see Table 5.4.84).

- More than 80 percent of establishments did not conduct ongoing verification testing of their finished product, and only 1 percent used FSIS "best practices" (see Table 5.4.85).

- Two percent of establishments cleaned and sanitized after mechanically tenderizing components from each supplier (see Table 5.4.91).

- Thirty-two percent of establishments were creating bench trim that could be used as a raw beef component and was not specifically accounted for in a robust testing program.[9]

The Safe Food Coalition is very concerned that over 80% of the plants that produce MT beef do not test either the source or the final product and that the bench trim (created from MT beef) for ground beef products are not routinely included in a robust testing program. This is especially disturbing since the pathogen of concern – *E. coli* O157:H7 – has a zero tolerance if it is detected!

The Safe Food Coalition strongly believes that the lack of labeling of MT beef products, along with FSIS' low recommended cooking guidelines and temperatures for intact beef products, poses a serious and unnecessary threat to public health. Given this potential health hazard to consumers, the Agency must act quickly and publicly to recommend that all beef products be carefully handled and thoroughly cooked.

The Agency has an obligation to immediately inform consumers and retail outlets (including restaurants) that its recommended temperature (145°F instant read for consumers and 145° instant read with a 3minute stand time for restaurants) for intact beef products is not safe for all beef products that have the appearance of being intact. Further, given that the Agency has recommended to consumers that145º F (instant read) is a safe internal temperature for intact beef for many years (throughout many of its publications), the Agency needs to proactively re-educate all consumers and retail purchasers about the importance of cooking MT beef to a higher internal temperature.

The Safe Food Coalition appreciates the seriousness of what we are asking the Agency to do. However, we are confident that FSIS will fulfill its mandate to protect public health.

The Safe Food Coalition expects FSIS to:

- Issue a press release as soon as possible indicating that the current cooking guidelines and temperatures for intact beef products are not safe for all beef products that look intact.

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[9] *Ibid.*, pp.93-4.

- Take immediate steps to develop regulation that will require labeling to clearly identify mechanically tenderized, nonintact beef and pork products for all processing facilities, retail purchasers and consumers.
- Initiate a FSIS program to assess the effectiveness of public health messaging, so that effective food safety messages can be delivered to all food safety stakeholders.

In addition to these three immediate steps, SFC also expects FSIS to:

- Develop data on the contamination rates of different processes, technologies and practices involved in MT products, including blade and needle tenderization, sterilization of blades and needles between each piercing, use of marinades with antimicrobial properties, etc.
- In conjunction with the development of the above data, FSIS should investigate processes in which it can be demonstrated that contamination of the product does not occur or is substantially reduced. When studies document such findings, FSIS should consider different labeling and cooking instructions.
- Develop an educational outreach campaign, based on the above research, to inform retail purchasers about the risk of MT meat products, with particular effort aimed at informing purchasers who prepare food for those populations most likely to develop serious foodborne disease.
- Initiate a FSIS program, in conjunction with the FDA, that would require restaurants to specify on their menus that MT beef and pork products require higher cooking temperatures and/or longer stand times to ensure the safety of the product.
- Develop a similar educational outreach program for public health officials to improve the accuracy and timeliness of their foodborne illness reporting.
- Develop, in conjunction with the CDC and state public health departments, a user-friendly reporting system that medical providers can employ when cases of foodborne illness are identified.

We appreciate USDA's prompt response to this very serious public health issue.

SAFE FOOD COALITION

1620 I Street, NW, Suite 200, Washington, DC 20006 202-797-8551

August 24, 2012

The Honorable Tom Vilsack
Secretary
U.S. Department of Agriculture
1400 Independence Avenue, SW
Washington, D.C. 20520

Dear Secretary Vilsack,

The undersigned members of the Safe Food Coalition write to urge you to expedite and approve a proposal before your office to label mechanically tenderized beef products. Labeling of these products is an important first step so that consumers can make informed decisions about their food purchases and understand what steps are necessary when handling or preparing these higher-risk products. As you know, mechanically tenderized products (such as steaks and roasts) have been treated with a process that repeatedly inserts small needles or blades into the product. These needles or blades pierce the surface of the product increasing the risk that any pathogens located on the surface of the product can be transferred to the interior. Consumers need to be provided with labeling information so that they can make appropriate selections and take the necessary steps in handling and cooking these products.

Members of the Safe Food Coalition have been waiting for over three years for USDA to label mechanically tenderized meat products. On June 12, 2009, the Safe Food Coalition wrote you and asked USDA to address the important public health threat presented by these treated and non-intact products. The coalition also issued a press release in January 2010 urging the Department to act after the December 24, 2009 recall of 248,000 pounds of mechanically tenderized steaks that sickened twenty-one people in 16 states. Since then, the Safe Food Coalition has had multiple meetings with the Food Safety and Inspection Service (FSIS) about mechanically tenderized meat products and has routinely asked about the progress of the labeling proposal at the monthly Safe Food Coalition meetings with FSIS.

Other food safety stakeholders are also interested in this issue. In June 2010, the Conference for Food Protection petitioned FSIS to "promulgate regulations requiring that packers or processors of mechanically tenderized beef cuts label these products to identify that they have been pinned, bladed or otherwise mechanically manipulated in a way that tenderizes the meat by penetrating the intact muscle." [1]

USDA is well-aware of the potential threat that these products pose to consumers. As early as 1999, USDA publicly stated that mechanically tenderized meat products were not considered "intact products" and needed to have more specific cooking instruction included on their labels.[2] In USDA's 2004 *Fulfilling the Vision*, FSIS once again clearly identified mechanically tenderized meat as a non-intact product that needed special attention.

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[1] Conference for Food Protection Petition. USDA/FSIS Regulations & Policies. June 17, 2010. http://www.fsis.usda.gov/Regulations_&_Policies/Petitions/index.asp

[2] U.S. Department of Agriculture, Food Safety and Inspection Service. Beef products contaminated with *E. coli* O157:H7 (64 FR 2803) January 19, 1999. http://www.fsis.usda.gov/oppde/rdad/FRPubs/99-060Npm.htm

Further, since 2003, FSIS and the Centers for Disease Control and Prevention have tracked multiple meat recalls and foodborne illness outbreaks associated with non-intact, mechanically tenderized products, [3] [4] and USDA's Agricultural Research Service has conducted multiple studies, showing that translocation of pathogenic material from the surface of an intact steak or roast to the interior can occur during blade and needle tenderization processes and recommends thorough cooking to offset this risk. [5] [6] [7]

However, to date, all of this work has had little impact on reducing foodborne illness because the public has not been informed about the potential risk that these products pose. Without a label to identify mechanically treated meat products, along with information to help mitigate the risk, the unsuspecting purchasers of these products – whether they are restaurant cooks or consumers – will have no idea that the product that they have selected needs additional protective handling and preparation.

Given USDA's food safety goals and its acknowledgement of mechanically tenderized meat as a non-intact and higher risk product capable of causing illness – along with the research that documents translocation of all types of pathogens into the interior of these products – it is past time for USDA to require labeling of all mechanically tenderized products.

We urge you to immediately approve the proposal to label mechanically tenderized beef products. Further delays are unacceptable.

Sincerely,

Center for Foodborne Illness Research & Prevention

Center for Science in the Public Interest

Consumer Federation of America

Consumers Union

Food & Water Watch

Government Accountability Project

National Consumers League

STOP Foodborne Illness

US Public Interest Research Group

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[3] U.S. Department of Agriculture, Food Safety and Inspection Service. 2007. Michigan firm recalls beef products due to possible *E. coli* O157:H7 contamination. Recall included boxes of mechanically tenderized steaks and ground beef of varying weights. Davis Creek Meats and Seafood, Kalamazoo, MI, May 11, 2007. http://www.fsis.usda.gov/fsis_recalls/Recall_Case_Archive_2007/index.asp

[4] 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.

[5] Luchansky, J., R. Phebus, H. Thippareddi, J. Call. Translocation of Surface-Inoculated *Escherichia coli* O157:H7 into Beef Subprimals following Blade Tenderiaztion. 2008. *Journal of Food Protection,* Vol. 71, No. 11, pp. 2190–219.

[6] Luchansky, J., A. Porto-Fett; B. Shoyer, R. Phebus, H. Thippareddi, J. Call. Thermal Inactivation of *Escherichia coli* O157:H7 in Blade-Tenderized Beef Steaks Cooked on a Commercial Open-Flame Gas Grill. 2009. *Journal of Food Protection,* Vol. 72, No. 7, pp. 1404–1411.

[7] Luchansky, J., A. Porto-Fett, P. Shoyer, J. Call, W. Schlosser, W. Shaw, N. Bauer, H. Latimer. 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. 2012. *Journal of Food Protection,* Vol. 75, No. 1, pp. 62–70.

SAFE FOOD COALITION

1620 I Street, NW, Suite 200, Washington, DC 20006 202-797-8551

FOR IMMEDIATE RELEASE August 24, 2012

Consumer Groups Urge USDA to Immediately Approve Labeling of Mechanically Tenderized Meat Products

Members of the Safe Food Coalition wrote today to USDA Secretary Tom Vilsack urging him to immediately approve a proposal to label mechanically tenderized beef products. The proposal must be approved by the Secretary before it is sent to the Office of Management and Budget for review. The letter is available here.

Often used on less expensive cuts of meat to increase tenderness, mechanical tenderization is a process by which small needles or blades are repeatedly inserted into the product. These needles or blades pierce the surface of the product increasing the risk that any pathogens, such as *E. coli* or *Salmonella*, located on the surface of the product can be transferred to the interior. In order to kill pathogens which may be located on the interior of these products, consumers must cook these products differently than they would intact steaks and roasts. Without labeling to identify these products as mechanically tenderized and non-intact products, and information on how to properly cook these products, consumers may be unknowingly at risk for foodborne illness. Labeling of mechanically tenderized products would allow consumers to identify these products in the supermarket.

Based on estimates from USDA's Food Safety and Inspection Service's 2008 Beef Checklist, approximately 18% of all beef steaks and roasts sold in the U.S. are mechanically tenderized. This means that approximately 50 million pounds of mechanically tenderized products are produced each month.

USDA has known about this potential threat for many years. As early as 1999, USDA/FSIS publicly stated that mechanically tenderized meat products were considered non-intact products because the product had been pierced and surface pathogens could have been translocated to the interior of the product. USDA/FSIS further stated, "As a result, customary cooking of these products may not be adequate to kill the pathogens." At that time, USDA/FSIS said that they would not require a label for these products but strongly encouraged industry to label all non-intact, mechanically tenderized meat products with safe food handling guidance. To date, industry labeling of these products is rare.

In June 2009, members of the Safe Food Coalition wrote to USDA urging the mandatory labeling of these products. Consumer groups raised the issue again in January 2010 following the December 24, 2009 recall of 248,000 pounds of mechanically tenderized steaks that sickened twenty-one people in 16 states. In June 2010, the Conference for Food Protection petitioned FSIS to put forward regulations that would require mechanically tenderized products to be labeled.

The groups – Center for Foodborne Illness Research & Prevention, Center for Science in the Public Interest, Consumer Federation of America, Consumers Union, Food & Water Watch, Government Accountability Project, National Consumers League, STOP Foodborne Illness, and US PIRG – are asking the Secretary of Vilsack to immediately approve the labeling of mechanically tenderized beef products and send the proposal to OMB for review. USDA should also develop and implement a sampling program for the detection of pathogens in non-intact beef products. And USDA should implement an educational outreach campaign to inform the public and food service meat purchasers about the proper cooking and handling procedures necessary to reduce the risk of foodborne illness from mechanically tenderized beef products.

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The Safe Food Coalition is made up of consumer groups, public health groups, groups representing victims of foodborne illness, and labor organizations dedicated to reducing the burden of foodborne illness in the United States by improving government food inspection programs.

The Safe Food Coalition letter to Secretary Vilsack is available here:
http://www.consumerfed.org/pdfs/Comments.SFC.Vilsack.Mech.Tenderized.Meat8.23.12.pdf