Validation of Cooking Times and Temperatures for Thermal Inactivation of *Yersinia pestis* Strains KIM5 and CDC-A1122 in Irradiated Ground Beef†

ANNA C. S. PORTO-FETT,1 VIJAY K. JUNEJA,1 MARK L. TAMPLIN,2 AND JOHN B. LUCHANSKY†*

1U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Microbial Food Safety Research Unit, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA; and 2Tasmanian Institute of Agricultural Research, School of Agricultural Science, University of Tasmania, Private Bag 54, Hobart, Tasmania 7001, Australia

MS 08-196: Received 25 April 2008/Accepted 19 October 2008

ABSTRACT

Irradiated ground beef samples (ca. 3-g portions with ca. 25% fat) inoculated with *Yersina pestis* strain KIM5 (ca. 6.7 log CFU/g) were heated in a circulating water bath stabilized at 48.9, 50, 52.5, 55, 57.5, or 60°C (120, 122, 126.5, 131, 135.5, and 140°F, respectively). Average D-values were 192.17, 34.38, 17.11, 3.87, 1.32, and 0.56 min, respectively, with a corresponding z-value of 4.67°C (8.4°F). In related experiments, irradiated ground beef patties (ca. 95 g per patty with ca. 25% fat) were inoculated with *Y. pestis* strains KIM5 or CDC-A1122 (ca. 6.0 log CFU/g) and cooked on an open-flame gas grill or on a clam-shell type electric grill to internal target temperatures of 48.9, 60, and 71.1°C (120, 140, and 160°F, respectively). For patties cooked on the gas grill, strain KIM5 populations decreased from ca. 6.24 to 4.32, 3.51, and ≤0.7 log CFU/g at 48.9, 60, and 71.1°C, respectively, and strain CDC-A1122 populations decreased to 3.46 log CFU/g at 48.9°C and to ≤0.7 log CFU/g at both 60 and 71.1°C. For patties cooked on the clam-shell grill, strain KIM5 populations decreased from ca. 5.96 to 2.53 log CFU/g at 48.9°C and to ≤0.7 log CFU/g at 60 or 71.1°C, and strain CDC-A1122 populations decreased from ca. 5.98 to ≤0.7 log CFU/g at all three cooking temperatures. These data confirm that cooking ground beef on an open-flame gas grill or on a clam-shell type electric grill to the temperatures and times recommended by the U.S. Department of Agriculture and the U.S. Food and Drug Administration Food Code, appreciably lessens the likelihood, severity, and/or magnitude of consumer illness if the ground beef were purposefully contaminated even with relatively high levels of *Y. pestis*.

In recent years, there has been increased concern from public health organizations worldwide about the potential use of *Yersinia pestis* as an agent of bioterrorism (8, 18, 19). Although gastrointestinal plague is extremely rare, Christie et al. (10) reported that consumption of camel meat that was naturally contaminated with *Y. pestis* caused 13 illnesses and six deaths in a village in Lyibia in 1976. Bin Saeed et al. (4) reported five cases of illness and two deaths from the pharyngeal and gastrointestinal forms of the disease in 1994 due to ingestion of contaminated raw camel liver. These reports suggest a possible role of *Y. pestis* in outbreaks of gastrointestinal plague acquired via consumption of contaminated raw or undercooked meat. These reports also emphasize the need for the development and validation of interventions to eliminate the pathogen in anticipation of situations in which natural or deliberate contamination of food has occurred, particularly food produced, distributed, and/or consumed on a large scale, such as ground beef.

Annually, each American consumes an average of ca. 28 lb (12.7 kg) of ground beef (63% of all the fresh beef consumed in the home), and ca. 7.5 billion pounds (3.4 billion kilograms) of ground beef are produced by the ca. 1,000 meat processing plants in the United States (2). In the last 10 years, the consumption of ground beef patties increased by ca. 51%, with an estimated consumption in commercial restaurants of nearly 8.2 billion hamburger patties in 2004 compared with 5.4 billion consumed in 1996 (29). Because the consumption of undercooked ground beef patties has been linked epidemiologically to foodborne outbreaks, particularly those caused by strains of *Escherichia coli* O157:H7, several studies have been published on the effectiveness of cooking to reduce the risk of foodborne illness (1, 12, 13, 31, 33, 35). Despite the health risks associated with consumption of undercooked or raw meat, food preparers often use unreliable procedures to estimate end-point cooking temperatures and/or doneness, such as visually inspecting the color and/or appearance of the meat (39). However, these parameters are not trustworthy because during cooking, browning may occur before the meat reaches internal temperatures sufficient to eliminate foodborne pathogens (28, 34). The U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) (41) and the U.S. Food and Drug Administration (FDA) (45) have established guidelines for both consumers and the food service industry for the safe handling and preparation of ground beef patties. According to USDA-FSIS, a mini-
mum instantaneous internal cooking temperature of 71.1°C (160°F) is recommended to assure that ground beef patties are cooked sufficiently to achieve at least a 5.0-log reduction of *E. coli* O157:H7 (44) and/or a 6.5-log reduction of *Salmonella* (43), whereas according to the FDA Food Code, a minimum instantaneous internal cooking temperature of 70°C (158°F) or an internal cooking temperature of 68.5°C (155°F) for 15 s is recommended (45). The so-called patty rule also provided for an 8.0-log reduction in numbers of Shiga-toxin–producing *E. coli* (42). As an aside, the time-temperature combinations listed in the patty rule would only provide for a 1.88- to 3.47-log reduction of *Listeria monocytogenes* and, ostensibly, would need to be validated for lethality toward vegetative cells of other foodborne pathogens for a decision as to its sufficiency.

Although the association of *Yersinia enterocolitica* and possibly *Yersinia pseudotuberculosis* with sporadic cases and outbreaks of foodborne illness has been well documented (17, 21, 22, 30, 36), there is essentially no information available on the viability of *Y. pestis* in food matrices or on interventions that may be effective against this species of *Yersinia*. Because of illnesses due to gastrointestinal plague and the potential for use of this pathogen as a biological weapon, it is important to gain a more complete understanding of the behavior and control of *Y. pestis* in food matrices. In a previous study using sterile and non-sterile ground beef, we found that *Y. pestis* strain KIM5 replicated at temperatures of 0 to 40°C (32 to 104°F) and reached maximum population densities of ca. 7.5 to 10 log CFU/g at corresponding growth rates of 0.0014 to 0.2887 CFU/h (40). The purpose of the present study was (i) to evaluate the thermal inactivation of *Y. pestis* strains KIM5 and CDC-A1122 in ground beef in a model system by heating 3-g samples in a temperature-controlled water bath and (ii) to validate recommended cooking regimens for ground beef patties using two types of commercial grills.

**MATERIALS AND METHODS**

**Bacterial strains.** Avirulent *Y. pestis* strains KIM5 (15) (Dr. Susan Straley, Department of Microbiology and Immunology, University of Kentucky, Lexington) and CDC-A1122 (14) (Dr. Robert Phillips, USDA-FSIS, Microbial Outbreaks and Special Projects Laboratory, Athens, GA) were used in this study to inoculate ground beef. Stocks of both strains were maintained at 20°C in brain heart infusion (BHI; Becton Dickinson, Sparks, MD) broth plus 10% glycerol. Before inoculation of ground beef, the strains were individually grown in 10 ml of BHI broth for 18 h at 28°C with shaking (100 rpm).

**Ground beef.** Raw ground beef (25% ± 1% fat) was purchased at a local supermarket. The two batches (50 lb [22.7 kg] each) were divided into 5-lb (2.3-kg) portions, vacuum sealed in stomacher bags (Koch Industries, Kansas City, MO) to 950 mBar with a Multivac A300/16 vacuum-packaging unit (Sepp Hagemüller KG, Wolfertschwenden, Germany), frozen at −20°C, and shipped to a commercial facility for irradiation to an absorbed dose of approximately 30 kGy at −20°C. The irradiated ground beef was stored at −20°C for up to 15 days. Before inoculation, the meat was thawed at 4°C for approximately 36 h.

**Thermal inactivation of *Y. pestis* in ground beef heated in a temperature-controlled water bath.** Irradiated ground beef was inoculated with an appropriate dilution of *Y. pestis* to achieve an average target level of ca. 6.7 log CFU/g. The inoculated ground beef was first mixed by hand and then macerated for 5 min with a stomacher (Stomacher 400, Seward, Cincinnati, OH). Three-gram samples were aseptically weighed and placed into plastic filter stomacher bags (Spiral Biotech, Norwood, MA) with a sterile spatula, and the bags were vacuum sealed. Thermal inactivation was conducted in a temperature-controlled water bath (Nestlab RTE17, Newington, NH) stabilized at 48.9, 50, 52.5, 55, 57.5, or 60°C (120, 122, 126.5, 131, 135.5, and 140°F, respectively) (±0.3°C [0.5°F]). The temperature of the water bath was monitored with a type K thermocouple (Omega Engineering Inc., Stamford, CT) connected to a temperature data logger (Dickson SP150, Dickson, Addison, IL). Two bags for each of the three trials were then removed at designated time intervals. Sampling frequency was based on the heating temperature, with more frequent sampling at the higher temperatures. The come-up times (CUT) needed to achieve the target temperatures were not monitored; however, the CUT was most likely negligible because of the small size and relative thinness of the samples. Total heating times ranged from 360 min at 48.9°C to 5 min at 60°C. After removal, samples were immediately plunged into an ice-water bath and analyzed within 30 min.

**Thermal inactivation of *Y. pestis* in ground beef cooked on commercial grills.** Irradiated ground beef was aseptically transferred to a sterile stainless steel food processing bowl and separately inoculated with *Y. pestis* strain KIM5 or strain CDC-A1122 to achieve an average target level of ca. 6.0 log CFU/g. The inoculated ground beef was mechanically mixed in a food mixer (model KP26M1XBS, KitchenAid, St. Joseph, MI) for 2 min at room temperature (22 ± 1°C [71.6 ± 1.8°F]) to ensure even distribution of the inoculum. Portions (ca. 95 g each) of the inoculated ground beef were placed in an aluminum mold to form a ground beef patty with uniform thickness (1 cm) and diameter (10 cm). All patties were stored at 4°C for ca. 1 h before cooking. Patties that were inoculated with the pathogen, but not cooked, served as controls. The inoculated ground beef patties were cooked on a commercial open-flame gas grill (model XXE-4, Baker's Pride, New Rochelle, NY) or on a commercial clam-shell type electric grill (model GR14, Star Manufacturing International Inc., St. Louis, MO). Both the upper and lower platen surfaces of the clam-shell grill were set at 176.7°C (350°F), and all four gas jets were fully opened to subsequently achieve target internal temperatures of either 48.9, 60, or 71.1°C. The grilling height between the top platen and the bottom platen was adjusted to the approximate thickness of a typical patty, ca. 1 cm. The top platen was fitted with a counterbalance system that when placed on the top of the hamburger patty applied 3 to 4 lb (1.4 to 1.8 kg) of pressure. To ensure that each side of the patty received about the same amount of heat when they were cooked on a gas grill, the patties were turned over with a sterile stainless steel spatula at the approximate midpoint between the initial patty temperature, ca. 12.5 ± 0.5°C (54.5 ± 0.9°F), and the final target temperature. When patties were cooked on the commercial clam-shell grill, it was not necessary to turn them over because the heat was simultaneously and evenly distributed to both sides of the patties. On both types of grill, three patties were positioned side by side and cooked simultaneously without adding any oil to the surface of either grill. The internal temperature of each patty was monitored with a calibrated stainless steel type K thermocouple (Omega Engineering) that was inserted into the approximate geometric center of each
patty. The thermocouple from each patty was individually connected to a six-channel digital panel temperature indicator (model 500T, Doric Instruments, VAS Engineering Inc., San Diego, CA), and readings were taken at 10-s intervals. When the target temperature was reached, patties were removed from the grill with a sterile spatula and transferred to plastic filter stomacher bags that were immediately placed into an ice-water bath. The patties were sampled within ca. 30 min.

The purge from each patty at each cooking temperature was collected by placing small aluminum foil containers directly below each patty on the bottom of the gas grill or in the grease drawer for patties cooked on a clam-shell type grill. For each of the three trials and each of the three temperatures, three patties were cooked, and samples of the cooked patties and their purge were tested.

**Enumeration of *Y. pestis* from ground beef.** For recovery of *Y. pestis* strains KIM5 and CDC-A1122 from both cooked and uncooked ground beef, 3 ml (for 3-g portions) or 100 ml (for 95-g patties) of 0.1% sterile peptone water was added to the filter bags, which were then macerated for 2 min in a stomacher. Three- and 12-ml samples of the filtrate from each portion and patty, respectively, were transferred to a sterile 15-ml screw-cap conical centrifuge tube. *Y. pestis* populations were determined by diluting the filtrate as needed and surface plating onto duplicate Congo red magnesium oxalate (CRMOX) agar plates, prepared as described by Bhaduri (3), which were incubated at 37°C for 48 h. The detection limit for direct plating was ≤0.7 log CFU/g.

Because the purge was dry or solid because of its proximity to the heat from the grills, it was necessary to add 10 ml of 0.1% sterile peptone water to each aluminum foil container drawer and use a sterile cotton swab to rehydrate and recover the purge and transfer it to sterile 15-ml screw-cap conical centrifuge tubes. *Y. pestis* was enumerated by direct plating 100 μl of the rehydrated purge onto duplicate CRMOX agar plates and incubating at 37°C for 48 h. The detection limit for direct plating was ≤1.3 log CFU/ml.

**Data analyses.** D-values were determined from the inactivation rates calculated with the DMFit curve fitting software (www.ifr.ac.uk/safety/DMFit; Dr. Joszef Baranyi, Institute of Food Research, Norwich, UK). The calculated D-values represented the absolute value of the inverse of the linear inactivation rate of the cell fraction that survived after heating in a water bath system. Only survival curves with more than five values in the straight line portion of the curve and with a correlation coefficient (r²) of >0.94 were used to calculate a D-value. The z-value was calculated as the absolute value of the inverse of the linear regression of D-values versus the corresponding temperatures. The SAS system (version 8.0, SAS Institute, Cary, NC) was used to determine the significance of differences among strains, temperatures, and cooking methods. Means and standard deviations (SD) in the cooking experiments were calculated from individual sets of data for each of the three trials at each of the three temperatures tested, with triplicate samples at each time interval. An analysis of variance was used to determine the effects and interactions of the factors on the log reduction values. The means were separated using the Bonferroni least significant difference method to determine the significance of differences (P < 0.05) among means.

**RESULTS**

**Thermal inactivation of *Y. pestis* in ground beef cooked with two types of commercial grills.** When ground beef patties inoculated with *Y. pestis* strains KIM5 or CDC-A1122 were cooked on a grill, the average times required (CUT) to reach the target internal temperatures of 48.9, 60, and 71.1°C were ca. 4.7, 6.1, and 6.9 min (mean CUT, 6 min) on the gas grill and ca. 2.4, 3.0, or 3.6 min (mean CUT, 3 min) on the clam-shell grill, respectively. Thus, cooking times required to reach the target internal temperature were significantly higher (P < 0.05) for patties cooked on the gas grill than for patties cooked on the clam-shell grill. For ground beef patties inoculated with *Y. pestis* strain KIM5, using the gas grill and heating to an internal temperature of either 48.9 or 60°C reduced pathogen numbers by at least 5.26 log CFU/g. Using the clam-shell grill, cooking patties to an internal temperature of 48.9°C reduced pathogen numbers by at least 5.26 log CFU/g.
grill to a target internal temperature of 48.9, 60, or 71.1°C, there was no significant difference ($P > 0.05$) between the two strains in the extent of thermal inactivation. With the exception of cooking to 60°C wherein the clam-shell grill was significantly more effective ($P < 0.05$) than the gas grill for inactivating strain KIM5, in general no significant differences ($P > 0.05$) were observed between the two types of grills for inactivation of $Y. pestis$ in ground beef patties. $Y. pestis$ strains KIM5 and CDC-A1122 were not detected ($\leq 1.3$ log CFU/ml) by direct plating the purge from the grease drawer of the clam-shell grill and from the bottom of the gas grill (three trials, three samples per trial per temperature for each of the two grill types).

**DISCUSSION**

Although reports of $Y. pestis$ as a foodborne pathogen are rare, this bacterium is being viewed as a significant public health threat because of its potential for use as an agent of agroterrorism. $Y. pestis$ is classified by the Centers for Disease Control and Prevention as a category A microorganism because it can be easily disseminated and has a high potential for causing massive causalities (6–8). Thus,
it is crucial to evaluate the behavior of this pathogen in food matrices, particularly in foods such as ground beef that are produced in high volume and/or that are consumed by a significant portion of the general population.

Consumption of raw or improperly prepared meat often has been implicated in human cases of yersiniosis (21). Although Y. enterocolitica and Y. pseudotuberculosis are foodborne pathogens, there is a general lack of information about the presence and viability of Y. pestis in food matrices. However, Y. pestis has been associated with gastrointestinal plague due to consumption of raw or undercooked contaminated camel meat (4, 10). In a survey of fresh, frozen, and ready-to-eat meat and fish products in India, Khare et al. (26) recovered Y. pestis from 14 (36.8%) of 38 samples and from 35 (11.6%) of 302 of the isolates retained from these 14 samples. Using a two-stage enrichment procedure followed by direct plating onto cefsulodin-irgasan-novobiocin agar, these authors reported that levels of Y. pestis isolated from the naturally contaminated meat and fish products ranged from 3.0 to 6.0 log CFU/g. These findings establish the potential for foodborne yersiniosis caused by Y. pestis and suggest that it could be used as a biological threat agent.

Adequate cooking is the most important and the most effective barrier against illness from pathogens that may be present in raw meat (23). Thus, the primary objective of the present study was to evaluate the thermal stability of Y. pestis in ground beef. As one component of this study, we evaluated the viability of Y. pestis strain KIM5 in 3-g portions of ground beef after thermal challenge in a temperature-controlled water bath at temperatures and times sufficiently high to generate D-values, but probably not sufficient to meet the USDA-FSIS or FDA recommended cooking guidelines. With the possible exception of results obtained following heating for ≥6 min at 57.5°C and for ≥20 min at 55°C (Fig. 1), the kinetics of inactivation were essentially linear. The presence of a subpopulation of more heat resistant bacteria, as indicated by the tailing of the survival curves from deviations in linear survivor curves, may be due to the variability in heat resistance within a population of cells. A tailing effect probably would have been observed for the other temperatures tested if the sampling times had been extended; it can be assumed that a relatively small subpopulation of more resistant bacteria would die at a slower rate for a given temperature when the heating time is extended (9, 16, 38). We observed maximal reductions ranging from ca. 2.0 log CFU/g within ca. 360 min at 48.9°C to ca. 7.1 log CFU/g within ca. 5 min at 60°C, with corresponding D-values of ca. 192 to 0.5 min, respectively.

In a previous study, we found that Y. pestis exhibited growth in sterile and nonsterile raw ground beef during storage at 0 to 40°C, but was inactivated during storage at −20°C or at temperatures ≥41°C (40). In that study, we also observed that growth was appreciably inhibited by the indigenous flora within the raw ground beef. To our knowledge, there have been no studies published on the viability of Y. pestis in foods after thermal challenge. However, some reports have included details of the thermal stability of Y. enterocolitica in food systems that can be compared with the results reported herein for Y. pestis. Doherty et al. (11) reported D-values of 25 and 1.28 min at 50 and 55°C, respectively, for Y. enterocolitica GER serotype O:3 inoculated into minced beef (ca. 2.4% fat) and heated in a water bath system. Bolton et al. (5) found that for Y. enterocolitica GER serotype O:3 in minced beef (ca. 2.8% fat) that was heated in a water bath, the D-values were 1.06 and 0.55 min at 55 and 60°C, respectively. Likewise, for E. coli O157:H7 in 3-g portions of lean ground beef (4% fat) that

![FIGURE 2. Thermal death time curve (z-value) for Y. pestis strain KIM5 in ground beef for the temperature range of 48.9 to 60°C. The D-values were calculated from the inactivation rates obtained from the DMFit curve fitting software. The means of two samples for each of the three trials were obtained based on survivors on the recovery media and were used to determine the z-value.](image-url)
were heated in a temperature-controlled water bath, the
D-values were 21.13 and 4.95 min at 55 and 57.5°C, respec-
tively (25). Overall, these results are in general agree-
ment with the data obtained in the present study after heat-
ing a 3-g portion of ground beef in a water bath system. Thus, E. coli O157:H7 is generally more heat tolerant than
either Y. enterocolitica or Y. pestis following thermal chal-
lenge. Further studies are needed to confirm these obser-
vations.

As another component of this study, we used com-
mercial grills to establish the thermal stability of Y. pestis
directly in ground beef and to compare these data with other
lethality data, including results obtained with the water bath
system used in this study. In general, the times needed to
achieve the target cooking temperatures were significantly
longer (P < 0.05) for the gas grill than for the clam-shell
grill. Differences between the grills may be attributed to a
higher heat penetration rate, a shorter cooking time, and/or
less variation of the internal temperature of the meat when
ground beef patties are cooked on a double-sided clam-shell
grill compared with a single-sided gas grill (32, 35). Al-
though the target internal temperatures for cooked patties
were 48.9, 60, and 71.1°C, the meat continued to cook dur-
ing the ca. 1.5 (±0.5) min that transpired from the time
when the patties were removed from the grill until when they
were chilled and sampled. Thus, when target internal
temperatures were 48.9, 60, and 71.1°C, the final average
temperatures of patties cooked on the gas grill were 52.8,
62.4, and 77.6°C, respectively, and those for patties cooked
on the clam-shell grill were 60.6, 61.1, and 77.6°C, respec-
tively. This added increase in the internal temperature of
the meat would most likely provide an additional margin
of safety (13).

The thermal resistance of pathogens in foods may in-
crease somewhat with an increase in the fat content of the
food matrix, probably because of the reduced water activity
and/or the reduced heat penetration rate (lower heat con-
ductivities) through the heating menstruum (24). Although
patties with a relatively high fat level (25%) were used in
this study, our results are in general agreement with thermal
data previously reported for E. coli O157:H7 in ground beef
patties with fat levels of 15 to 27%; we found about a 2.5-
to 5.5-log reduction of Y. pestis following cooking of
ground beef patties on commercial grills, whereas other in-
vestigators reported ca. 1.5- to 6.0-log reductions of E. coli
O157:H7 under similar conditions (13, 20, 23, 27, 35).

Despite the risk of foodborne diseases associated with
consumption of raw or undercooked ground beef, 16% of
consumers still cook or order their hamburger patties either
rare (internal temperature of ca. 60°C) or medium rare (in-
ternal temperature of ca. 63°C [145.4°F]) (32). In the pres-
ent study, we used two methods of cooking, i.e., heating in
a temperature-controlled water bath and cooking on a com-
mercial grill, to quantify lethality for Y. pestis. The method
of cooking and the meat composition, particularly the fat
content, affects both the rate and the extent of pathogen
inactivation because the heat transfer coefficient from the
heat source to the meat is nearly 20 times greater when the
product is heated in a water bath than when it is cooked on
a grill (37). Based on the results obtained from 3-g portions
of 75% lean ground beef heated in a water bath, to achieve
the 5.0-log reduction of E. coli O157:H7 required by the
USDA-FSIS under conditions likely to be used by food
service, retail establishments, and consumers, and based on
the D-values reported in Table 1, theoretically it would be
necessary to heat or cook these ground beef portions for at
least 2.8 min after the meat reached an internal temperature
of 60°C or for at least 6.6 min at 57.5°C. For ground beef
patties, with the possible exception of patties containing
strain KIM5 that were heated on the gas grill to 60°C
(which would be considered rare for ground beef (30)), it
was possible to achieve a 5.0-log reduction for Y. pestis
strains KIM5 and CDC-A1122 by heating to 60 or 71.1°C
on either the gas grill, which required ca. 6.5 min to reach
the target internal temperatures, or the clam-shell grill,
which required ca. 3.3 min to reach the target internal

Table 2. Inactivation of two Yersinia pestis strains in ground beef patties cooked with two types of commercial grills

<table>
<thead>
<tr>
<th>Temp, °C (°F)</th>
<th>Open-flame gas grill</th>
<th>Clam-shell grill</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KIM5 (log CFU/g)</td>
<td>CDC-A1122 (log CFU/g)</td>
</tr>
<tr>
<td>Before cooking</td>
<td>6.24 ± 0.04</td>
<td>6.22 ± 0.05</td>
</tr>
<tr>
<td>After cooking</td>
<td>48.9 (120)</td>
<td>1.92 ± 1.28 &lt;c&gt;</td>
</tr>
<tr>
<td></td>
<td>60.0 (140)</td>
<td>2.73 ± 0.90 &lt;bc&gt;</td>
</tr>
<tr>
<td></td>
<td>71.1 (160)</td>
<td>5.54 ± 0.28 &lt;A&gt;</td>
</tr>
</tbody>
</table>

a Within each row and each column, means with the same letters are not significantly different (P > 0.05).
b Mean ± SD of three trials, with three samples per trial.
c Mean ± SD of two trials, with three samples per trial.
d An SD of 0.0 was obtained when the pathogen levels were below the detection limit for all trials.
for heating temperatures that were less than 60°C, which required far more time to achieve the desired lethality in the water bath system than on the commercial grills.

Our findings confirm that the target internal temperature of 71.1°C recommended by the USDA-FSIS and the target internal temperatures of 68.3°C for 15 s or 70°C instantaneously (inclusive of the CUT) recommended in the FDA Food Code resulted in at least a 5.0-log reduction of Y. pestis strains KIM5 and CDC-A1122 when 95-g ground beef patties were cooked on commercial gas or clam-shell grills. These data also confirm that the cooking temperatures recommended by the USDA-FSIS and FDA for achieving at least a 5.0-log reduction of E. coli O157:H7 are sufficient to appreciably lessen the likelihood of foodborne illness due to consumption of ground beef that may be purposefully contaminated with relatively high levels of Y. pestis.

ACKNOWLEDGMENTS

We extend our appreciation to the following individuals at the USDA Agricultural Research Service (ARS) Eastern Regional Research Center (ERRC) who in large measure contributed to the successful completion of this study by sharing their time, talents, resources, and/or opinions: Brad Shoyer, Rosemary Martinjuk, John Cherry, Angie Martinez, Ellen Sanders, Jeff Call, Saunya Bhaduri, John Phillips, and the ERRC Bio-Security Special Projects Team. For these same reasons, we are extremely grateful to our colleagues at the USDA-FSIS: Lynda Kelley and Robert Phillips (Athens, GA) and Tim Mohr (Washington, DC). This research was supported in part by the National Biodfense Analysis and Countermeasures Center and by the USDA-FSIS.

REFERENCES