

# Impact of Cooking, Cooling, and Subsequent Refrigeration on the Growth or Survival of *Clostridium perfringens* in Cooked Meat and Poultry Products

ROBIN M. KALINOWSKI,<sup>1</sup>\* R. BRUCE TOMPKIN,<sup>1</sup> PETER W. BODNARUK,<sup>2</sup> AND W. PAYTON PRUETT, JR.<sup>1</sup>

<sup>1</sup>ConAgra Refrigerated Foods Group, 3131 Woodcreek Drive, Downers Grove, Illinois 60515; and <sup>2</sup>ABC Research Corporation, 3437 SW 24th Avenue, Gainesville, Florida 32607, USA

MS 02-419: Received 15 November 2002/Accepted 14 February 2003

## ABSTRACT

In January 1999, the Food Safety and Inspection Service (FSIS) finalized performance standards for the cooking and chilling of meat and poultry products in federally inspected establishments. More restrictive chilling (stabilization) requirements were adopted despite the lack of strong evidence of a public health risk posed by industry practices employing the original May 1988 guidelines (U.S. Department of Agriculture FSIS Directive 7110.3). Baseline data led the FSIS to estimate a “worst case” of  $10^4$  *Clostridium perfringens* cells per g in raw meat products. The rationale for the FSIS performance standards was based on this estimate and the assumption that the numbers detected in the baseline study were spores that could survive cooking. The assumptions underlying the regulation stimulated work in our laboratory to help address why there have been so few documented outbreaks of *C. perfringens* illness associated with the consumption of commercially processed cooked meat and poultry products. Our research took into account the numbers of *C. perfringens* spores in both raw and cooked products. One hundred ninety-seven raw comminuted meat samples were cooked to 73.9°C and analyzed for *C. perfringens* levels. All but two samples had undetectable levels (<3 spores per g). Two ground pork samples contained 3.3 and 66 spores per g. Research was also conducted to determine the effect of chilling on the outgrowth of *C. perfringens* spores in cured and uncured turkey. Raw meat blends inoculated with *C. perfringens* spores, cooked to 73.9°C, and chilled according to current guidelines or under abuse conditions yielded increases of 2.25 and 2.44 log<sub>10</sub> CFU/g for uncured turkey chilled for 6 h and an increase of 3.07 log<sub>10</sub> CFU/g for cured turkey chilled for 24 h. No growth occurred in cured turkey during a 6-h cooling period. Furthermore, the fate of *C. perfringens* in cooked cured and uncured turkey held at refrigeration temperatures was investigated. *C. perfringens* levels decreased by 2.52, 2.54, and 2.75 log<sub>10</sub> CFU/g in cured turkey held at 0.6, 4.4, and 10°C, respectively, for 7 days. Finally, 48 production lots of ready-to-eat meat products that had deviated from FSIS guidelines were analyzed for *C. perfringens* levels. To date, 456 samples have been tested, and all but 25 (ranging from 100 to 710 CFU/g) of the samples contained *C. perfringens* at levels of <100 CFU/g. These results further support historical food safety data that suggest a very low public health risk associated with *C. perfringens* in commercially processed ready-to-eat meat and poultry products.

According to the Centers for Disease Control, *C. perfringens* accounted for 2.1% of the outbreaks and 3.2% of the cases of foodborne illnesses reported from 1993 to 1997 (6). No deaths were attributed to *C. perfringens* foodborne illnesses during this period. Foods primarily responsible for such illnesses are cooked beef and poultry dishes that are usually prepared with gravy (4, 5, 10). These foods are typically mishandled in foodservice settings, where they are improperly cooled or held at room temperature for extended periods prior to serving.

Illness caused by *C. perfringens* occurs when the heat-resistant spores of the organism survive thermal processing and then germinate into vegetative cells. Once in the intestinal tract, the vegetative cells sporulate and release enterotoxin upon sporangial autolysis. Large numbers of *C. perfringens* cells (e.g.,  $\geq 10^6$  CFU/g of food) are typically required to cause illness (19).

Current U.S. Department of Agriculture (USDA)

guidelines require the cooling of cooked uncured meat and poultry products from 54.4 to 26.7°C within 1.5 h and from 26.7 to 4.4°C within 5 h (preferred) or from 48.9 to 12.8°C in 6 h (alternative), followed by additional cooling to 4.4°C prior to packing (28). For cooked cured meat and poultry items, the guidelines state that cooling from 54.4 to 26.7°C should occur within 5 h and cooling from 26.7 to 7.2°C should occur within 10 h. Products meeting these requirements have not been linked to foodborne illness.

Studies have shown that *C. perfringens* levels can significantly increase during simulated cooling cycles associated with cooked meat and poultry processes (15, 16, 22, 26). In these experiments, the increase in *C. perfringens* numbers was dependent on such factors as cooling temperature and rate, as well as the concentration of antimicrobial ingredients (e.g., salt, pyrophosphate). However, previous research has not generally considered the overall impact of initial *C. perfringens* levels in raw materials, thermal processing, cooling, and subsequent refrigerated storage on the growth and survival of this pathogen in commercially processed ready-to-eat (RTE) meat and poultry products.

\* Author for correspondence. Tel: 630-512-1087; Fax: 630-512-1125;  
 E-mail: rkalinowski@crfc.com.

To better understand why RTE meat and poultry products produced under federal inspection have had a favorable history with regard to the control of *C. perfringens*, a more comprehensive evaluation of the multiple stages involved in processing is essential. The objectives of this research were (i) to determine the levels of *C. perfringens* spores in a variety of raw meat blends, (ii) to evaluate the behavior of spores after cooking and cooling, (iii) to ascertain the effect of subsequent refrigeration on the viability of cells, and (iv) to determine the levels of *C. perfringens* in products that have not met USDA cooling requirements.

## MATERIALS AND METHODS

### Enumeration of *C. perfringens* spores in raw meat blends.

Raw ground-meat emulsion samples were obtained from federally inspected meat and poultry processing facilities. Products analyzed included pork sausage emulsion, ground turkey, ground pork, and ground beef. Fifty-gram portions of each sample were aseptically weighed into individual sterile Stomacher bags. Each sample was flattened to ca. 3-mm thickness to facilitate better heat transfer and was then vacuum sealed with a packaging machine (AGW 756, MultiVac, West Germany). To determine the levels of spores, products were cooked to a minimum internal temperature of 73.9°C by placing a single layer of packages in a flowing steam chamber (Arthur S. LaPine & Co., Chicago, Ill.). Immediately after cooking, packages were immersed in ice water. Each 50-g cooked sample was diluted with 100 ml of Butterfield's phosphate diluent (BPD) and pummeled in a Stomacher (Seward, London, England) for 30 s. A 0.1-ml aliquot of each diluted sample was spread plated onto tryptone-sulfite-cycloserine (TSC) agar without egg yolk (17). Plates were covered with an additional 8 to 10 ml of TSC agar and incubated at 35°C for 48 h in an anaerobic environment. Anaerobic conditions were generated by vacuum sealing plates into 3 mil high-barrier Nylon/EVOH/PE pouches (Koch, Kansas City, Mo.) that contained AnaeroGen sachets (Oxoid Ltd., Basingstoke, Hampshire, UK). All black colonies on TSC plates were counted as presumptive. Presumptive colonies were confirmed to be *C. perfringens* on the basis of Gram staining, cell morphology, lactose fermentation, gelatin liquefaction, nitrate reduction, and motility reactions.

**Inoculated-pack studies: spore preparation.** Four strains of *C. perfringens*, NCTC 8239, NCTC 8798, NCTC 8449, and ATCC 13124, were used in all inoculated-pack studies. Stock cultures were maintained in Microbank cryovials (Prolab Inc., Ontario, Canada) at -20°C. The four *C. perfringens* cultures were grown individually in fresh fluid thioglycollate medium incubated at 35°C for 18 h. A 100- $\mu$ l aliquot of each culture was added to separate tubes of Duncan and Strong medium with caffeine (200  $\mu$ g/ml). All cultures were then incubated at 35°C for 24 h. Spores were observed microscopically and enumerated (13).

**Inoculated-pack studies: sample preparation and inoculation.** Raw uncured or cured turkey breast meat formulated with 156  $\mu$ g of sodium nitrite per ml was used. All *C. perfringens* cultures were pooled and diluted in BPD to achieve a final level of ca. 100 spores per g when added to the raw turkey emulsion and mixed for 5 min in a Hobart mixer (A200, Hobart Mfg. Co., Troy, Ohio). The inoculated turkey emulsion was weighed into 100-g portions and aseptically dispensed into 3 mil high-barrier Nylon/EVOH/PE pouches and vacuum sealed.

**Inoculated-pack studies: cooking.** Prior to cooking, a thermocouple was inserted into one package to monitor the temper-

ature during cooking and cooling. Each pouch was flattened to ca. 3-mm thickness and cooked in the flowing steam chamber until the product reached a minimum temperature of 73.9°C.

**Inoculated-pack studies: influence of cooling rate on outgrowth of spores in cured and uncured turkey.** The potential for the outgrowth of *C. perfringens* spores during cooling was investigated after cooking by placing the pouches in a water bath set at 48.9 or 54.4°C. After the product temperature reached the water bath temperature, the cooling rate was controlled so that the temperature decreased from 48.9 to 12.8°C in 6 h for uncured turkey. For cured turkey, the temperature decreased from 48.9 to 26.7°C in 6 h or from 54.4 to 7.2°C in 24 h.

**Inoculated-pack studies: effect of temperature abuse on the outgrowth of spores in cured and uncured turkey.** The effect of holding products at abuse temperatures on the outgrowth of spores was investigated. After cooking, pouches were placed in a water bath set at one of five selected abuse temperatures (26.7, 32.2, 37.8, 43.3, or 48.9°C) and held for up to 6 h.

**Inoculated-pack studies: effect of salt level on outgrowth of spores in cured and uncured turkey.** The effect of salt concentration on the outgrowth of spores was investigated for cured and uncured turkey formulated with 1, 2, or 3% NaCl. After cooking, pouches were placed in a water bath set at 43.3°C.

**Inoculated-pack studies: survival of *C. perfringens* in cooked cured and uncured turkey during refrigerated storage.** After cooking, pouches were placed in a water bath set at 42°C and held for 2 h; these pouches were then held at 0.6, 4.4, or 10°C for 7 days. Duplicate samples of cooked turkey were analyzed daily for the first 4 days. A final set of samples was analyzed on the seventh day.

**Inoculated-pack studies: enumeration.** Unless otherwise noted, one package of cooked turkey was removed from the water bath at 1-h intervals, placed in ice water, and analyzed according to the following procedure. Analytical units (25 g each) were removed, diluted 1:10 in BPD, and pummeled for 30 s in a Stomacher. Serial dilutions were prepared in BPD, and 0.1-ml portions were spread plated directly onto TSC plates without egg yolk. All plates were covered with an additional 8 to 10 ml of TSC agar and incubated anaerobically at 35°C for 24 h.

**Analysis of products not meeting USDA chilling requirements.** From time to time, for a variety of reasons, RTE meat and poultry products do not meet the USDA time-temperature requirements for chilling. When deviations from these guidelines occurred, samples from across the lot were analyzed for microbial growth to determine the disposition of the lot. Forty-eight production lots were analyzed. Although sampling plans varied depending on the nature of the deviation, 10 samples were typically collected from across the lot and analyzed for aerobic plate count, anaerobic plate count, and *C. perfringens* count. Analytical units (25 g each) were aseptically removed from each product sample and diluted 1:10 in BPD. Samples were pummeled in a Stomacher for 30 s. Aliquots (1 or 0.1 ml) were spread plated onto TSC agar without egg yolk and pour plated on standard methods agar (Difco, Becton Dickinson Microbiology Systems, Sparks, Md.) for the aerobic plate count and onto tryptone-peptone-glucose-yeast extract (TPGY) agar for the anaerobic plate count. TSC plates were covered with an additional 8 to 10 ml of TSC agar and incubated anaerobically at 35°C for 48 h. Standard methods agar plates were incubated aerobically at 35°C for 48 h. TPGY plates were covered

TABLE 1. *C. perfringens* spore levels in raw meat blends

Product type	No. of samples	No. of samples with <i>C. perfringens</i> level <sup>a</sup>		
		<3 spores/g	3–100 spores/g	>100 spores/g
Ground turkey	154	154	0	0
Ground pork	11	9	2	0
Ground beef	6	6	0	0
Pork sausage	26	26	0	0
Total	197	195	2	0

<sup>a</sup> Biochemically confirmed.

with an additional 8 to 10 ml of TPGY agar and incubated anaerobically at 35°C for up to 72 h.

**RESULTS**

**Spore levels in raw meat blends.** One hundred ninety-seven raw ground-meat samples were tested for *C. perfringens* spores (Table 1). All but two of the samples contained no detectable levels of *C. perfringens* spores (<3 spores per g). Two ground-pork samples contained low levels of spores, with 3.3 and 66 spores per g being detected.

**Effect of cooling.** In experiments 1 and 2, 2.44- and 2.25-log increases in *C. perfringens* levels were observed for uncured turkey during the 6-h chilling process (Table 2). An additional study (experiment 3) was conducted to determine whether the increases in *C. perfringens* levels observed in experiments 1 and 2 were a function of a high initial inoculum level. The target inoculum level for the third study was 10 spores per g. In this experiment, a 0.83-log increase was observed during the chilling process.

No *C. perfringens* growth occurred in cured turkey during a 6-h cooling period. However, a 3.07-log increase in *C. perfringens* levels occurred in cured turkey over a 24-h cooling period (Table 3). Growth reached the early exponential phase after 6 h of cooling, when the product temperature was 35°C. Over the next 12 h (cooling from 35 to 11.1°C) a 2.66-log increase was observed. This experiment was repeated (Table 3), with a 2.52-log increase in *C. perfringens* counts over the entire 24-h cooling period being observed.

**Effects of temperature and salt level.** The proliferation of *C. perfringens* in uncured turkey samples held at various abuse temperatures is illustrated in Table 4. A lag phase of 2 to 3 h was observed at 26.7 and 32.2°C, and a

TABLE 2. Behavior of *C. perfringens* in uncured turkey cooled from 48.9 to 12.8°C in 6 h

Experiment	<i>C. perfringens</i> level (log <sub>10</sub> CFU/g) at storage time						
	0 h	1 h	2 h	3 h	4 h	5 h	6 h
1	2.86	2.96	2.1	2.8	3.4	3.8	5.3
2	3.23	3.06	0.7	3.04	4.65	5.24	5.48
3	1.66	2.04	2.04	2.04	2.48	2.41	2.49

TABLE 3. Behavior of *C. perfringens* in cured turkey cooled from 54.4 to 7.2°C in 24 h

Experiment	<i>C. perfringens</i> level (log <sub>10</sub> CFU/g) at storage time						
	0 h	3 h	6 h	9 h	12 h	18 h	24 h
1	0.97	0.63	1.38	1.66	3.04	4.04	4.04
2	2.36	1.78	3.06	4.68	5.52	4.47	4.88

lag phase of 2 h was observed at 37.8, 43.3, and 48.9°C. Proliferation occurred at all temperatures, with increases of 1.37, 3.27, 5.21, 6.20, and 5.18 log<sub>10</sub> CFU/g being observed at 26.7, 32.2, 37.8, 43.3, and 48.9°C, respectively.

The growth of *C. perfringens* in cured turkey is shown in Table 5. No increase in counts was observed at 26.7 and 32.2°C, with levels remaining relatively constant for 6 h. Lag phases of 2, 3, and 2 h with *C. perfringens* increases of 2.58, 5.60, and 4.00 log<sub>10</sub> CFU/g were observed when samples were held at 37.8, 43.3, and 48.9°C, respectively.

An additional experiment was conducted with cured and uncured turkey samples containing 2 or 3% salt at 43.3°C. For uncured turkey (without the effects of the curing ingredients), a 2-h lag phase was observed for all salt levels tested. At 43.3°C, *C. perfringens* levels increased to 8.40, 7.32, and 6.26 CFU/g after 6 h for products containing 1, 2, and 3% salt, respectively. For cured turkey containing 1 and 2% salt, a 3-h lag phase followed by growth to levels of 7.60 and 6.65 CFU/g after 6 h at 43.3°C was observed. No increases in *C. perfringens* levels were observed for cured turkey samples containing 3% salt, with levels dropping below the detection limit after 1 h. As the salt level increased, the *C. perfringens* growth rate decreased for cured and uncured turkey samples. The combination of 3% salt and 156 µg of sodium nitrite per ml prevented the growth of *C. perfringens* in cooked turkey.

**Survival of *C. perfringens* during refrigerated storage.** *C. perfringens* levels in the cooked turkey samples decreased during refrigerated storage (Table 6). Reductions of 1.25, 1.22, and 1.58 log CFU/g were observed after 24 h at 0.6, 4.4, and 10°C, respectively. *C. perfringens* counts continued to decrease at all storage temperatures, with reductions of 2.52, 2.54, and 2.75 log CFU/g occurring after 7 days for samples stored at 0.6, 4.4, and 10°C, respectively. Reductions in *C. perfringens* levels in uncured turkey were similar (Table 6). Initial reductions of 1.91, 1.83, and

TABLE 4. Behavior of *C. perfringens* in cooked uncured turkey containing 1% salt

Temperature (°C)	<i>C. perfringens</i> level (log <sub>10</sub> CFU/g) at storage time						
	0 h	1 h	2 h	3 h	4 h	5 h	6 h
26.7	3.56	3.38	3.63	3.78	4.81	5.22	4.93
32.2	3.62	3.60	3.90	4.10	5.02	5.46	6.89
37.8	2.40	2.10	2.58	4.52	5.80	6.66	7.61
43.3	2.20	2.04	2.60	5.07	6.80	7.80	8.40
48.9	2.80	2.50	3.00	4.10	5.90	7.40	7.98

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/66/7/1227/16172707/0362-028x-66\_7\_1227.pdf by guest on 10 August 2022

TABLE 5. Behavior of *C. perfringens* in cooked cured turkey containing 1% salt

Temperature (°C)	<i>C. perfringens</i> level (log <sub>10</sub> CFU/g) at storage time							
	0 h	1 h	2 h	3 h	4 h	5 h	6 h	24 h
26.7	2.97	3.59	3.45	3.63	3.20	3.18	3.60	2.78
32.2	3.21	3.20	3.36	3.49	3.59	3.36	3.30	2.59
37.8	3.45	2.93	3.10	4.02	4.77	6.03	5.27	4.14
43.3	2.00	2.85	2.85	2.70	6.09	6.38	7.60	NT <sup>a</sup>
48.9	3.34	2.90	3.88	4.42	5.02	6.64	7.34	6.10

<sup>a</sup> NT, not tested.

1.55 log CFU/g were observed after 24 h of storage at 0.6, 4.4, and 10°C, respectively. Numbers continued to decrease at all storage temperatures. After 7 days at 0.6, 4.4, and 10°C, the corresponding total reductions in *C. perfringens* levels were 2.42, 2.45, and 2.31 log CFU/g, respectively.

***C. perfringens* levels in product not meeting USDA cooling requirements.** Table 7 shows *C. perfringens* levels for 456 samples obtained from 48 lots of product not meeting USDA cooling requirements (28). Levels for 431 samples were <100 CFU/g, and levels for 25 samples ranged from 110 to 710 CFU/g.

## DISCUSSION

The FSIS proposed rule for cooling was predicated on baseline studies of the numbers of *C. perfringens* cells detected on raw ground-meat and poultry samples (8). The analysis, however, did not specifically determine the number of spores and did not include a confirmation procedure for presumptive *C. perfringens* colonies. Therefore, the data from the baseline studies do not represent a valid estimate of the levels of *C. perfringens* spores in raw meat and poultry and should not be used to estimate the number of *C. perfringens* spores in freshly cooked meat and poultry products. Historical data indicate that no raw turkey breast roast product samples tested have contained *C. perfringens* at confirmed levels exceeding 10<sup>2</sup> CFU/g prior to inoculation (22). Hall and Angelotti (9) found *C. perfringens* in 43.1% of their raw, unprocessed meat samples. These investigators did not analyze for the presence of spores, since enumerations were carried out without the heating of samples. Quantitative determinations were carried out for only 36 of 262 samples tested, with levels ranging from 1 to 760 CFU/g. Most samples evaluated contained 1 to 100 CFU/g of raw meat.

The results presented here indicate that raw meat samples contain low levels of *C. perfringens*. Substantial changes in slaughtering and processing conditions over the past 30 years have led to lower levels of *C. perfringens*.

Steele and Wright (22) investigated the effect of cooling rate on the outgrowth of *C. perfringens* in cooked uncured RTE turkey breast roasts. Cooling times of 6 and 8 h did not violate the FSIS cooling requirement of no greater than 1-log<sub>10</sub> multiplication of *C. perfringens* during a decrease from 48.9 to 12.8°C. On the basis of a 95% tolerance interval, a cooling period of ≤8.9 h was recommended.

TABLE 6. Behavior of *C. perfringens* in cooked cured and uncured turkey at 0.6, 4.4, and 10°C

Type of product	Temperature (°C)	<i>C. perfringens</i> level (log <sub>10</sub> CFU/g) at storage time					
		0 days	1 day	2 days	3 days	4 days	7 days
Cured	0.6	4.91	3.66	3.26	2.33	2.37	2.39
	4.4	4.91	3.69	3.64	2.42	2.40	2.37
	10	4.91	3.33	2.39	2.20	2.00	2.16
Uncured	0.6	5.68	3.77	3.61	3.57	3.20	3.26
	4.4	5.68	3.85	3.65	3.69	3.47	3.23
	10	5.68	4.13	3.88	3.79	3.63	3.37

Research on cooked ground beef indicated that cooling to 7.2°C in ≤15 h was necessary to prevent an increase in *C. perfringens* levels (16). Both studies involved low initial inoculum levels (<1 spore per g for turkey breast (22) and 1.5 log<sub>10</sub> CFU/g for ground beef (16)). These results agree with those of our cooling study when a low inoculum level (10 spores per g) was used.

Our data indicate that no *C. perfringens* growth occurred in cured turkey held at 26.7 and 32.2°C for 6 h. Blankenship et al. (1) reported similar results for cooked chili, with no *C. perfringens* growth being observed over 6 h at 26.7°C. Lag times of 3.3, 2.2, 2.0, and 2.0 h were observed for samples held at 32.2, 37.8, 43.3, and 48.9°C, respectively.

Juneja and Marmer (15) investigated the fate of *C. perfringens* in ground turkey containing 0.3% sodium pyrophosphate and 0, 1, 2, or 3% salt. The product was cooked, chilled, and held at 28°C. Lag times of 7.3, 10.6, 11.6, and 8.0 h were observed for salt levels of 0, 1, 2 and 3%, respectively. The combination of a salt level of 3% and a pH of 5.5 inhibited *C. perfringens* outgrowth in cook-in-bag ground beef containing 0.3% sodium pyrophosphate at 15 and 28°C (14). Each treatment was found to be inhibi-

TABLE 7. Clostridium perfringens levels in various cooked RTE meat and poultry products not meeting FSIS stabilization requirements<sup>a</sup> from 1998 to 2001

Product category	No. of lots	No. of samples	No. of samples with <i>C. perfringens</i> level			
			<10 <sup>b</sup> CFU/g	<100 <sup>b</sup> CFU/g	11–100 CFU/g	>100 CFU/g
Cured meats	6	60	54	6	0	0
Cured poultry	2	15	15	0	0	0
Noncured poultry	15	151	106	45	0	0
Roast beef	3	21	15	6	0	0
Chili	5	47	47	0	0	0
BBQ	12	116	81	0	10	25
Beef brisket	2	16	14	0	2	0
Cooked ground taco beef	3	30	30	0	0	0
Total	48	456	362	57	12	25 <sup>c</sup>

<sup>a</sup> The lengths of the deviations ranged from 0.66 to 13 h.

<sup>b</sup> Two detection limits were reported for some sample sets.

<sup>c</sup> Levels for these samples were 110 to 710 CFU/g.

tory when tested separately, but the combination of treatments completely suppressed the growth of *C. perfringens* for 21 days at 15°C and delayed growth for 24 h at 28°C. The addition of 3% salt to cook-in-bag ground turkey containing 0.3% sodium pyrophosphate was effective in delaying proliferation for 12 h at 28°C and completely inhibited the outgrowth of *C. perfringens* spores at 15°C (15).

*C. perfringens* was able to grow in media containing up to 6% salt at 35°C (21). A level of 300 µg/ml sodium nitrite (unheated) was required to prevent *C. perfringens* growth in media containing 3% salt. Labbe and Duncan (18) found that heat-injured spores were more sensitive to the effects of nitrite than non-heat-injured spores. Concentrations of 200 and 1,000 µg/ml nitrite were necessary to prevent outgrowth at pH 6 and at pH 7, respectively (18).

The nitrite levels employed in the aforementioned studies were higher than the allowable levels of nitrite for meat and poultry products. Results obtained in the present study indicate that nitrite at a concentration of 156 µg/ml in cooked turkey, alone or in combination with salt, is more inhibitory to *C. perfringens* outgrowth than has been reported in the literature for laboratory media.

Our results indicate that *C. perfringens* dies during refrigerated storage. This finding is consistent with previous research indicating that *C. perfringens* vegetative cells remain stable or decline during refrigerated storage (7, 9, 23, 25). With the exception of short holding times, refrigerated storage is more lethal to *C. perfringens* vegetative cells and spores than freezing is (7, 24).

When *C. perfringens* spores are inoculated into raw meat or poultry products and cooked in the laboratory, they can survive and, depending on the temperature profile during cooling and the product composition, may multiply. This is a situation in which research indicates a public health need for a certain degree of control, but commercial experience indicates otherwise. The FSIS has expressed the opinion that the risk of *C. perfringens* illness is best controlled through processes based on challenge tests and predictive modeling. These approaches to estimating risk have merit, but they are not a replacement for other sources of information. A comprehensive evaluation that takes into account historical commercial experience and a critical review of epidemiological data is needed to place such research into perspective.

On the basis of the results obtained in this study, as well as the historical safety of RTE products produced under federal inspection, a requirement of no more than a 1-log<sub>10</sub> increase in the *C. perfringens* level may be unnecessarily restrictive. Even minor deviations from the chilling requirements have required extensive management and laboratory effort to demonstrate that affected products are safe, wholesome, and not adulterated. The economic impact of destroying questionable but safe and wholesome products was not considered in the development of the cooling regulation.

While a pathogen may have the potential to grow (on the basis of theory, inoculation challenge studies, and/or modeling) in a given processed food, in reality this does

not mean the product is hazardous to consumers. Several factors should be considered in determining the likelihood of a public health risk: prevalence and/or levels of the pathogen in raw ingredients, historical association with foodborne illness, product composition, packaging environment, postprocessing growth potential as influenced by cooling rate and subsequent storage temperatures, and customary cooking or reheating practices carried out prior to serving.

In addition to the low numbers of spores in today's raw meat and poultry supply, an additional inherent safety feature is the decline in viable cells during subsequent refrigerated storage. The addition of certain spices and other ingredients during the preparation of foods in the home or at the foodservice level may be associated with a greater risk. Also, from 1970 to 1996 in the United Kingdom, 94% of the 1,525 reported outbreaks were due to mishandling at the foodservice level (2, 3). In Australia, "nearly all outbreaks have been associated with the ingestion of meat or poultry dishes prepared and cooked 24 h or more before consumption, then allowed to cool slowly. Most outbreaks have occurred in large eating establishments where large joints, roasts and batches of food are prepared and served" (20). On the basis of epidemiologic data, it was concluded that outbreaks of foodborne illness in the United States and Canada prior to 1980 occurred most frequently during the final stages in the food chain (i.e., the home and foodservice stages) (11).

The accumulated data indicate that the performance standard should be changed from "no more than 1-log<sub>10</sub> multiplication of *C. perfringens*" (28) to "no greater than a 2-log increase or no greater than 500/g at the time the product is released for shipment." Furthermore, in the event of a deviation from the cooling guidelines, sampling of the suspect lot is a valid option. For example, the sampling protocol could be based on a scientifically recognized plan, such as one proposed by the International Commission on Microbiological Specifications for Foods (12).

## REFERENCES

- Blankenship, L. C., S. E. Craven, R. G. Leffler, and C. Custer. 1988. Growth of *Clostridium perfringens* in cooked chili during cooling. *Appl. Environ. Microbiol.* 54:1104-1108.
- Brett, M. M. 1998. 1566 outbreaks of *Clostridium perfringens* food poisoning, 1970-1996. *Proc. 4th World Congr. Foodborne Infect. Intox. Berlin Ger.* 1:243-244.
- Brett, M. M., and R. J. Gilbert. 1997. 1525 outbreaks of *Clostridium perfringens* food poisoning, 1970-1996. *Rev. Med. Microbiol.* 8(Suppl. 1):64-65.
- Bryan, F. L. 1988. Risks associated with vehicles of foodborne pathogens and toxins. *J. Food Prot.* 51:498-508.
- Bryan, F. L., and T. W. McKinley. 1979. Hazard analysis and control of roast beef preparation in foodservice establishments. *J. Food Prot.* 42:1-18.
- Centers for Disease Control. 2000. Surveillance for foodborne disease outbreaks—United States, 1993-1997. *Morb. Mortal. Wkly. Rep.* 49:1-51.
- Craven, S. E. 1980. Growth and sporulation of *Clostridium perfringens* in foods. *Food Technol.* 34:80-87.
- Food Safety and Inspection Service. 1998. Lethality and stabilization performance standards for certain meat and poultry products: technical paper. U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, D.C.

9. Hall, H. E., and R. Angelotti. 1965. *Clostridium perfringens* in meat and meat products. *Appl. Microbiol.* 13:352–357.
10. Hobbs, B. C., M. E. Smith, C. L. Oakley, G. H. Warrack, and J. C. Cruickshank. 1953. *Clostridium welchii* food poisoning. *J. Hyg. Camb.* 51:75–101.
11. International Commission on Microbiological Specifications for Foods. 1988. Microbiological control of foods—the case for using HACCP, p. 3–21. *In HACCP in microbiological safety and quality*. Blackwell Scientific Publications, Boston.
12. International Commission on Microbiological Specifications for Foods. 2002. Sampling plans, p. 123–143. *In Microbiological testing in food safety management*. Kluwer Academic/Plenum Publishers, New York.
13. Juneja, V. K., J. E. Call, and A. J. Miller. 1993. Evaluation of methylxanthines and related compounds to enhance *Clostridium perfringens* sporulation using a modified Duncan and Strong medium. *J. Rapid Methods Automation Microbiol.* 2:203–218.
14. Juneja, V. K., and W. M. Majka. 1995. Outgrowth of *Clostridium perfringens* spores in cook-in-bag beef products. *J. Food Saf.* 15: 21–34.
15. Juneja, V. K., and B. S. Marmar. 1996. Growth of *Clostridium perfringens* from spore inocula in *sous-vide* turkey products. *Int. J. Food Microbiol.* 32:115–123.
16. Juneja, V. K., O. P. Snyder, and M. Cygnarowicz-Provost. 1994. Influence of cooling rate on outgrowth of *Clostridium perfringens* spores in cooked ground beef. *J. Food Prot.* 57:1063–1067.
17. Labbe, R. G. 2001. *Clostridium perfringens*, p. 325–330. *In F. P. Downes and K. Ito (ed.), Compendium of Methods for the microbiological examination of foods*, 4th ed. American Public Health Association, Washington, D.C.
18. Labbe, R. G., and C. L. Duncan. 1970. Growth from spores of *Clostridium perfringens* in the presence of sodium nitrite. *Appl. Microbiol.* 19:353–359.
19. McClane, B. A. 1992. *Clostridium perfringens* enterotoxin: structure, action and detection. *J. Food Saf.* 12: 237–252.
20. Murrell, W. G. 1989. *Clostridium perfringens*, p. 209–232. *In K. A. Buckle (ed.), Foodborne microorganisms of public health significance*, 4th ed. Australian Institute of Food Science and Technology Ltd. (NSW Branch) Food Microbiology Group, New South Wales, Australia.
21. Roberts, T. A., and C. M. Derrick. 1978. The effect of curing salts on the growth of *Clostridium perfringens (welchii)* in a laboratory medium. *J. Food Technol.* 13:349–353.
22. Steele, F. M., and K. H. Wright. 2001. Cooling rate effect on outgrowth of *Clostridium perfringens* in cooked, ready-to-eat turkey breast roasts. *Poult. Sci.* 80:813–816.
23. Stiles, M. E., and L. K. Ng. 1979. Fate of pathogens inoculated onto vacuum-packaged sliced hams to simulate contamination during packaging. *J. Food Prot.* 42:464–469.
24. Strong, D. H., K. F. Weiss, and L. W. Higgins. 1966. Survival of *Clostridium perfringens* in starch pastes. *J. Am. Diet. Assoc.* 49:191–195.
25. Traci, P. A., and C. L. Duncan. 1974. Cold shock lethality and injury in *Clostridium perfringens*. *Appl. Microbiol.* 28:815–821.
26. Tuomi, S., M. E. Matthews, and E. H. Marth. 1974. Behavior of *Clostridium perfringens* in precooked chilled ground beef gravy during cooling, holding, and reheating. *J. Milk Food Technol.* 37:494–498.
27. U.S. Department of Agriculture. 1988. Time/temperature guidelines for cooling heated products. Food Safety and Inspection Service Directive 7110.3. U.S. Department of Agriculture, Washington, D.C.
28. U.S. Department of Agriculture. 1999. Performance standards for the production of certain meat and poultry products. 9 CFR 301, 317, 318, 320, 381. Office of Federal Register, National Archives and Records Administration, Washington, D.C.