Survival of Campylobacter jejuni in Fresh and Heated Red Meat

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ABSTRACT

Studies were done to assess the ability of Campylobacter jejuni to survive in fresh ground beef during refrigerated storage and to identify time-temperature treatments needed to inactivate Campylobacter in ground and cubed red meat. The organism survived well in refrigerated ground beef containing large numbers of indigenous bacteria. Relatively little death (1.2-log10 reduction) occurred for 7 of 8 strains during 14 d at 4°C. C. jejuni inoculated into ground beef and cubed lamb meat was quite sensitive to heat treatment. D-values for inactivation of campylobacters in ground beef ranged from 5.9 to 6.3 min at 50°C and from 12 to 21 s at 58°C. D-values were generally greater when campylobacters were heated in lamb meat, ranging from 5.9 to 13.3 min and 12.5 to 15.8 s at 50 and 60°C, respectively. All strains of C. jejuni were more sensitive to heat than salmonellae, hence meat heated to a temperature sufficient to inactivate Salmonella spp. should be free of viable campylobacters.

Campylobacter jejuni is now an established enteropathogen that has been responsible for several food-associated disease outbreaks (3). The organism is frequently present in the intestinal tract of domestic animals and has been isolated from carcasses of slaughtered beef, sheep and swine (12,15,16). At least two reports have implicated beef as a vehicle of campylobacter infections. In one instance, a presumptive outbreak of campylobacter enteritis was attributed to consumption of campylobacter-contaminated raw hamburger (13). In the other occurrence, illness resulted following consumption of campylobacter-contaminated steak (filet mignon) (1). Analysis of frozen steaks (approx. 120 g each) obtained from the same box as the implicated steak found C. jejuni at a level of 4 organisms per gram, suggesting an infective dose of about 500 cells.

The association of campylobacter enteritis with consumption of beef has prompted the need to determine the survival characteristics of C. jejuni in beef under storage and cooking conditions. Christopher et al. (5) have studied the fate of the organism at several temperatures in beef cubes and ground beef. The meat was prepared aseptically to exclude microbial contaminants. The two strains evaluated experienced a 2- to 3-log10 reduction by day 14 at either 1 or 10°C, suggesting that the organism survives well in beef containing relatively few competitive microorganisms and held at refrigeration temperature. Additionally, these investigators studied the heat resistance of C. jejuni in beef roasts (5). Roasts were heated in an oven at 177°C (350°F) until their internal temperature reached 50 or 55°C. Roasts reaching a final internal temperature of 57°C had no survivors (initially 6.6 x 10⁶ campylobacters/g inoculated at 2 depths). In another study, Stern and Kotula (17) found that heating C. jejuni-inoculated ground beef in an oven at 190 or 218°C inactivated approximately 10² campylobacters/g in less than 10 min after the meat reached an internal temperature of 60°C. These data indicate that C. jejuni is sensitive to heating in beef, but fail to identify times at specific temperatures necessary to inactivate the organism.

The purpose of this study was to determine the survival characteristics of C. jejuni in refrigerated ground beef containing high levels of indigenous microorganisms, and to establish D-values (the time required to inactivate 90% of the cells at a specific temperature) for C. jejuni in beef so that a specific time-temperature relationship for thermal inactivation of the organism can be identified.

MATERIALS AND METHODS

Survival of Campylobacter strains in raw ground beef

Fresh ground beef (approx. 20% fat content) was obtained from a local retail market and held at 4°C until inoculated (within 2 d). Portions (25 g) of the meat to be inoculated were screened for C. jejuni by selective enrichment (8); none was positive. Seven strains of C. jejuni, including FRI-CF3, FRI-CF6, FRI-CF8, FRI-CF33P, FRI-CF74C, FRI-CF145B and FRI-CF147B, and one nalidixic acid-resistant thermophilic Campylobacter (NARTC), FRI-CF31P, were used for these studies. The source of each strain was described previously (7). Each strain was grown to late-log phase in brucella broth supplemented with 0.3% sodium succinate and 0.01% cysteine-hydrochloride as described previously (6). Each strain was studied individually by inoculation of 0.3 ml of 10⁻¹-diluted (0.1% peptone) culture into each of a total of 15 10-g portions of ground beef. The inoculum was homogeneously dispersed throughout each 10-g sample by vigorously hand mixing the sample in a Whirl-Pak bag for 30 s. Meat was held at 4°C and sampled at appropriate intervals. At each sampling, ground beef was assayed for Campylobacter strains, aerobic plate count (APC) and pH. Surviving campylobacters were enumerated by

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serial-diluting (1:10) meat in 0.1% peptone, macerating the initial dilution by stomaching 2 min (Seward Lab-Blender 400, London), and surface plating 0.1-ml portions in duplicate onto Campy-BAP. Plates were incubated in an atmosphere of 5% O₂, 10% CO₂, 85% N₂ for 48 h at 42°C, and colonies typical of C. jejuni were counted. Representative colonies were confirmed to be C. jejuni or NARC by the method described previously (7). APC's were determined by procedures described in the "Bacteriological Analytical Manual" (9).

**Thermal inactivation of C. jejuni in ground beef**

Three cultures of C. jejuni (FRI-CF6, FRI-CF8 and FRI-CF404S) were individually heated in ground beef (aseptically prepared as described by Christopher et al. (5)) at 50, 56 and 58°C. Ground beef-heating menstrua were prepared by placing 1-g portions of meat into sterile 10 x 75-mm Pyrex test tubes, inoculating each portion with 0.01 ml of Campylobacter culture, mixing meat and culture for 20 s with the wooden end of a sterile swab, tightly Packing the meat to obtain a compact mass, and capping the tube with a rubber stopper. Culture inocula were grown in supplemented brucella broth as described previously (6). Tubes were completely submerged in an ice-water bath and duplicate tubes were withdrawn at appropriate intervals, beginning once the meat reached the desired temperature ("zero time"). At each sampling interval, tubes were immediately immersed in an ice-water bath and meat was enumerated for CFU/g, using methods described previously (6). Each experiment was performed in duplicate.

**Thermal inactivation of C. jejuni in lamb meat**

Six cultures of C. jejuni, including four strains isolated from fecal specimens of apparently healthy lambs (FRI-CF401S, FRI-CF402S, FRI-CF403S and FRI-CF404S) and FRI-CF8 and FRI-CF31P, were heated in lamb meat singly or as a composite at 4°C. Lamb meat (leg) was prepared by flaming the surface and removing the outermost 1- to 1.5-cm portion with a sterile knife. The remaining meat was aseptically cut into cubes (2.2 x 2.2 x 2.2 cm), each weighing approx. 10 g. One cube each was placed into 1675 x 100-mm Whirl-Pak bags, submerged in a heated water bath, and duplicate tubes were withdrawn at appropriate intervals, beginning once the meat reached the desired temperature. Thermocouples were inserted into the center of reference cubes to monitor temperature. Once the cubes had reached the desired temperature, C. jejuni (0.1 ml of appropriately diluted culture prepared as described above) was injected through the bag into the center of each cube. The time required to inoculate each cube was taken into consideration when calculating thermal death times. Duplicate samples were removed from the water bath at specified times and immediately blended in a Waring Blender with 90 ml of 0.1% peptone (approx. 10°C). Survivors were enumerated using methods described previously (6). Each experiment was performed in duplicate.

**RESULTS AND DISCUSSION**

**Survival in raw ground beef**

Survival of the different Campylobacter strains in refrigerated raw ground beef was remarkably consistent (Fig. 1). Relatively little death (<1.2-log₁₀ reduction) occurred for 7 of 8 strains during 14 d at 4°C. The exception was strain CF8 for which a >5-log₁₀ reduction occurred to the undetectable level (<100 CFU/g) by day 14. Strain CF6 was studied up to 34 d, by which time only an ~1.5-log₁₀ reduction had occurred. By this time, the meat was well past consumer acceptance. The initial APC of the ground beef ranged from 1.4 x 10¹⁰ to 2.0 x 10¹⁰ CFU/g, and after 14 d at 4°C increased to 2.1 x 10¹⁰ to 2.1 x 10¹⁰ CFU/g (Table 1), indicating that the large population of psychrotrophic microbes in ground beef had little adverse effect on the survival of campylobacters. The initial pH of the meat ranged from 5.7 to 6.2 and after 14 d increased to pH 6.5 to 6.9, close to the optimal pH for survival and growth of C. jejuni (6).

![Figure 1. Survival of C. jejuni and NARC in uncooked ground beef held at 4°C. ---, No campylobacters were detected at the <100-CFU/g level (minimum level of sensitivity) in the final sampling.](http://meridian.allenpress.com/jfp/article-pdf/46/9/771/1650791/0362-028x-46_9_771.pdf)

These results contrast the findings of similar studies done in unpasteurized milk using the same Campylobacter strains (7). In unpasteurized milk, >4-log₁₀ reduction occurred for 7 of 8 strains during 14 d at 4°C, with three of the strains being inactivated to the undetectable level (<10 CFU/ml), i.e., >6-log₁₀ reduction, between 7 and 12 d. Several reasons may be suggested to explain why campylobacters survive better in ground beef than in milk. One factor is that the microbial population of milk is likely different than that of ground beef, hence metabolites produced by microorganisms growing in milk may be more toxic to campylobacters than microbial metabolites produced in meat. Differences observed in the changes of pH of milk and meat during storage indicate that different metabolites are being produced in the two foods. In milk, after 14 d the pH decreased from 6.6-6.7 to 6.0-6.2, whereas in ground beef the pH increased from 5.7-6.2 at day 0 to 6.5-6.9 at day 14. These changes in pH may reflect fermentation of lactose to lactic acid in milk and deamination of amino acids in ground beef.

The presence of some anti-campylobacter properties in unpasteurized milk, such as the lactoperoxidase system (7), may also contribute to the organism’s increased rate of death. Lactoperoxidase, in combination with H₂O₂ and SCN⁻ , produces metabolites that are bactericidal to many gram-negative bacteria. Another consideration may be that certain components of ground beef, such as iron porphyrins (heme), have a survival-promoting effect on campylobacters. Border et al. (4) have reported that incorporation of hematin in a complex medium considerably enhances the growth of Campylobacter fetus. Razi and Park (14) also found that adding hematin to nutrient agar ensured the...
compounds promote the growth of Campylobacter sp., perhaps they also enhance the organism's survival.

As in ground beef, *C. jejuni* also survives well on uncooked chicken (2) and in ground beef liver (11) stored at 4°C. Blankenship and Craven (2) reported an approximate 2-log_{10} decrease of *C. jejuni* per cm² of raw chicken after 14 to 21 d at 4°C. Survival was even greater when the meat was packaged in an atmosphere of CO₂ and stored at the same temperature. Hänninen (11) found that storing ground beef liver at 4°C for 6 d had little effect on the survival of five strains of *C. jejuni/coli*. Less than a 0.5-log_{10} decrease of Campylobacter cells occurred by day 6, whereas the APC and lactobacilli counts increased to 1.8 X 10⁷ and 1.0 X 10⁷ CFU/g, respectively. The pH of the liver had decreased from an initial pH of 6.3 to pH 5.75 by day 6. By day 6, the liver had deteriorated to such a state that it was no longer suitable for human consumption.

### TABLE 2. D-values for different strains of *C. jejuni* in ground beef.

<table>
<thead>
<tr>
<th>Strain</th>
<th>50°C (min)</th>
<th>56°C (s)</th>
<th>58°C (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRI-CF6</td>
<td>5.90</td>
<td>37.2</td>
<td>12.3</td>
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<tr>
<td>FRI-CF8</td>
<td>6.17</td>
<td>37.1</td>
<td>17.1</td>
</tr>
<tr>
<td>FRI-404S</td>
<td>6.28</td>
<td>57.8</td>
<td>21.1</td>
</tr>
</tbody>
</table>

### TABLE 3. D-values for different strains of *C. jejuni* and NARTC in cubes (2.2 X 2.2 X 2.2 cm) of lamb meat.

<table>
<thead>
<tr>
<th>Strain</th>
<th>50°C (min)</th>
<th>55°C (min)</th>
<th>60°C (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRI-CF8</td>
<td>5.88</td>
<td>0.96</td>
<td>12.5</td>
</tr>
<tr>
<td>FRI-CF31P</td>
<td>11.2</td>
<td>1.20</td>
<td>13.1</td>
</tr>
<tr>
<td>FRI-CF401S</td>
<td>13.2</td>
<td>1.26</td>
<td>13.8</td>
</tr>
<tr>
<td>FRI-CF402S</td>
<td>8.96</td>
<td>1.23</td>
<td>15.8</td>
</tr>
<tr>
<td>FRI-CF404S</td>
<td>13.3</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Composite</td>
<td>1.26</td>
<td>13.8</td>
<td></td>
</tr>
</tbody>
</table>

\[^{a,b}\text{Composite comprised of approximately equal numbers of FRI-CF401S, CF402S and CF403S.}\]

Thermal inactivation in ground beef and lamb meat

D-values for *C. jejuni* inactivation in ground beef ranged from 5.9 to 6.3 min at 50°C and from 12 to 21 s at 58°C (Table 2). D-values were generally greater when campylobacters were heated in cubes of lamb meat. In lamb meat, D-values ranged from 12.5 to 21 s at 50°C and from 12.5 to 15.8 s at 60°C (Table 3). These D-values are somewhat less than those reported by Blankenship and Craven (2) for the thermal death times of a composite of five strains of *C. jejuni* in autoclaved ground chicken breast meat. They reported D-values at 55 and 57°C of 2.25 and 0.98 min, respectively. This suggests that our strains may be more heat sensitive than those used by
Blankenship and Craven or perhaps campylobacters are more sensitive to heat in ground beef and lamb meat than in ground chicken breast meat.

Goodfellow and Brown (10) reported D-values at 57.2°C of 3.8 to 4.2 min for a composite of five strains of Salmonella spp. in ground beef. Our results indicate that C. jejuni is more sensitive to heat than salmonellae, hence ground beef heated to a temperature sufficient to inactivate Salmonella spp. should be free of viable campylobacters. Ground beef or lamb meat held for 2.0 min at 58 or 60°C (internal temperature), respectively, should be sufficient treatment to inactivate approximately 10⁶ campylobacters per gram.

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REFERENCES