

## Effects of Selected Cooking Procedures on the Survival of *Escherichia coli* O157:H7 in Inoculated Steaks Cooked on a Hot Plate or Gas Barbecue Grill

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### ABSTRACT

Beef steaks (2 cm thick) were each inoculated at three sites in the central plane with *Escherichia coli* O157:H7 at  $5.9 \pm 0.3$  log CFU per site. Temperatures at steak centers were monitored during cooking on a hot plate or the grill of a gas barbecue. Steaks were cooked in groups of five using the same procedures and cooking each steak to the same temperature, and surviving *E. coli* O157:H7 at each site was enumerated. When steaks cooked on the hot plate were turned over every 2 or 4 min during cooking to between 56 and 62°C, no *E. coli* O157:H7 was recovered from steaks cooked to  $\geq 58$  or 62°C, respectively. When steaks were cooked to  $\leq 71$ °C and turned over once during cooking, *E. coli* O157:H7 was recovered from steaks in groups turned over after  $\leq 8$  min but not from steaks turned over after 10 or 12 min. *E. coli* O157:H7 was recovered in similar numbers from steaks that were not held or were held for 3 min after cooking when steaks were turned over once after 4 or 6 min during cooking. When steaks were cooked on the grill with the barbecue lid open and turned over every 2 or 4 min during cooking to 63 or 56°C, *E. coli* O157:H7 was recovered from only those steaks turned over at 4-min intervals and cooked to 56°C. *E. coli* O157:H7 was recovered from some steaks turned over once during cooking on the grill and held or not held after cooking to 63°C. *E. coli* O157:H7 was not recovered from steaks turned over after 4 min during cooking to 60°C on the grill with the barbecue lid closed or when the lid was closed after 6 min. Apparently, the microbiological safety of mechanically tenderized steaks can be assured by turning steaks over at intervals of about 2 min during cooking to  $\geq 60$ °C in an open skillet or on a barbecue grill. When steaks are turned over only once during cooking to  $\geq 60$ °C, microbiological safety may be assured by covering the skillet or grill with a lid during at least the final minutes of cooking.

In North America, beef steaks are often prepared from cuts of meat that have been mechanically tenderized (6, 7). Mechanical tenderizing is commonly accomplished by incising the meat with multiple thin blades or needles, without injection of brine (17). Such blade or needle tenderizing treatments will allow some bacteria, including pathogens such as *Salmonella* or *Escherichia coli* O157:H7, from the meat surface to be carried into the previously sterile deep tissues (10). Pathogens in deep tissues can survive and infect consumers when some or all contaminated tissues are not adequately heated during cooking. The increased risks to human health as a result of mechanical tenderizing of beef were initially assessed as small (2, 22). However, outbreaks of *E. coli* O157:H7 infection associated with consumption of contaminated mechanically tenderized beef (5, 6, 12) has prompted reevaluation of these risks. In recent evaluations, the risks, although small, were greater than previously thought (4, 23). Consequently, North American regulatory authorities have identified a need for labeling mechanically tenderized beef and providing

instructions for cooking to ensure that the cooked meat is microbiologically safe (24).

The U.S. Department of Agriculture (USDA), Food Safety and Inspection Service recommends that to ensure microbiological safety mechanically tenderized beef should be cooked to 160°F (71.1°C) or to 145°F (62.8°C) and then held for 3 min before consumption (25). These conditions are considered sufficient to reduce the numbers of *Salmonella* or *E. coli* O157:H7 at any point in a portion of beef by at least 5 log units (24). However, these instructions alone may be inadequate to ensure appropriate cooking. Therefore, the USDA (24, 25) has proposed that every mechanically tenderized product be labeled with cooking instructions that have been validated with respect to their efficacy for destroying pathogens throughout the product.

A number of researchers have studied the effects of cooking conditions on the survival of *E. coli* O157:H7 in mechanically tenderized beef steaks that were not moisture enhanced. These studies have variously involved cooking surrogate steaks composed of beef strips (1), steaks prepared from cuts blade tenderized after inoculation of their surfaces (15, 16, 21), or steaks inoculated at internal points (8).

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Steaks were cooked on electric skillets or hot plates, in electric ovens, or on gas grills to center temperatures ranging from 49 to 71°C. Regardless of the type or thickness of the steaks, the method of cooking, and the temperature at the center at the end of the cooking time, *E. coli* O157:H7 reductions in most instances varied by  $\geq 2$  log units and rarely exceeded 4 log units. These results indicate that with all methods of cooking the heating of the steaks was uneven, and consequently bacteria survived at cold spots within steaks (15).

Most steaks are cooked by heating one side at a time on a hot skillet surface or grill. In three of the reported studies (15, 18, 21), steaks cooked in this manner were turned over only once, about midway during cooking, as is commonly recommended in culinary instructions (3, 19, 27). Heating of hamburger patties is more even when they are turned over frequently rather than only once during cooking (9, 20). Frequent turning of steaks during cooking would then be expected to reduce the occurrence of cold spots within the meat. In one study, surrogate steaks composed of inoculated beef slices were cooked to 60°C and turned every 2 min during cooking (1). However, *E. coli* O157:H7 reductions were variable and  $< 4$  log CFU. In another study, *E. coli* O157:H7 was inoculated at  $> 5$  log CFU at points within the central plane of otherwise intact steaks (8). Various numbers of pathogen cells were recovered from inoculated points in some steaks that were turned over only once during cooking to temperatures up to 71°C. However, no *E. coli* O157:H7 was recovered from steaks that were turned over twice or more during cooking to 63°C.

The results of that study suggest that  $> 5$ -log reductions in *E. coli* O157:H7 might be obtained with cooking temperatures  $\geq 63^\circ\text{C}$  if steaks were turned over more than once during cooking. Temperature histories obtained from steaks during cooking suggested that holding for 3 min after cooking might enhance reduction of *E. coli* O157:H7 that survived cooking to temperatures of  $\geq 63^\circ\text{C}$ , but this hypothesis was not investigated directly. The findings also suggested the possibility that closing the lids of barbecues during grilling might be another means of obtaining even heating of steaks to ensure *E. coli* O157:H7 reductions of  $> 5$  log CFU at all points in steaks cooked to  $\geq 63^\circ\text{C}$ . These three issues of turning, holding, and closing the lid of the barbecue were investigated because of their possible bearing on instructions for safe cooking of mechanically tenderized steaks.

## MATERIALS AND METHODS

**Preparation and cooking of steaks.** Vacuum-packaged eye of round (semitendinosus) beef primal cuts weighing 2.3 to 3.0 kg were obtained from a beef packing plant and stored at  $1 \pm 1^\circ\text{C}$ . Cuts and raw steaks prepared from them were held in a refrigerator at 4°C until used. Steaks 2 cm thick were prepared from cuts in groups of six. One steak in each group was inoculated with a suspension of *E. coli* O157:H7, and then all steaks were returned to the refrigerator. After 15 min, the inoculated steak was removed from the refrigerator and cooked. After sampling of the cooked steak, four more steaks were sequentially inoculated, held in the refrigerator for 15 min, and then cooked. This procedure was

adopted to avoid large differences between the temperatures of steaks when cooking commenced. The sixth steak was inoculated and then sampled without being cooked.

Steaks were cooked on a sheet of aluminum foil covering a hot plate (Data-plate model 720A, Bamstead International, Dubuque, IA) operated at  $200 \pm 2^\circ\text{C}$  or on the grill of a portable gas barbecue (Weber Q100, Weber-Stephens Products, Palatine, IL) rated at 8,500 BTU/h (2.49 kW). The grill was operated at the highest setting with the barbecue lid open or closed for all or part of the cooking time as required. During cooking, the temperatures at a point at the approximate center of each steak were recorded with a stainless steel probe (20 cm long, 1.5 mm in diameter; no. U-08505-63, Cole-Parmer Instruments, Vernon Hill, IL) inserted through the side of the steak. The probe carried a type T thermocouple sensor with a response time of 10 s. Probe output was processed through a thermocouple thermometer (Dualog R, Cole-Parmer) connected to a computer to record temperatures at 10-s intervals. The probe was sterilized by immersion in ethanol before insertion into a steak.

Steaks were removed from the hot plate or grill when a specified temperature at the center was attained. The times at which each steak was placed on and removed from the hot plate or grill were noted. The duration of cooking time was recorded to the nearest minute. During cooking, steaks were turned over as required using tongs with smooth gripping surfaces.

**Preparation of inocula and inoculation of steaks.** Five strains of nontoxigenic *E. coli* O157:H7 (strains 0304, 0627, 0648, 1840, and 3581) obtained from the culture collection of the Department of Food Science at the University of Manitoba (Winnipeg, Manitoba, Canada) and used in previous studies of steak cooking (8) were maintained on slants of nutrient agar (Difco, BD, Sparks, MD). To prepare each inoculum, each strain was grown in half-strength brain heart infusion (BHI; Difco, BD) overnight at 35°C. Each culture of stationary-phase cells was diluted in BHI to obtain a suspension containing 8 to 9 log CFU/ml, and 2 ml of each suspension was mixed to obtain a five-strain cocktail containing  $> 8$  log CFU/ml plus 1 ml of a 2,000-ppm solution in BHI of the food dye FDC Blue #1 (Calico Food Ingredients, Kingston, Ontario, Canada).

Each steak was inoculated at the geometric center and at points in the central plane 1.0 and 0.5 cm from the steak edge. Tissue at each point to be inoculated was disrupted using a sterile toothpick wrapped with autoclave tape to leave 1 cm uncovered. The toothpick was inserted vertically into the meat until the tape edge rested on the meat surface. It was then inclined about  $10^\circ$  from the vertical and rotated around the point of insertion to disrupt tissue at the tip. The toothpick was removed, and the tip of a mechanical pipette was inserted into the tract created by the toothpick to inoculate 10  $\mu\text{l}$  of the *E. coli* O157:H7 cocktail into the disrupted tissue. This procedure was adopted because bacteria inoculated into intact muscle tissue will disperse into large volumes of tissue (26). Disruption of the tissue prevents dispersion of the inoculum, and inclusion of dye in the inoculum allowed location and excision of all inoculated tissue.

**Excision of inoculated tissue and enumeration of *E. coli* O157:H7.** For excision of inoculated tissue from cooked or raw steaks, the surface of each steak was first seared to a depth of about 3 mm using a heated stainless steel plate. About 1 g of meat, which included all dyed tissue, was then excised from each inoculated point by cutting through the seared surface with sterile instruments. Each portion of excised tissue was placed with 10 ml of 0.1% peptone water (Difco, BD) in a stomacher bag fitted with a filter

TABLE 1. Conditions used for cooking groups of five steaks inoculated with *Escherichia coli* O157:H7 on a hot plate operated at 200°C

No. of groups	Time for turning meat during cooking	Cooked to temp (°C)	Held after cooking <sup>a</sup>
4	Every 2 min	62, 60, 58, 56	No
4	Every 4 min	62, 60, 58, 56	No
4	Once after 4 min	60, 63, 67, 71	No
4	Once after 4 min	60, 63, 67, 71	Yes
4	Once after 6 min	60, 63, 67, 71	No
4	Once after 6 min	60, 63, 67, 71	Yes
1	Once after 8 min	71	No
1	Once after 10 min	71	No
1	Once after 12 min	71	No

<sup>a</sup> Inoculated tissues were excised immediately after cooking ended, or steaks were held at room temperature for 3 min before tissues were excised.

sleeve and pummeled with a stomacher. The stomacher fluid was withdrawn from inside the filter sleeve, and 1 ml was used to prepare 10-fold dilutions to 10<sup>-2</sup> in 0.1% peptone water. All undiluted stomacher fluid and all of each dilution were filtered through a hydrophobic grid membrane filter (Oxoid, Nepean, Ontario, Canada). Each filter was placed on a plate of lactose monensin glucuronate agar (LMG; Oxoid) and incubated at 35°C for 18 to 24 h. Coliforms form blue colonies on LMG, and all coliforms recovered from inoculated tissues were assumed to be *E. coli* O157:H7. Squares containing blue colonies on a suitable filter prepared from each stomacher fluid sample were counted. A most probable number (MPN) of *E. coli* O157:H7 in the filtered fluid was calculated from the count of squares using the formula  $MPN = N \times \log_n(N/N - X)$ , where  $N$  is the total number of squares on a filter and  $X$  is the count of squares containing blue colonies.

**Cooking procedures.** Groups of five steaks cooked on the hot plate or barbecue grill were turned over repeatedly at intervals of 2 or 4 min or once after 4, 6, 8, or 10 min during cooking to temperatures of 56 to 71°C (inclusive) at the center. Inoculated tissues were excised from each steak immediately after cooking ended, or steaks were held at room temperature for 3 min before tissues were excised. The barbecue was operated with the lid open or closed throughout cooking or open for 6 min and then closed, with steaks turned over after cooking for 4 min. The various

conditions used during cooking of groups of steaks on the hot plate or grill are listed in Tables 1 and 2, respectively.

**Analysis of cooking time and microbiological data.** Counts of *E. coli* O157:H7 recovered from inoculated sites were transformed to log values (log CFU per inoculated site). Cooking times or numbers of surviving *E. coli* O157:H7 were obtained from one to four sets of steaks. For numbers of surviving *E. coli* O157:H7, a value of -0.5 log CFU per site was assigned for sites from which no *E. coli* O157:H7 was recovered.

A Shapiro-Wilk test for normal distribution was applied to each set of cooking times or log bacterial counts. When all or most of the sets of times or log counts to be compared were normally distributed, mean values for the sets were separated using a Tukey test. When all or most of the sets to be compared were not normally distributed, median values for the sets were separated by pairwise application of a Kruskal-Wallis test. All tests were performed using SAS, version 12 (SAS Institute, Cary, NC).

## RESULTS

The temperatures at the centers of steaks when cooking started ranged from 5.5 to 10.0°C (mean = 7.6°C). *E. coli* O157:H7 recovered from inoculated sites in raw steaks ranged from 5.49 to 6.21 log CFU per site (mean = 5.93 log CFU per site).

When steaks were turned over every 2 min during cooking on the hot plate to temperatures of 62 to 56°C, the mean cooking times for each temperature at the end of cooking were similar (Table 3). *E. coli* O157:H7 was recovered from some steaks cooked to 56°C but not from steaks cooked to higher temperatures. When steaks were turned over every 4 min during cooking, mean cooking times were shorter for steaks cooked to lower temperatures than for those cooked to higher temperatures. *E. coli* O157:H7 was recovered from two steaks cooked to 60°C and from four steaks in each of the groups cooked to 58 and 56°C. Most of the steak sites from which *E. coli* O157:H7 was recovered were centers.

Times for cooking steaks to temperatures of 62 to 56°C with turning every 2 or 4 min were normally distributed ( $P > 0.05$ ). Mean cooking times for these steaks were 10.1 and 11.2 min, respectively, and were significantly different ( $P < 0.05$ ). The counts for *E. coli* O157:H7

TABLE 2. Conditions for cooking groups of five steaks inoculated with *Escherichia coli* O157:H7 on a gas barbecue grill operated at the highest setting

No. of groups	Time for turning meat during cooking	Cooked to temp (°C)	Held after cooking <sup>a</sup>	Position of lid
2	Every 2 min	63, 56	No	Open
2	Every 4 min	63, 56	No	Open
1	Once after 4 min	63	No	Open
1	Once after 4 min	63	Yes	Open
2	Once after 4 min	63	No	Closed
2	Once after 4 min	63	No	Closed after 6 min
1	Once after 6 min	63	No	Open
1	Once after 6 min	63	Yes	Open
3	Once after 4 min	60, 58, 56	No	Closed
3	Once after 4 min	60, 58, 56	No	Closed after 6 min

<sup>a</sup> Inoculated tissues were excised immediately after cooking ended, or steaks were held at room temperature for 3 min before tissues were excised.

TABLE 3. Cooking times and survival of *Escherichia coli* O157:H7 at inoculated sites in groups of five steaks cooked on a hot plate and turned over every 2 or 4 min during cooking to temperatures of 62 to 56°C

Turning over time (min)	Cooked to temp (°C)	<i>E. coli</i> O157:H7 in raw steaks (log CFU/site) <sup>a</sup>			No. of positive samples			<i>E. coli</i> O157:H7 at positive sites (log CFU/site)			
		Cooking time (min)		Mean	Range	Center	1.0 cm from edge	0.5 cm from edge	Center	1.0 cm from edge	0.5 cm from edge
		Mean	Range								
2	62	11.0	10–12	5.67	5.61–5.80	0	0	0	ND <sup>b</sup>	ND	ND
	60	9.6	8–11	6.07	5.90–6.19	0	0	0	ND	ND	ND
	58	10.0	9–11	6.03	5.89–6.13	0	0	0	ND	ND	ND
4	56	9.8	7–12	6.05	5.91–6.15	1	1	3	3.93–>5.00	2.27	ND
	62	12.2	10–13	6.07	5.94–6.15	0	0	0	ND	ND	ND
	60	11.2	10–13	5.77	5.61–6.01	2	0	1	0.30–2.55	ND	2.51
	58	10.8	8–13	5.82	5.75–5.86	1	1	2	1.04–>5.00	3.84	0.00–>5.00
	56	10.2	10–12	6.18	6.14–6.21	4	1	0	0.30–2.46	2.30	ND

<sup>a</sup> Inoculated sites were the geometric center and in the central plane 1.0 or 0.5 cm from the edge of each steak.

<sup>b</sup> ND, not detected.

recovered from all inoculated sites in each of the groups of steaks cooked to 56°C with turning every 2 min or cooked to 58 or 56°C with turning every 4 min were not normally distributed ( $P < 0.05$ ). The median values for all three of those groups of steaks were  $-0.5$  log CFU per site and were not significantly different ( $P > 0.05$ ).

Most times for cooking steaks on the hot plate to a temperature of 60 to 71°C with turning once after 4 or 6 min were normally distributed ( $P > 0.05$ ; Table 4). Most mean cooking times for these steaks were not significantly different ( $P > 0.05$ ). However, mean times for cooking to higher temperatures were generally significantly longer ( $P < 0.05$ ) than mean times for cooking to lower temperatures.

*E. coli* O157:H7 was recovered from most inoculated sites in steaks cooked to temperatures of 60, 63, 67, and 71°C when steaks were turned over once after cooking for 4 or 6 min and were or were not held for 3 min before excision of tissues for analysis (Table 5). Most counts from the same site in four groups of steaks turned over after the same time during cooking to temperatures of 60 to 71°C were not normally distributed ( $P < 0.05$ ; Table 6). The median values for pairs of *E. coli* O157:H7 recovered from the centers of steaks turned over after 4 min during cooking and not held or held for 3 min after cooking were not significantly different ( $P > 0.05$ ). Those values were significantly greater ( $P < 0.05$ ) than the median values for *E. coli* O157:H7 recovered from the other sites in those groups of steaks or from each of the sites in groups of steaks turned over after cooking for 6 min and not held or held for 3 min after cooking. The median values for *E. coli* O157:H7 recovered from the centers of sets of steaks turned over after 6 min and not held or held for 3 min after cooking and median values for *E. coli* O157:H7 recovered from the other sites in steaks turned over after 4 or 6 min and not held or held for 3 min after cooking were not significantly different ( $P > 0.05$ ).

Cooking times for groups of five steaks cooked on the hot plate and turned over after 8, 10, or 12 min during cooking to 71°C ranged from 12 to 19 min, 13 to 21 min, and 17 to 26 min, respectively. The mean cooking times were 14.4, 15.6, and 21.6 min, respectively. *E. coli* O157:H7 was recovered from one or more inoculated sites in each of the steaks turned over after 8 min at 2.57 and  $>5.00$  log CFU per site for two sites at steak centers, 0.30 and 3.13 for two sites 1.0 cm from steak edges, and 0.00 to 3.77 log CFU per site for five sites 0.5 cm from steak edges. No *E. coli* O157:H7 was recovered from steaks turned over once after 10 or 12 min during cooking to 71°C.

Cooking times for groups of steaks turned over at 2 or 4 min during cooking on the gas barbecue grill to 63 or 56°C ranged from 7 to 10 min, with a mean of 8.9 min. No *E. coli* O157:H7 was recovered from steaks turned over every 2 min during cooking to 63 or 56°C or every 4 min during cooking to 63°C. *E. coli* O157:H7 was recovered at 1.23 to 3.05 log CFU per site from the centers of four steaks turned over every 4 min during cooking to 56°C.

For groups of steaks cooked on the grill and turned over once after 4 or 6 min during cooking to 63°C, the mean time for cooking with the barbecue lid closed and turning over

TABLE 4. Cooking times for sets of five steaks turned over once after 4 or 6 min during cooking on a hot plate to 60, 63, 67, or 71 °C

Time turned over (min)	Cooked to temp (°C)	Cooking time (min)			
		Mean <sup>a</sup>	SD	Median	Range
4	60	15.5 CB	2.64	16	10–18
	63	11.9 D	2.02	12.5	9–15
	67	15.5 CB	2.80	15.5	11–19
	71	18.5 AB	2.64	19.5	14–22
6	60	12.0 D	1.05	12	11–14
	63	13.0 CD <sup>b</sup>	1.63	13	11–17
	67	16.4 AB	1.71	16.5	13–18
	71	18.6 A	2.55	19	14–22

<sup>a</sup> Mean values with the same letter are not significantly different ( $P > 0.05$ ).

<sup>b</sup> Cooking times are not normally distributed.

after 4 min was significantly shorter ( $P < 0.05$ ) than the mean times for cooking with the lid open and turning over after 4 or 6 min or turning over after 4 min during cooking with the lid open and then closing the lid after 6 min (Table 7). *E. coli* O157:H7 was recovered from some steaks turned over after 4 min during cooking with the lid open whether or not the steaks were held for 3 min after cooking (Table 8). The bacteria were recovered more frequently from sites at steak edges than from steak centers. *E. coli* O157:H7 was recovered from two steaks turned over after 6 min during cooking with the barbeque lid open and not held after cooking but not from any steaks cooked in that way but held for 3 min before excision of tissue for analysis.

For groups of steaks cooked on the grill with the barbeque lid closed and turned over once after 4 min during cooking to 60, 58, or 56 °C, cooking times ranged from 5 to 8 min, with a mean of 6.0 min. *E. coli* O157:H7 was

recovered from only two of those steaks, which were cooked to 58 °C. *E. coli* O157:H7 was recovered from the centers of these steaks at 1.86 and 2.51 log CFU per site. For groups of steaks that were cooked on the grill with the barbeque lid open for 6 min and then closed and were turned over once during cooking to 60, 58, or 56 °C, cooking times ranged from 7 to 10 min, with a mean of 8.1 min. *E. coli* O157:H7 at 3.73 log CFU per site was recovered from the center of only one of these steaks, which was cooked to 56 °C.

### DISCUSSION

Steak size and composition, the equipment used for cooking and the way it is operated, and manipulation of the steak during cooking can all affect the temperature of any point within a steak and thus possibly affect the survival of bacteria in deep tissues. Because of practical limits, only a small number of combinations of steak type, cooking

TABLE 5. Inoculated sites from which *Escherichia coli* O157:H7 was recovered and ranges of levels recovered from groups of five steaks cooked on a hot plate, turned over once after cooking for 4 or 6 min during cooking to 60 to 71 °C, and held for 3 min or not held after cooking

Time turned over (min)	Held after cooking	Cooked to temp (°C)	No. of positive sites <sup>a</sup>			<i>E. coli</i> O157:H7 at positive sites (log CFU/site)		
			Center	1.0 cm from edge	0.5 cm from edge	Center	1.0 cm from edge	0.5 cm from edge
4	No	60	4	4	5	4.10–4.97	0.48–3.38	1.11–3.94
		63	5	3	5	2.46–4.30	0.48–4.09	2.53–3.06
		67	5	5	5	2.93–4.91	2.36–4.38	2.48–4.36
		71	4	3	2	2.87–3.63	2.92–4.06	0.00–1.15
	Yes	60	4	5	5	3.07–4.54	2.32–4.76	3.14–3.81
		63	5	5	4	1.04–3.91	1.76–3.25	0.48–2.59
		67	4	5	5	0.30–4.03	0.30–3.98	1.63–3.91
		71	4	5	5	2.37–3.64	0.30–3.88	0.48–2.74
6	No	60	5	4	4	2.53–5.00	1.36–3.58	0.83–3.59
		63	4	2	3	2.48–3.83	2.05–2.18	0.48–2.35
		67	5	5	4	1.32–3.69	0.30–3.59	1.00–3.91
		71	0	2	3	ND <sup>b</sup>	0.30–2.79	1.04–2.83
	Yes	60	4	3	5	1.34–4.51	1.58–3.81	0.30–2.72
		63	5	4	4	1.26–2.82	1.28–3.68	0.48–3.55
		67	4	3	2	1.82–3.71	1.30–2.85	1.79–2.53
		71	4	1	2	0.30–3.18	3.43	0.00–3.16

<sup>a</sup> *E. coli* O157:H7 was inoculated at a mean of 5.98 log CFU per site. Inoculated sites were the geometric center and in the central plane 1.0 or 0.5 cm from the edge of each steak.

<sup>b</sup> ND, not detected.

TABLE 6. Median values for *Escherichia coli* O157:H7 recovered from inoculated sites in groups of five steaks that were cooked on a hot plate, turned over once after cooking for 4 or 6 min during cooking to 60 to 71°C, and held for 3 min or not held after cooking<sup>a</sup>

Time turned over (min)	Held after cooking	Median <i>E. coli</i> O157:H7 (log CFU/site) <sup>b</sup>		
		Center	1.0 cm from edge	0.5 cm from edge
4	No	3.49 A	2.34 B	2.52 B
	Yes	3.28 A	2.88 B <sup>c</sup>	2.52 B <sup>c</sup>
6	No	2.69 B	1.71 B	1.98 B
	Yes	2.61 B <sup>c</sup>	1.29 B	0.67 B

<sup>a</sup> Steaks in each set were cooked to 60, 63, 67, or 71°C, with all steaks in a group cooked to one of those temperatures.

<sup>b</sup> *E. coli* O157:H7 was inoculated at a mean of 5.98 log CFU per site. Inoculated sites were the geometric center and in the central plane 1.0 and 0.5 cm from the edge of each steak. Median values with the same letter are not significantly different ( $P > 0.05$ ).

<sup>c</sup> The set of log-transformed counts was normally distributed ( $P > 0.05$ ).

equipment, and cooking procedures can be investigated at one time. The steaks used for this study were devoid of fat cover and of relatively uniform shape. Steaks 2 cm thick were used because recent studies indicate that most beef steaks for retail sale in North America are  $\leq 2$  cm thick (11). Steaks were cooked on a hot plate to approximate cooking in an open skillet or on a barbecue grill to take into account the two types of equipment mostly used for cooking steaks (12, 17). Steaks were turned over one or more times during cooking and were held for 3 min or not held after cooking to investigate how these handling practices affect survival of deep tissue contaminants. Steaks were inoculated with *E. coli* O157:H7 at both the steak centers and points near the edges of the steaks because points near steak edges can sometimes remain cooler than steak centers (8). Adequate heating of those points is particularly important because tissues near the edges of steaks cut from primal cuts that were surface incised during mechanical tenderizing will generally be more heavily contaminated with bacteria than tissues further from the incised surface (13).

For steaks turned over more than once during cooking on the hot plate,  $>5$  log CFU of *E. coli* O157:H7 could be eliminated from all the inoculated points when steaks were cooked to  $<63^\circ\text{C}$ . Turning over every 2 min resulted in *E. coli* O157:H7 being eliminated in steaks with lower temperatures at the center compared with steaks turned over every 4 min. Turning over more frequently seems to assure inactivation of *E. coli* O157:H7 at cooking temperatures lower than are needed when steaks are turned over less frequently. The extent to which cooking temperatures for assured inactivation of *E. coli* O157:H7 can be reduced by increasing the frequency of turning over and the minimum temperature for assured inactivation of large numbers of *E. coli* O157:H7 will differ with different types of cooking equipment. Thus, the limited findings for steaks turned over more than once during cooking on the

TABLE 7. Cooking times for groups of five steaks cooked on a gas barbecue grill with the lid open or closed throughout cooking or closed 2 min after steaks were turned over once after cooking for 4 or 6 min during cooking to 63°C

Time turned over (min)	Lid position	Cooking time (min) <sup>a</sup>			
		Mean	SD	Median	Range
4	Open	10.6 A	1.58	11	8–14
	Closed	7.8 B	0.92	8	6–9
	Closed after 6 min	9.6 A	1.71	9.5	7–12
6	Open	10.2 A	0.79	10	9–11

<sup>a</sup> Mean values with the same letter are not significantly different ( $P > 0.05$ ). Cooking times are not normally distributed.

gas grill with the lid open again indicated a large reduction in *E. coli* O157:H7 at lower temperatures with more frequent turning over of steaks. However, those findings also indicate that with the same frequency of turning steaks over, the minimum temperature to which steaks can be cooked with uniform attainment of large reductions in *E. coli* O157:H7 would be lower for steaks cooked on the grill than for steaks cooked on the hot plate. This finding suggests that steaks were heated more efficiently and evenly on the grill than on the hot plate.

When steaks were turned over only once during cooking on the hot plate or grill with the lid open, the time for cooking individual steaks to the same temperature differed considerably. For some groups of steaks, the shortest cooking time was  $<60\%$  of the longest time. Nevertheless, cooking times tended to increase with an increase in the temperature to which steaks were cooked, as expected. For steaks cooked to the same temperature, cooking times were longer for steaks cooked on the hot plate than those cooked on the grill, which again suggests that heating steaks on the grill was more efficient than heating them on the hot plate. However, the point during cooking at which steaks were turned over did not obviously affect the time of cooking to a particular temperature probably because the temperatures at steak centers tended to be at least maintained after steaks were turned over before rising as tissues above the newly heated surface became warm.

Culinary instructions for cooking steaks commonly recommend that steaks be turned over only once during cooking (3, 19, 25, 27). The recommended time for cooking before turning over steaks usually is relatively short. The common recommendation is that a 2.5-cm (1-in.)-thick steak be turned over after 4 or 5 min. In the present study, cooking in such a manner to  $\leq 71^\circ\text{C}$  on the hot plate or barbecue grill with the lid open did not result in a uniformly large reduction in *E. coli* O157:H7 at all points in the steaks. This finding is in agreement with those of other reports of *E. coli* O157:H7 survival in steaks turned over only once during cooking (8, 14, 15, 20). The limited findings for steaks turned over once after 10 or 12 min during cooking to  $71^\circ\text{C}$  indicate that turning over once during cooking to  $71^\circ\text{C}$  can be effective for inactivating large numbers of *E. coli* O157:H7 when turning is delayed for longer times. Presumably, a suitably longer time before turning allows

TABLE 8. Inoculated sites from which *Escherichia coli* O157:H7 was recovered and ranges of levels recovered from groups of five steaks cooked on a gas barbecue grill with the lid open, turned over once after cooking for 4 or 6 min during cooking to 63°C, and held for 3 min or not held after cooking

Time turned over (min)	Held after cooking	No. of positive sites <sup>a</sup>			<i>E. coli</i> O157:H7 at positive sites (log CFU/site)		
		Center	1.0 cm from edge	0.5 cm from edge	Center	1.0 cm from edge	0.5 cm from edge
4	No	1	3	2	1.92	0.60–2.29	0.00–2.54
	Yes	3	4	5	1.11–1.38	0.00–3.45	0.00–5.00
6	No	1	0	1	1.49	ND <sup>b</sup>	1.92
	Yes	0	0	0	ND	ND	ND

<sup>a</sup> *E. coli* O157:H7 was inoculated at a mean of 5.80 log CFU per site. Inoculated sites were the geometric center and in the central plane 1.0 or 0.5 cm from the edge of each steak.

<sup>b</sup> ND, none detected.

all tissues below the central plane to attain temperatures at which large numbers of *E. coli* O157:H7 cells would be inactivated within the cooking time. Holding steaks at ambient temperatures after cooking will at best compensate little and uncertainly for inadequate heating of tissues during cooking.

Closing the barbecue lid during cooking will result in warming of surfaces that are not directly heated which can reduce the time for cooking to a specified temperature, as was observed. Even heating of all steak surfaces also can reduce the number of persistent cold spots within steaks and so enhance inactivation of *E. coli* O157:H7 in the tissues. The present findings verified inactivation of high numbers of *E. coli* O157:H7 at steak centers after cooking to relatively low temperatures, with lid closure and turning steaks over every 2 min producing similar results. The findings also indicate that closing the lid for a short time at the end of cooking can be sufficient for equilibration of the temperature needed to ensure inactivation of large numbers of *E. coli* O157:H7 throughout the steak.

In this study, the fate of *E. coli* O157:H7 inoculated into only the central plane of steaks was investigated, whereas with mechanical tenderizing bacteria would be distributed throughout the meat, and the steaks used in this study were composed of mostly muscle tissue. The effects of cooking conditions on *E. coli* O157:H7 in steaks with large fractions of fat or other tissues might then be somewhat different. However, some broad conclusions about cooking practices that should ensure the microbiological safety of mechanically tenderized beef steaks can be drawn from the findings of this study. When steaks are turned over only once during cooking in an open skillet or barbecue, cooking to 71°C or to 63°C with holding for 3 min after cooking will not reliably ensure that temperatures at all points in a steak will be high enough for inactivation of large numbers of *E. coli* O157:H7. Turning steaks over twice at suitable intervals during cooking to 63°C was found previously to be sufficient for inactivation of large numbers of *E. coli* O157:H7 throughout steaks (8), which is in agreement with the current finding that *E. coli* O157:H7 was eliminated from inoculated steaks turned over every 4 min during cooking for 10 to 13 min to 62°C. However, turning steak over more frequently during cooking in an open skillet or barbecue can apparently ensure conditions sufficient for

inactivation of large numbers of *E. coli* O157:H7 throughout steaks cooked to temperatures  $\geq 60^\circ\text{C}$ . Similar results can be obtained when steaks are turned over only once but the barbecue lid is closed for a few minutes at the end of the cooking time. Placing a lid on a skillet during the final minutes of cooking may be as effective as closing the barbecue lid. Use of such cooking practices and cooking to 63°C should then be generally effective for ensuring the microbiological safety of mechanically tenderized beef steaks. These results should be considered during formulation of recommendations for cooking mechanically tenderized steaks.

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