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Survival of *Campylobacter jejuni* Inoculated into Ground Beef

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Ground beef was inoculated with mixed cultures of *Campylobacter jejuni*, and the samples were subjected to various cooking and cold-storage temperatures. When samples were heated in an oven at either 190 or 218°C, approximately 10^7 cells of *C. jejuni* per g were inactivated (<30 cells per g) in less than 10 min after the ground beef reached an internal temperature of 70°C. When the samples were held at −15°C over 14 days of storage, the numbers of *C. jejuni* declined by 3 $\log_{10}$. When inoculated samples were stored with an equal amount of Cary-Blair diluent at 4°C, no changes in viability were observed over 14 days of storage. Twenty-five times as much *C. jejuni* was recovered from inoculated ground beef when either 10% glycerol or 10% dimethyl sulfoxide was added to an equal amount of ground beef before freezing as was recovered from peptone-diluted ground beef. Twice as much inoculated *C. jejuni* was recovered from ground beef plus Cary-Blair diluent as was recovered from ground beef plus peptone diluent.

Clinical microbiologists have demonstrated that *Campylobacter jejuni* is a significant human enteropathogen. Rates of isolation from human diarrheal samples indicate that the organism is at least as prevalent a causative agent of gastroenteritis as *Salmonella* spp. (1). Undercooked ground beef has been implicated as the likely vehicle of infection in a large outbreak among military personnel (J. Oosterom, Abstr. Public Health Lab. Serv. Int. Workshop *Campylobacter* Infect. 1981, p. 37.) Other food sources have yielded *C. jejuni* upon investigation. Isolation rates of 2% from beef, 24% from sheep, and 38% from pig carcasses have been reported (11). Park and co-workers (8) recovered the organism from 62 and 54% of chickens sampled in Ontario and Ohio, respectively.

Doyle and Roman (3) stated that "the times and temperatures used to pasteurize milk should be sufficient to free milk of even unusually large numbers of viable cells of *C. fetus* subsp. *jejuni*." This statement was based on data that showed that in a skim milk-heating menstruum, *D*-values ranged from 0.74 to 1.00 min at 55°C. Because nonhomogenous fat distribution in foods may serve to create microenvironments that may favor survival of the organism, the effects of oven cooking temperatures on *C. jejuni* inoculated into ground beef were assessed. Additionally, in an ongoing study at the U.S. Department of Agriculture, we have been involved with screening meat samples from various slaughterhouses for the presence of this pathogen. The initial protocol of that study requires that meat samples be shipped frozen to the participating laboratories for analysis. We report the results of tests to determine the effect of freezing on *C. jejuni*.

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MATERIALS AND METHODS

Cooking study. Five strains of *C. jejuni* were used in this study. ATCC 29428, FRI-CF3 (supplied by M. P. Doyle, Food Research Institute, Madison, Wis.), and USN 312 (supplied by J. C. Coolbaugh, Naval Medical Research Institute, Bethesda, Md.) were isolated from clinical sources; Lamb 49-3B was isolated from a swabbed lamb carcass; and Pork 15-1 was isolated from a swabbed pig carcass. Each strain was streaked onto blood agar plates, and isolated colonies were checked for characteristic morphology and motion with phase-contrast microscopy. Pure cultures were transferred to 250-ml screw-capped Erlenmeyer flasks containing 100 ml of brucella broth supplemented with 0.025% each of ferrous sulfate, sodium metabisulfite, and sodium pyruvate (FBP) (4). These inoculated cultures were grown to the late log phase at 42°C for 24 h in a shaker water bath at 40 oscillations per min (10). The FBP supplement enabled *C. jejuni* to tolerate the oxygen concentration of air (4).

Five-milliliter aliquots of each of these strains were mixed together and counted to determine the cell numbers per milliliter in the inoculum. Nine milliliters of the mixed culture was inoculated into about 890 g of ground beef (with about 25% fat content) purchased from a retail market. The inoculum was homogeneously dispersed throughout the ground beef sample by vigorously hand mixing the sample in a sterile plastic bag for 3 min. The homogeneity of dispersal was evaluated by counting *C. jejuni* in subsamples of the ground beef (12). Meatballs of 100 g each were asepti-

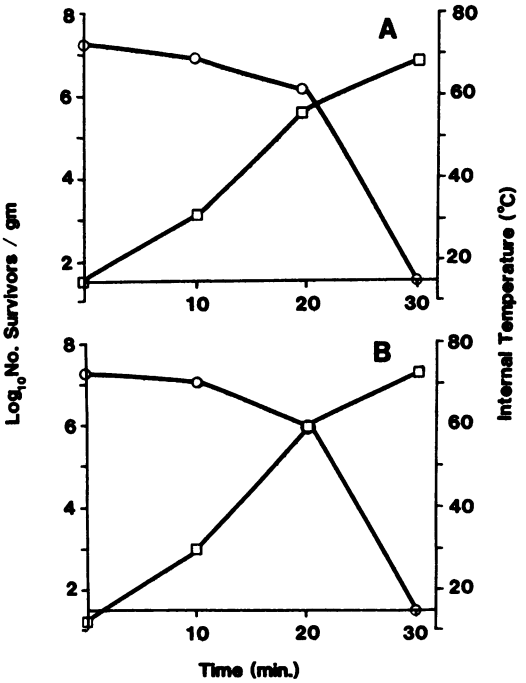

FIG. 1. Thermal inactivation of a mixed *C. jejuni* culture inoculated into ground beef and cooked in an oven at either 375 (190°C) (A) or 425°F (218°C) (B). Detection sensitivity was limited to numbers 300/g (1.48 $\log_{10}$). Symbols: □, internal temperature; ○, surviving organisms.

cally formed from the inoculated ground beef. The meatballs were placed on oven racks that were then set into an oven equilibrated at either 375 or 425°F (190 or 218°C). The internal temperatures of the cooking meatballs were monitored with thermocouples. The number of culturable *C. jejuni* per gram of meatball was determined at 0, 10, 20, and 30 min with Cary-Blair diluent (6) without agar and Campy-BAP (2) as a selective, enumerative medium. At each sampling time, a meatball was removed from the oven and blended immediately in 200 ml of the diluent with a stomacher, thus tempering and dissipating the heat so that the blended slurry had a temperature of about 30°C in about 5 s or less. The slurry was diluted and spread plated for enumeration on Campy-BAP. The diluent consisted of 9-ml FBP-supplemented brucella broth blanks. Because of the initial 1:3 dilution and application of 0.1 ml of sample, the direct spread plating of the slurry resulted in a minimum sensitivity of 30 cells per g of ground beef. The plates were incubated at 42°C for 48 h in an atmosphere of 5% O_2–10% CO_2–85% N_2 with a piece of filter paper impregnated with glycerol humectant (12). Five separate replicate trials were carried out for each oven temperature.

Cold-temperature studies. Five strains of *C. jejuni* were used in these studies. These included FRI-CF3, ATCC 29428, Beef 1, Pork 8-3, and Lamb 46-1, the last three of which were isolated from carcass swabs. For each aspect of this study, strains were individually grown in diphasic brain heart infusion agar/broth supplemented with FBP for 16 h at 42°C (4, 9). The supernatant growth of each culture was drawn off, centrifuged at 15,000 × *g* for 10 min, washed twice, and suspended in 0.1% peptone water. The cultures were then combined and enumerated as described previously (12). One milliliter of the mixed culture was added to each of 10 samples consisting of about 100 g of ground beef purchased from a retail market, and the inocula were distributed as previously described (12). These inoculated samples were placed into sterile stomacher bags, and 11-g subsamples were removed and used to determine both the initial numbers of *C. jejuni* per gram and the aerobic plate counts at 25°C (Trypticase soy agar [BBL Microbiology Systems, Cockeysville, Md.] 72 h). The remaining portions of the samples were placed in a −15°C freezer. Aerobic plate counts and *C. jejuni* plate counts were made initially and after 3, 7, and 14 days of storage at −15°C.

Strains of *C. jejuni* were grown, inoculated, and enumerated as described above, except that 5 ml of the mixed culture was inoculated into 495 g of ground beef, and after dispersal, the sample was subdivided into 100-g subsamples. To the subsamples an equal amount of Cary-Blair diluent (two subsamples), an equal amount of 0.1% peptone diluent (control), and an equal amount of 10% dimethyl sulfoxide or 10% glycerol were added to each of the subsamples from the original inoculated sample. Six replicate inoculated samples were treated with these four supplements. One inoculated subsample supplemented with Cary-Blair diluent was refrigerated at 4°C; the other was placed in a −15°C freezer along with the three remaining subsamples. These subsamples were each enumerated for *C. jejuni* plate counts on cultures and aerobic plate counts in the manner and at the time intervals described above.

RESULTS AND DISCUSSION

Cooking study. Because of the recurring recovery of *C. jejuni* from meat products, the effect of cooking temperatures on the organism in ground beef was considered important. Even when exceedingly high levels of the organism were inoculated into the ground beef, commonly employed cooking times and temperature inacti-

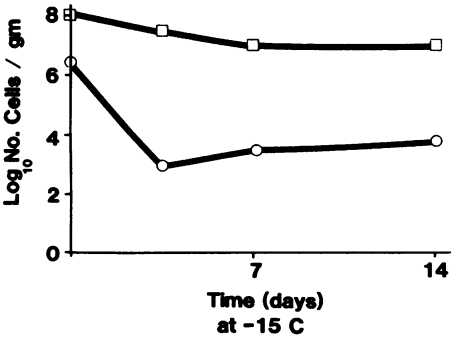

FIG. 2. Effect of storage at −15°C upon both the numbers of inoculated *C. jejuni* (○) and the aerobic plate counts (□) in ground beef.

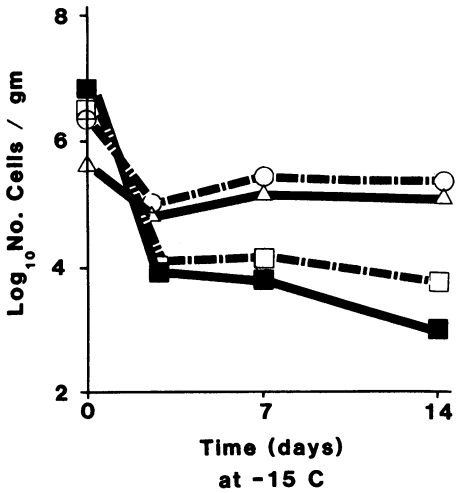

FIG. 3. Viability of *C. jejuni* inoculated into ground beef and suspended in an equal amount of four diluents before storage at −15°C. The diluents used were 10% dimethyl sulfoxide (○), 10% glycerol (△), Cary-Blair diluent (■), and 0.1% peptone (□).

vated this potential pathogen (Fig. 1). The survival curves generated at 375 and 425°F (190 and 218°C) were very similar, with a slightly more rapid increase in the internal temperature of the meatballs cooked at 425°F. At either oven temperature, complete inactivation (<30 cells per g) occurred after the internal temperature reached approximately 70°C. Thus, it appears that sanitary preparation and cooking to a temperature which will destroy other foodborne pathogens should remove the health hazards associated with *C. jejuni* indigenous to foods.

Cold-temperature studies. *C. jejuni* is highly sensitive to freezing conditions (Fig. 2). The number of organisms declined by 3 orders of magnitude after 3 days of frozen storage and, subsequently, viability remained relatively constant. In contrast, aerobic plate counts dropped slowly and consistently throughout the 14-day study, declining only 1 $\log_{10}$. The extensive reduction in the counts of *C. jejuni* stored at −15°C indicates that foods to be assessed for the pathogen should not be frozen before analysis. However, a freeze-thaw cycle has potential as a method of significantly reducing foodborne transmission of *C. jejuni*. Similar findings for reducing the numbers of *Salmonella* spp. on poultry products were presented by Olson and co-workers (7).

After the initial data were gathered and interpreted, we decided to consider methods that might prove useful for protecting the organism at freezing temperatures. Kotula and co-workers recommended the use of cryoprotective agents to improve the survival of bacteria during transport of ground beef samples (5). The viability of *C. jejuni* in frozen ground beef can be improved through the addition of either dimethyl sulfoxide or glycerol cryoprotective agents (Fig. 3). With either agent at freezing temperatures, the number of organisms declined by 1 to 2 $\log_{10}$ during the 14-day trial, about half the $\log_{10}$ decline seen with 0.1% peptone. The inspection service of the U.S. Department of Agriculture currently transports samples under freezing conditions before microbiological analysis. Therefore, the addition of dimethyl sulfoxide or glycerol could be considered before freezing foods to be assessed for the presence of *C. jejuni*. The use of Cary-Blair diluent resulted in an average initial count of 6.0×10^6 *C. jejuni* cells per g, an amount significantly ($P < 0.05$) greater than the 2.8×10^6 cells per g enumerated with 0.1% peptone diluent. The initial count of *C. jejuni* was significantly ($P < 0.05$) reduced with glycerol diluent, rather than the other diluents, was used.

When ground beef was inoculated with *C. jejuni*, diluted with Cary-Blair diluent, and then stored at either 4 or −15°C, the levels of *C. jejuni* remained constant during storage at 4°C but declined drastically during storage at −15°C (Fig. 4). This finding parallels those of Luechtefeld and co-workers (6), who used Cary-Blair diluent at refrigeration temperatures for transporting fecal specimens. Initial levels of the organism were generally high in such diarrheal samples from infected individuals, as was also the case in the present study. Although Cary-Blair diluent at 4°C maintains the viability of *C. jejuni* in foods, it should be noted that competing psychrotrophic bacteria could further proliferate and potentially make it more difficult to detect the pathogen under these storage conditions. Therefore, it is advisable to assess foods for the

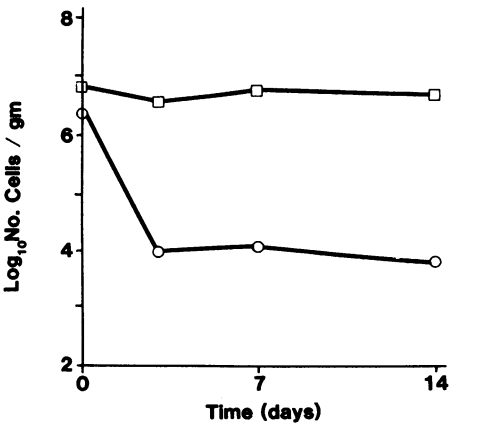

FIG. 4. Viability of *C. jejuni* inoculated into ground beef and mixed at a 1:1 ratio with Cary-Blair diluent. Samples were stored at either 4°C (□) or −15°C (○).

presence of *C. jejuni* as soon as samples arrive at the laboratory. If foods are to be analyzed for the presence of the pathogen, freezing should be avoided, or cryoprotective measures should be taken before the specimens are frozen.

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