Survival of *Campylobacter jejuni* at Different Temperatures in Broth, Beef, Chicken and Cod Supplemented with Sodium Chloride

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ABSTRACT

Growth and survival of *Campylobacter jejuni* strains ATCC 33250 and ATCC 29428 with NaCl levels ranging from 0 to 3% were studied in brucella broth at -18, 6, 10 and 42°C. Both strains grew under microaerobic conditions at 42°C with 0 to 1% NaCl, but counts declined sharply with higher salt levels. At -18°C, there was a large initial decline in counts with little subsequent change during 5 d of storage and no appreciable effect of NaCl. At 6 or 10°C, counts decreased with increasing NaCl concentration over the 5-d period, but the organism survived in substantial numbers even with 3% NaCl. Survival of *C. jejuni* in ground chicken, beef and cod with 0, 1 and 2% added NaCl was determined at -18, 6 and 10°C over a 5-d period. At -18°C, survival of *C. jejuni* was similar in all three types of flesh, whether raw or previously cooked, and *C. jejuni* counts during frozen storage were not affected by the level of NaCl. Survival patterns of *C. jejuni* at 6 and 10°C in raw and cooked chicken and beef were very similar. Statistically significant decreases in counts occurred with increasing NaCl concentration, but the differences were slight. Substantial decreases in counts occurred in refrigerated raw cod, but less in the cooked fish. In the raw cod, counts decreased significantly with increasing NaCl concentration and the decline was more pronounced during storage at 10°C than at 6°C.

Studies throughout the world have implicated *Campylobacter jejuni* as the causative agent of acute gastroenteritis in 3 to 14% of cases requiring medical attention (3). Infections have been associated with the consumption of raw milk, contaminated water, undercooked chicken, beef and pork, and raw clams (3,5).

*C. jejuni* is commensal in the intestines of sheep, pigs, cattle, goats, chickens, turkeys and wild birds (17), and the organism frequently contaminates carcasses of these animals during slaughter (12,14,18,21). The organism has been detected in fresh and frozen chicken and turkey obtained from supermarket shelves (10,16), and it is able to survive for extended periods in frozen or refrigerated meat (2,7,9,19). While *C. jejuni* is killed by ordinary cooking temperatures (2,19), cross-contamination from knives or cutting boards onto foods that are not subsequently heated may be a source of infection.

Sodium chloride is commonly added to foods, and although *C. jejuni* is unable to grow in a medium containing 3.5% NaCl (17), investigators have found that the injurious effects of NaCl on the organism are substantially reduced at lowered temperatures in brucella broth (6,8). The purpose of this study was to further examine the effects of low levels of NaCl on the survival of *C. jejuni* in broth, and to extend the studies to the food systems of ground beef, chicken and cod.

MATERIALS AND METHODS

**Strains of *C. jejuni***

*C. jejuni* strains ATCC 33250 and ATCC 29428 were obtained from the American Type Culture Collection. Cultures were maintained on brucella agar (Difco) containing 5% citrated sheep blood (BBL) and incubated at 42°C under microaerobic conditions established in a vacuum chamber by removing 3/4 of the air and replacing it with carbon dioxide. This provided an atmosphere of 5% O₂, 75% CO₂ and 20% N₂.

**Preparation of inoculum**

Inoculum was prepared by transferring isolated colonies of *C. jejuni* from streaked brucella/blood agar plates into 10.0 ml of brucella broth (Difco). Two-day-old colonies of ATCC 33250 were used for the transfer, and the broth tubes were incubated at 42°C for 48 h before use as inoculum. ATCC 29428 was slower growing, and 3-d-old colonies were transferred into the broth and allowed to incubate 72 h before inoculation.

**Preparation of brucella broth samples**

Experiments were performed to determine the effects of NaCl on the growth and survival of strains ATCC 29428 and ATCC 33250 at -18, 6, 10 and 42°C in brucella broth. Salt levels of 0, 0.5, 1, 2 and 3% were tested. Since preformulated brucella broth (Difco) contains 0.5% NaCl, the medium for these experiments was prepared without this NaCl, using the following ingredients per liter: 10 g tryptone (Difco); 10 g peptamin (Difco); 2 g yeast extract (Difco); 1 g dextrose (Difco); 0.1 g sodium bisulfite; and the appropriate amount of NaCl to produce each desired salt level.

Tubes containing 9.0 ml of the formulated broths were inoculated with 0.1 ml of *C. jejuni* broth culture. After Vortex mixing, the tubes were stored at the desired temperature, except for one tube containing no NaCl which was sampled immediately to provide initial cell counts.
For the 42°C-growth studies, loosely capped test tubes were kept under the microaerobic conditions described above. The refrigerated and frozen broth tubes for determination of survival were stored in an unmodified, aerobic atmosphere. Samples from each salt concentration were taken every 12 h for 2 d at 42°C, and every 24 h for 5 d at the other temperatures. Replicate frozen tubes were individually thawed at room temperature (ca. 30 min) before sampling to avoid repeat freeze-thaw microbial damage.

Preparation of chicken, beef and cod samples
Survival of *C. jejuni* at -18, 6 and 10°C (abusive refrigeration) was tested in both raw and cooked beef, chicken and cod with 0, 1 and 2% added NaCl. *C. jejuni* ATCC 33250 was used throughout these experiments.

Fresh ground beef, boneless chicken breasts and cod fillets were purchased from local supermarkets. The chicken and fish were ground as needed for each experiment. The added salt was thoroughly mixed into each flesh using a sterile mortar and pestle. Samples (9.0 g) of each flesh/NaCl mixture were transferred to sterile test tubes and stoppered with foam plugs. For experiments with cooked samples, sealed tubes of ground beef, chicken or fish were steamed for 20 min at 100°C and refrigerated overnight before inoculation.

At the start of each experiment, 1.0 ml of ATCC 33250 broth culture was inoculated into each 9.0-g portion of ground flesh and thoroughly distributed with a sterile spatula. The samples were then refrigerated at 6 or 10°C or frozen at -18°C under aerobic conditions, except for one tube with no added NaCl which was used to determine initial cell counts.

At 24-h intervals, tubes at each NaCl level were removed and, if frozen, allowed to thaw at room temperature for ca. 30 min. The entire 10-g contents of each tube were transferred into a sterile Waring Blender, diluted with 90 ml of sterile 0.1% peptone water, and blended at low speed until homogeneous (ca. 30 s).

*C. jejuni* counts
Serial dilutions of the brucella broth and food samples were made in 0.1% peptone water, and duplicate 0.1-ml portions of three consecutive dilutions were spread plated on brucella agar containing 5% citrated sheep blood. Plates were incubated at 42°C under microaerobic conditions for 2 d in experiments with strain ATCC 33250 and for 3 d in experiments with strain ATCC 29428. Typical *Campylobacter* colonies were counted and counts were averaged. Such colonies, which were smooth, low convex, spherical, opaque, small, nonhemolytic and tannish pink, could be readily distinguished from background flora of food samples with experience and by visual and microscopic comparisons against pure cultures. Further, the 42°C-microaerobic incubation condition favored growth of *Campylobacter* over background flora and facilitated identification without the use of selective antibiotics.

Aerobic plate counts and pH determination
Aerobic plate counts were determined in the experiments with raw foods. At 24-h intervals, 0.1-ml portions of appropriate dilutions were streak-plated in duplicate on plate count agar (Difco) and incubated for 2 d at 30°C. Changes in pH in the refrigerated raw foods were determined by blending 50 g with 50 ml of distilled water and using a Corning Research pH meter.

Sodium chloride determinations
Triplicate samples of ground beef, chicken and cod were analyzed for their initial NaCl contents. AOAC method 24.010 was used for the beef and chicken, and method 18.035 was used for the cod (1).

Statistical analysis
The results of each experiment were analyzed using a two-factor analysis of variance, and 95% confidence intervals were calculated for the mean of each treatment over storage time within each experiment. All experiments were replicated and subsequent figures present typical data from at least duplicate tests.

RESULTS AND DISCUSSION

Broth studies
The effects of NaCl concentration on survival and growth of *C. jejuni* ATCC 29428 and 33250 at 42°C are shown in Figure 1. Both strains were able to grow in brucella broth with 0, 0.5 and 1% NaCl; however, rapid declines in cell counts occurred with 2 and 3% NaCl. There was no statistically significant difference in the growth of strain ATCC 33250 between 0 and 0.5% NaCl, but slightly less growth occurred with 1% NaCl. Strain ATCC 29428 grew equally well with 0, 0.5 and 1% NaCl and declined less rapidly with 2% NaCl than did ATCC 33250. Counts for strain ATCC 29428 at NaCl levels supporting growth were lower than those for strain ATCC 33250 after 48 h, reflecting its slower growth rate. These results on the growth or decline of *C. jejuni* at 42°C agree with those of Doyle and Roman (6).

The survival of *C. jejuni* ATCC 33250 at -18, 6 and 10°C in different NaCl concentrations is shown in Figure...
The organism was sensitive to the freezing process, and counts for this strain decreased by 1.5 to 2 log$_{10}$ during the first 24 h of storage at -18°C. However, during the remaining 4 d at -18°C, the decline in counts was more gradual. There were only slight differences in the survival of ATCC 33250 between 0 and 2% NaCl, but there was a significant decrease in counts with 3% NaCl. Strain ATCC 29428 behaved similarly (data not shown), although it showed a slightly smaller initial decline in counts and showed no significant differences in counts between NaCl levels after 5 d of storage at -18°C.

Survival patterns of *C. jejuni* at 6 and 10°C were very similar. At these temperatures, there were substantial declines in counts with increasing NaCl concentrations over the 5-d period. However, there was little or no difference in the survival of the organism between the 0 and 0.5% NaCl levels. Strain ATCC 29428 counts (data not shown) decreased more rapidly at the higher NaCl levels than strain ATCC 33250, but the results were otherwise similar.

These studies indicate that *C. jejuni* will survive appreciably better at low temperatures in NaCl concentrations that are lethal under conditions permitting growth of the organism (Fig. 1). Studies in brucella broth, however, may not give a good indication of what occurs in foods. Because animal products that have been implicated in cases of campylobacter enteritis are normally frozen or refrigerated, additional experiments were done to test the survival of *C. jejuni* at different levels of NaCl in foods.

**Muscle food studies**

Chicken, beef and cod were chosen because *C. jejuni* is frequently found on chicken meat, and is often present in the fecal contents of cattle. Although *C. jejuni* has not been associated with fish, raw clams were implicated in one outbreak of campylobacter enteritis and the organism has been isolated from sea water (11). NaCl concentrations of 0, 1 and 2% were added because significant differences were found between these levels in the broth studies, and they are levels that are commonly found in foods. Because both strains ATCC 33250 and ATCC 29428 gave similar results in their reactions to NaCl in the broth studies, further experiments were done using only one strain. Strain ATCC 33250 was chosen because of its more rapid growth rate and its more consistent behavior.

Initial NaCl contents of chicken, beef and cod were 0.06, 0.07, and 0.49%, respectively. The high value obtained for cod is probably due to light brining, a process used to prevent the formation of free liquid drip and one which causes the fish muscle to take up between 0.5 and 1.0% NaCl (20). In the food studies, the total NaCl concentration in cod was about 0.5% higher than the added salt concentrations of 0, 1 and 2% NaCl, whereas the NaCl levels for the chicken and beef were higher by only 0.06 and 0.07%, respectively.

Survival of *C. jejuni* in raw and cooked chicken with added NaCl stored at -18°C is illustrated in Figure 3. Very similar results were obtained in beef and cod (data not shown), and there was little variation between raw and cooked products. The largest decrease in counts occurred during the first 24 h of storage, but after this initial drop of 1 to 1.5 log$_{10}$, there was a more gradual decrease or leveling off of counts. Statistically significant differences (P<0.05) between the added NaCl levels were detected in cooked chicken and beef; however, the differences were small, with generally 0.5 log$_{10}$ variation or less between the highest and lowest levels. The remaining frozen products did not show significant differences between NaCl concentration and survival of *C. jejuni*. The initial decrease in counts during freezing was of less magnitude in the muscle foods than in brucella broth, and it has been reported that proteins or complex chemical substances have a protective effect on bacterial cells that are frozen and thawed (13).

Survival of *C. jejuni* at 6°C in beef with added NaCl is shown in Figure 4. Similar results were obtained in chicken (data not shown), and in these foods there was little difference between raw and cooked products or between storage temperatures of 6 and 10°C. There were
statistically significant differences (P<0.05) between the salt levels, which in most cases showed a decreasing survival of *C. jejuni* with increasing NaCl concentration. However, the differences were slight, and there was less than a 1-log₁₀ decrease in *C. jejuni* counts after 5 d of storage at any NaCl level. NaCl had a greater effect on aerobic plate counts than on *C. jejuni*.

The survival of *C. jejuni* in cod with added NaCl stored at 6 and 10°C is shown in Figures 5 and 6. Significant differences were apparent between the raw and cooked fish. The organism survived well in cooked cod, except for a significant decrease in counts with 2% added NaCl stored at 10°C. However, in raw cod *C. jejuni* counts decreased by 1 to 1.5 log₁₀ at 6°C and 1.75 to 2.5 log₁₀ at 10°C over the 5-d period. At 10°C, a statistically significant difference was apparent between the NaCl levels, with a greater decrease in counts as the NaCl concentration increased. At 6°C, there appeared to be less of an effect due to NaCl, although counts with 2% added NaCl were significantly lower than those with 0 or 1% added salt.

One explanation of these latter observations on raw vs. cooked cod is that *C. jejuni* may be affected by lipid hydroperoxides and other free radicals formed by autoxidation of fatty acids in the raw cod. The highly unsaturated fatty acids present in fish are extremely susceptible to autoxidation, and the rates of reactions increase with increasing temperature (14). Castell et al. (4) found that the addition of NaCl to blended cod muscle accelerated the development of oxidative rancidity. They also found that cod in which the proteins had been denatured by heat or by prolonged frozen storage was much less sensitive to the development of NaCl-catalyzed rancidity than was fresh, uncooked cod. Since *C. jejuni* is quite sensitive to hydrogen peroxide and free radicals, it would not seem unlikely that lipid hydroperoxides contributed to its poorer survival in the present raw cod systems.

Significant increases in aerobic plate counts in chicken, beef and cod during storage at 6 and 10°C did not seem to affect the survival of *C. jejuni*. There were statistically significant differences in aerobic plate counts at different NaCl levels, with higher counts occurring at lower NaCl concentrations. As for pH, there was little change in the pH of the chicken which remained close to 5.8 throughout the 5 d of storage at both 6 and 10°C at all NaCl levels. The pH of the beef dropped from about 5.8 to

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**Figure 3.** Survival of *C. jejuni* at -18°C in raw and cooked chicken with 0%, △; 1%, hexagon; and 2% added NaCl, □.

**Figure 4.** Survival of *C. jejuni* (open symbols) and growth of aerobes (closed symbols) at 6°C in ground beef with 0%, △, 1% open hexagon, closed hexagon; and 2% added NaCl, □.
5.5 at both temperatures during the 5-d period regardless of NaCl level. The beef had higher initial aerobic plate counts than the chicken, and this may have contributed to an increased production of acid in the meat. The pH of the cod usually remained fairly constant, except for a rise from 6.9 to 7.2 with 1% added NaCl and a drop from 7.0 to 6.7 with 2% added NaCl which occurred in raw cod stored at 10°C.

These studies indicate that NaCl, at moderate levels encountered in foods, can decrease survival of C. jejuni. The magnitude of the effect differed between foods, and in the systems studied was slightly greater at the abusive refrigeration temperature of 10°C than at 6°C over a 5-d period. In the same period, no differences in survival at the different NaCl levels was seen during frozen storage at -18°C.

REFERENCES


