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Campylobacter jejuni Survival in Chicken Meat as a Function of Temperature

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Recognition of *Campylobacter fetus* subsp. *jejuni* (referred to hereafter as *C. jejuni*) as an important human pathogen and its isolation from meat products indicate the need for knowledge of its survival characteristics in meats. Thermal death times (D-values) for a single strain and a five-strain composite were determined in 1% peptone and autoclaved ground chicken meat at temperatures ranging from 49 to 57°C. Survival was determined for three strains in chicken meat at 4, 23, 37, and 43°C. Survival was also determined on raw chicken drumsticks stored at 4°C in either an ambient or a CO_2 atmosphere. D-values were greater in chicken meat than in peptone in all cases. D-values in peptone for strain H-840 at 49, 51, 53, 55, and 57°C were 15.2, 4.90, 1.71, 0.64, and 0.25 min, respectively. The corresponding D-values in ground chicken meat were 20.5, 8.77, 4.85, 2.12, and 0.79 min, respectively. Similar results were obtained with a composite of five strains. When sterile ground chicken meat was inoculated with approximately 10^6 to 10^7 *C. jejuni* cells per g and stored at 37°C in an ambient atmosphere, a 1- to 2-log count increase occurred during the first 4 days, followed by a gradual decline of about 1 log during the remainder of the 17-day storage period. No growth was observed among similarly inoculated samples that were stored at 4, 23, and 43°C, but counts declined by about 1 to 2 logs at 4°C (17 days), by 2.5 to 5 logs at 23°C (17 days), and to undetectable levels at 43°C (between 10 and 16 days). Survival on raw chicken drumsticks stored at 4°C in CO_2 and in an ambient atmosphere declined by about 1.5 and 2.0 logs, respectively, during 21 days of storage. The effect of temperature on the survival of *C. jejuni* in chicken meat was similar to that reported in other natural and laboratory milieus. Ordinary cooking procedures that destroy salmonellae would be expected to destroy *C. jejuni*.

Campylobacter fetus subsp. *jejuni* (referred to hereafter as *C. jejuni*) is now well documented as an important cause of human gastroenteritis (3, 6, 8, 19, 22, 23). Evidence also indicates that the environmental reservoir of campylobacter may be large and varied and include food animals, such as chickens and swine (5, 14, 16, 21). A high incidence of *C. jejuni* has been reported among broiler chicken carcasses in a commercial slaughter plant (17), chickens from a live poultry market (12), and commercially processed turkeys (15). Undercooked poultry has been implicated as a possible source of human infection (7, 13). Furthermore, the organism has been shown to survive on chicken carcasses during refrigerated transport to a simulated retail point and during frozen storage (17).

Because chicken meat may be an important source of *C. jejuni*, we studied the thermal resistance characteristics of several strains in sterile chicken meat and in 1% peptone for comparison with other intestinal pathogens of known thermal resistance. Also, survival of several *C. jejuni* strains in sterile chicken meat during extended storage at temperatures ranging from 4 to 43°C and of one strain on raw poultry stored in air or CO_2 at 4°C was determined.

MATERIALS AND METHODS

Cultures and cultivation. The *C. jejuni* H-840 culture was obtained from N. A. Cox, Richard B. Russell Agricultural Research Center (originally obtained from R. M. Smibert, Virginia Polytechnic Institute). All other cultures were kindly supplied by Robert Weaver, Centers for Disease Control, and were originally human clinical isolates. Strains E5054, B8788, A7455, E2567, and C3692 were grown separately and then combined in approximately equal portions to form a five-strain composite for thermal death time experiments. Additionally, a nalidixic acid-resistant isolate was selected from strain B7315 for use in survival studies with raw chicken. Cultures were maintained in brucella broth (Difco Laboratories) containing 0.16% agar and supplemented with ferrous sulfate, sodium pyruvate, and sodium bisulfite (FPB) as described by George et al. (10). All strains used in this study conformed to the traits outlined by Skirrow and Benjamin (20) for classification as *C. jejuni*. These

TABLE 1. Thermal death times of several strains of *C. jejuni* in 1% peptone and autoclaved ground chicken breast meat

Medium	Death time (min) of following strain at indicated temp (°C)[a]									
	H-840					Composite[b]				
	49	51	53	55	57	49	51	53	55	57
Peptone	15.2	4.90	1.71	0.64	0.25	14.9	7.02	2.70	1.09	ND[c]
Chicken	20.5	8.77	4.85	2.12	0.79	ND	9.27	4.89	2.25	0.98

[a] Death times were determined from regression analysis of three replications.
[b] Composite of approximately equal numbers of strains E5054, B8788, A7455, E2567, and C3692.
[c] ND, Not determined.

traits included morphology and motility, oxidase positivity, catalase positivity, nitrate reduction, growth in 1% glycine, no growth in 1.5% NaCl, no growth at 25 or 30.5°C, growth at 35 and 43°C, sensitivity to 32 ppm nalidixic acid, and sensitivity to triphenyl tetrazolium chloride.

Cells used for inoculum in the various experiments were grown in Erlenmeyer flasks containing a diphasic medium system similar to that described by Simon (18) which consisted of brucella agar plus FPB base and thioglycolate broth (Difco) without agar overlay. Cultures were inoculated with a 1% inoculum from a 24-h culture and incubated for 24 h at 37°C in a reduced-oxygen atmosphere containing 5% O_2, 10% CO_2, and 85% N_2. Cells produced in this manner were in the early stationary phase. Survivors of treatments were detected by direct spread plating on brucella agar plus FPB, followed by incubation for 48 to 72 h at 37°C in a reduced-oxygen atmosphere. Brucella agar plus FPB containing 150 μg of nalidixic acid per ml was used to detect the nalidixic acid-resistant isolate from strain B7315 that was used for the survival experiment on raw chicken. All 10-fold serial dilutions were made with tubes containing 9.0 ml of 0.1% sterile peptone.

Thermal death time determination. Thermal death time in peptone was determined by quickly pipetting a 1-ml portion of the five-strain composite or strain H-840 suspension into 100 ml of sterile 1% peptone (final cell concentration, about 10^8/ml) in a magnetically stirred flask equilibrated at 49, 51, 53, 55, or 57°C ± 0.1°C in a water bath. Samples were taken periodically, serially diluted, and plated in duplicate.

For thermal death time determination in chicken meat, 4 ml of the composite or H-840 suspension was mixed with 40 g of autoclaved ground (meat grinder with 4-mm plate; Sears) chicken breast meat in a Colworth stomacher for 5 min. Samples (2 g) of the contaminated meat were weighed into small stomacher bags (12 by 18 cm) and spread out in a thin layer in the lower third of the bags. Bags were then clipped onto photo film holders, and the lower two-thirds of the bags were submerged for various times in a water bath equilibrated to the above temperatures. The temperature in the bags equilibrated with the water bath temperature within 10 s. Bags were removed from the water bath after heating for given periods, immediately mixed with 20 ml of room temperature, sterile 0.1% peptone by macerating for 2 min in a Colworth stomacher (model 80), serially diluted, and plated in duplicate. All thermal death time experiments in both peptone and chicken meat were replicated three times.

Survival of *C. jejuni* in stored, autoclaved chicken meat. Ground chicken breast meat samples (25 g) were autoclaved in 50-ml beakers covered with aluminum foil. Meat samples were surface inoculated with 0.1 ml of a 24-h culture and stored at 4, 23, 37, or 43°C in an ambient atmosphere. After storage, meat and juices were transferred to stomacher bags, mixed with 100 ml of sterile 0.1% peptone for 2 min in a stomacher (Colworth, model 400), diluted, and plated in duplicate. The minimum level of sensitivity was 10 cells per g of meat. Different strains of *C. jejuni* were tested in each of three trials.

***C. jejuni* survival on fresh chicken.** Fresh chicken drumsticks were placed in individual alcohol-flamed aluminum baking pans and inoculated by spreading 0.1 ml of a 24-h suspension (total inoculum, approximately 8 $\log_{10}$ cells) of the nalidixic acid-resistant isolate from strain B7315 over an approximate 12-cm^2 skin area with a bent glass rod. Pans with drumsticks were placed in plastic bags and stored at 4°C. Half of the bags were closed with twist-ties for ambient atmosphere storage, and the remaining bags were flushed three times by alternately inflating with CO_2 and collapsing the bags before heat sealing. After storage, 12.3-cm^2 samples of inoculated skin were excised and macerated with 20 ml of sterile 0.1% peptone in a Colworth model 80 stomacher, diluted, and plated in duplicate on brucella agar plus FPB containing 150 μg of nalidixic acid per ml. Three drumsticks from each treatment were sampled after each storage period.

RESULTS

Thermal death times (D-values) of *C. jejuni* H-840 and the five-strain composite were determined by regression analysis of survival data from three replicates for each temperature in 1% peptone and in autoclaved ground chicken breast meat (Table 1). D-values in chicken meat were greater than those in peptone and were similar for both the single strain and the composite. D-values for the composite in 1% peptone were generally greater than those for strain H-840. z-values (temperature change causing a 1-log change in thermal death time) for strain H-840 and the five-strain composite in 1% peptone were 4.62°C (8.12°F) and 5.07°C (9.12°F), respectively. z-values in sterile chicken meat were 5.91°C (10.63°F) and 6.35°C (11.43°F), respectively.

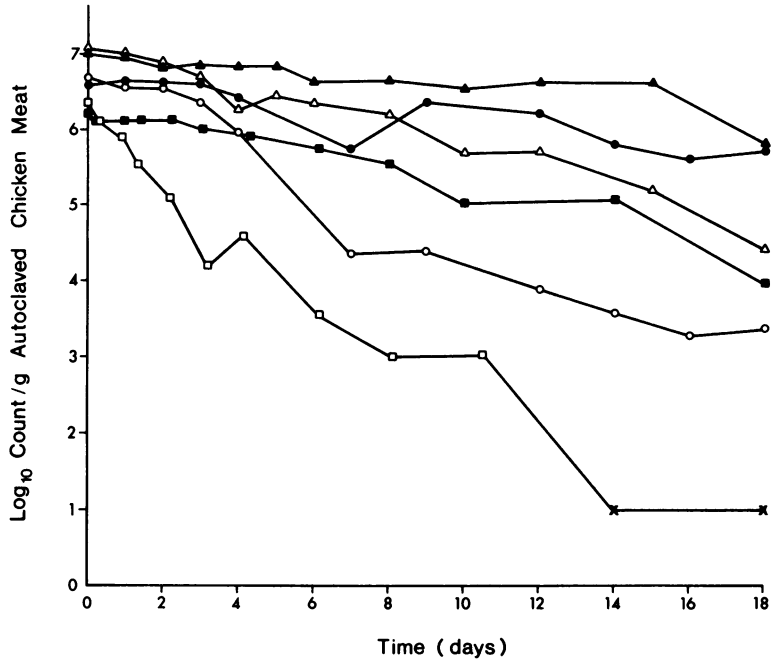

FIG. 1. Effect of temperature on survival of three strains of *C. jejuni* stored in sterile ground chicken meat in ambient atmosphere. Open symbols, 23°C storage; solid symbols, 4°C storage. (■, □) Strain H-840; (●, ○) strain B8852; (▲, △) strain B-8788; (×) count at or below minimum level of detection.

The effect of temperature on survival of three strains of *C. jejuni* stored in ground chicken meat at 4, 23, 37, and 43°C is shown in Fig. 1 and 2. Storage at 4°C resulted in a slow, gradual decline in colony-forming units of about 1 to 2 logs over 17 days, whereas at 23°C, the decline was 2.5 to 5 logs (Fig. 1). Growth (1- to 2-log colony-forming unit increase) occurred during the first 4 days of storage at 37°C, followed by a gradual 1-log decline by 17 days of storage. In contrast, no growth was observed at 43°C; instead, the colony-forming units declined to nondetectable levels between 10 and 16 days.

Survival of the nalidixic acid-resistant strain B7315 on raw chicken drumsticks stored at 4°C in a CO_2 or an air atmosphere is shown in Fig. 3. Survival was similar in both atmospheres through 21 days of storage but declined rapidly thereafter in air storage.

DISCUSSION

A comparison of z-values for *C. jejuni* strains tested in peptone (strain H-840, 5.91°C; composite strains, 5.07°C) with a similarly tested strain of *Salmonella typhimurium* (z-value, 5.58°C) (2) suggests that the organisms have similar heat resistance. Doyle and Roman (9) recently reported D-values for five strains of *C. jejuni* in milk that ranged from 1.56 to 1.95 min at 53°C and 0.74 to 1.0 min at 55°C. Our D-values (Table 1) for strain H-840 and the composite strains in chicken meat were somewhat higher at these temperatures. Differences in D-values between strain H-840 and the composite strains in peptone at 51, 53, and 55°C probably represent resistance variation among strains. Although greater heat resistance was observed in chicken meat than in peptone, the differences between the single strain and the composite strains in meat were negligible at these temperatures. Greater heat resistance of food-poisoning bacteria, such as salmonellae, in meat than in broth has been reported (11). Our results suggest that commonly practiced cooking methods that destroy salmonellae in meats would also be suitable to eliminate *C. jejuni*.

Because of possible cross-contamination of cooked food by contact with *C. jejuni*-contaminated raw product or food preparation surfaces, it was of interest to determine the growth and survival characteristics of *C. jejuni* in experimentally contaminated, sterile meat stored at several temperatures. Survival of *C. jejuni* strains stored in sterile, ground chicken meat provided some interesting contrasts. The growth and extended survival observed at 37°C with ambient-atmosphere incubation suggest that the test strains were readily able to locate favorable

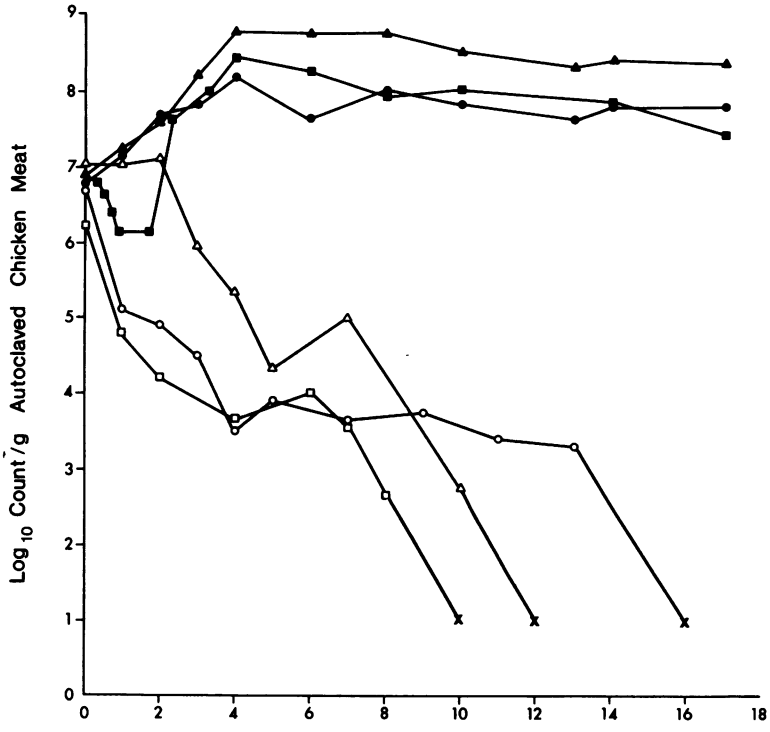

FIG. 2. Effect of temperature on survival of three strains of *C. jejuni* stored in sterile ground chicken meat in ambient atmosphere. Solid symbols, 37°C; open symbols, 43°C. (■, □) Strain H-840; (●, ○) strain B8852; (▲, △) strain B8788; (×) count at or below lower limit of detection. Numbers on the horizontal axis are the storage time in days.

microaerophilic conditions for multiplication within the ground meat after surface inoculation. Failure of the test strains to grow at 43°C in chicken meat was unexpected, since all strains grew readily at this temperature in laboratory media under microaerophilic conditions in the same incubator. The maximum incubator temperature variance was ±0.8°C. Survival characteristics of the test strains in chicken meat at 4 and 23°C were similar to those reported in other milieus, such as bile (4).

Because fresh, raw poultry has been reported to be contaminated by *C. jejuni* (12, 17), it was of interest to compare the effect of product storage atmosphere on its survival. Most fresh poultry is marketed in an air atmosphere, but some product is stored in CO_2 atmosphere, which extends shelf-life (1). The spoilage flora that develops on poultry during low-temperature air storage is predominantly *Pseudomonas* species, whereas in CO_2 storage, *Lactobacillus* species are most prevalent (1). Our results indicate that *C. jejuni* survived quite well with the spoilage flora that developed during both air and CO_2 atmosphere storage.

Estimates of the number of campylobacter

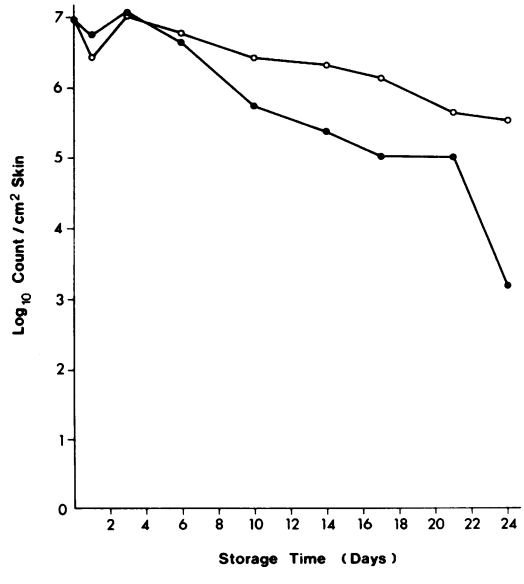

FIG. 3. Survival of *C. jejuni* B7315 on raw chicken drumsticks stored at 4°C and packaged in air (●) or CO_2 (○). Points represent averages of counts from three drumsticks.

cells commonly present on the average contaminated poultry carcass are currently not available, and the infective dose for humans is not presently well defined. Although it is apparent that customary cooking procedures can be expected to destroy contaminating campylobacter, good food-handling practice is indicated to avoid cross-contamination from contaminated raw foods to foods which are consumed without further heating.

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