

# Survival of *Campylobacter jejuni* and *Campylobacter coli* on Retail Broiler Meat Stored at $-20$ , $4$ , or $12^{\circ}\text{C}$ and Development of Weibull Models for Survival

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## ABSTRACT

Survival of *Campylobacter jejuni* and *Campylobacter coli* isolated from broiler meat was investigated and modeled on retail breast meat. Meat portions were inoculated with *C. jejuni* or *C. coli* at 6.4 to 6.8 log CFU/g followed by storage at  $-20^{\circ}\text{C}$  for 84 days or at  $4$  or  $12^{\circ}\text{C}$  for 14 days. Kinetic data within a species and temperature were fitted to the Weibull model. When  $\geq 70\%$  of the residuals were in an acceptable prediction zone from  $-1$  (fail-safe) to  $0.5$  (fail-dangerous) log units, the model was considered to have acceptable performance. Survival of *Campylobacter* was highest at  $4^{\circ}\text{C}$ , lowest at  $12^{\circ}\text{C}$ , and intermediate at  $-20^{\circ}\text{C}$ . Survival of *C. jejuni* and *C. coli* was similar at  $-20^{\circ}\text{C}$  but was lower ( $P < 0.05$ ) for *C. jejuni* than for *C. coli* at  $4$  and  $12^{\circ}\text{C}$ . The Weibull model provided acceptable predictions for four of six sets of dependent data with unacceptable performance for survival of *C. jejuni* at  $-20$  and  $12^{\circ}\text{C}$ . A difference in survival was observed between the two strains of *C. jejuni* tested. Comparison of Weibull model predictions with data for *C. jejuni* archived in ComBase revealed mostly unacceptable performance, indicating that *C. jejuni* and *C. coli* survival on raw broiler breast meat differs from published results for other strains and growth media. Variation in *Campylobacter* survival among replicate storage trials was high, indicating that performance of the models can be improved by collection of additional data to better define the survival response during storage at temperatures from  $-20$  to  $12^{\circ}\text{C}$ .

Although commercially processed broiler chickens go through a wide variety of steps during processing to reduce microbial contaminants (30), several studies have revealed that retail broiler meat is frequently contaminated with *Campylobacter* spp. (10, 31). This contamination occurs during processing when carcasses come in direct contact with fecal matter and then commingle in the chiller tank (22, 28, 42).

In an attempt to reduce contamination and improve the shelf life of broiler carcasses, rapid chilling methods have been developed by the poultry industry. In the United States, immersion chilling is the typical method used to reduce the carcass temperature. In other countries, air chilling and evaporative air chilling are more common (39). All three cooling methods are effective for rapidly reducing the temperature of the carcasses, which have similar prevalences of microbial contamination (4, 21, 26). However, El-Shibiny et al. (14) found that these methods may enhance the survival of foodborne bacterial pathogens,

including *Campylobacter*, during the shelf life of broiler meat.

Beyond the rapid chilling methods, retail broiler meat is subjected to variable freezing and refrigeration temperatures during storage, transportation, and display in retail outlets and in consumers' refrigerators. Because of the relatively high prevalence of *Campylobacter* spp. found in retail broilers and the low infective dose required to cause human disease (38), an understanding of the ability of *Campylobacter* spp. to survive refrigeration and freezing is directly relevant to designing new strategies to improve food safety and public health. The survival of *Campylobacter* spp. on broiler meat also may be an important factor for at-home contamination due to improper food handling.

Several researchers have reported the effects of refrigeration and freezing on the survival of *Campylobacter jejuni* (5, 24, 37), but all of these studies have included chicken skin as the product evaluated to determine survival. In one study, broth medium was used instead of poultry meat (7). Very few studies carried out in the last 20 years have addressed the survival of *C. jejuni* in broiler meat. Similarly, the survival of *Campylobacter coli* has been studied only in inoculated chicken skin (14). Although *C. coli* may represent 20% or more of all *Campylobacter*

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species isolated from retail broiler meat (25), there have been no studies in the last 15 years that have addressed the survival of this pathogen in retail broiler meat.

The objective of the present study was to investigate and model the survival rate of *C. jejuni* and *C. coli* isolates that were obtained from retail broilers, inoculated onto boneless, skinless broiler breast meat, and stored at 4, 12, and  $-20^{\circ}\text{C}$  for various time periods. Kinetic data within a species and temperature were fitted to the Weibull model, and performance of the models was assessed using the acceptable prediction zone (APZ) method, which classifies a model as providing acceptable predictions of the test data when  $\geq 70\%$  of the residuals fall in an APZ (29).

## MATERIALS AND METHODS

### Bacterial strains, culture conditions, and typing method.

*C. jejuni* 971 and 1065 and *C. coli* 947 and 956 were isolated from retail broiler meat and identified using described multiplex PCR assays (31). These isolates were recovered from stock cultures ( $-80^{\circ}\text{C}$  in *Brucella* broth supplemented with 30% glycerol and 5% lysed horse blood) by filtration through a 0.65- $\mu\text{m}$ -pore-size Millipore filter (Fisher Scientific, Billerica, MA) and onto modified Campy-Cefex (mCC) agar supplemented with 5% lysed horse blood (33). Cultures were incubated at  $42^{\circ}\text{C}$  for 48 h under microaerobic conditions (10%  $\text{CO}_2$ , 5%  $\text{O}_2$ , and 85%  $\text{N}_2$ ; Airgas, Radnor, PA), which were provided by an evacuation replacement system (MACSmics Jar Gassing System, Microbiology International, Frederick, MD) in anaerobic jars. All strains were typed using a pulsed-field gel electrophoresis (PFGE) protocol described elsewhere (32). During the trials, isolates were collected at the initial, middle, and final sampling points and were typed using the same PFGE protocol.

**Retail broiler meat and inoculum preparation.** Boneless, skinless broiler breast meat was purchased from a local retail store. The meat was aseptically cut into 30-g ( $\pm 1$  g) pieces and grouped into runs consisting of 16 pieces. Meat samples (16 pieces) were spread onto sanitized trays and allowed to dry in a biological safety II laminar flow cabinet for 20 min. Inocula were prepared using colonies grown on mCC agar plates for 24 h at  $42^{\circ}\text{C}$  under microaerobic conditions and then dissolved into 4.5 ml of phosphate-buffered saline (PBS). Suspension concentrations were standardized to optical densities of 1.5 ( $\pm 0.2$ ) at 600 nm and transferred into sanitized spray bottles. The inoculum was supplemented with 15.5 ml of sterile PBS to obtain a final volume of 20 ml, with a final level of approximately 7 log CFU/ml. The inoculum level was checked for each strain and for each replicate for each temperature.

Meat samples were evenly inoculated on all sides until the inoculum was exhausted, allowed to dry in a biological hood for 60 min, and then transferred to individual Ziploc freezer bags (Glad Products Company, Oakland, CA), which were stored at  $4^{\circ}\text{C}$ .

**Survival experiments.** Samples stored at 4 and  $12^{\circ}\text{C}$  were placed in an MIR 252 incubator (Sanyo North America Corporation, San Diego, CA), and two samples were removed from each trial for enumeration at day 0 and every 2 days for up to 14 days. Samples stored at  $-20^{\circ}\text{C}$  were placed in a freezer (Thermo-Kool, Laurel, MS). Two samples were then removed from each run for enumeration at day 0 and every 14 days for 84 days. Samples removed from  $-20^{\circ}\text{C}$  storage were allowed to thaw at room temperature ( $\sim 25^{\circ}\text{C}$ ) for 1 h. All samples were then

aseptically transferred to individual sterile plastic bags (Whirl-Pak, Nasco, Fort Atkinson, WI) and stomached for 1 min in a 1:2 (wt/vol) meat:broth ratio of Bolton broth supplemented with 5% lysed horse blood.

**Bacterial counts.** Surviving *Campylobacter* cells were enumerated by direct plating. Samples were serially diluted in sterile PBS (1:9) and spread plated on mCC agar in duplicate. The average of two duplicate plates and the average of two samples (two pieces of meat) were used to calculate the surviving number of cells per replicate. Enrichment samples and plates were incubated at  $42^{\circ}\text{C}$  under microaerobic conditions for 48 h. For enumeration by direct plating, CFUs for the last countable spread plate were recorded. If the enriched sample was positive and no *Campylobacter* colonies were found during enumeration, a value of 10 CFU/g of meat was assigned for that sample. For survival at  $-20^{\circ}\text{C}$ , three replicate experiments were run with *C. jejuni* 1065, and three replicates were run with *C. coli* 947. For survival at  $4^{\circ}\text{C}$ , three and one replicate experiments were run with *C. jejuni* 971 and 1065, respectively, and three and one replicate experiments were run with *C. coli* 947 and 956, respectively. For survival at  $12^{\circ}\text{C}$ , three and one replicate experiments were run with *C. jejuni* 1065 and 971, respectively, and three and one replicate experiments were run with *C. coli* 947 and 956, respectively. The time 0 count was determined right before placing the meat at the test temperature and right after taking the meat from a 24-h storage at  $4^{\circ}\text{C}$ .

**Typing of bacterial strains.** PFGE patterns of the inoculated strain and the strains surviving at the end of the experiment were compared. PFGE was performed as described elsewhere (32). *Salmonella* Choleraesuis Braenderup H9812 (ATCC BAA-664) cut with the restriction enzyme *Xba*I was used as the DNA size marker (1). Pair comparisons and cluster analyses were performed using the Dice correlation coefficient and the unweighted pair group with mathematical average clustering algorithm. The position tolerance for band analysis was set at 3%, and a cutoff of 90% DNA relatedness was used to determine whether isolates were similar.

**Survival modeling.** The GInaFit program was used to identify an appropriate survival model for the data (16). This program allowed comparison of 10 microbial survival models for their goodness of fit to the data. After this evaluation (results not shown), replicate data ( $n = 24$ ; eight samples for each of three storage trials) for survival of *C. jejuni* or *C. coli* on raw chicken breast meat stored at  $-20^{\circ}\text{C}$  for 0 to 84 days,  $4^{\circ}\text{C}$  for 0 to 14 days, or  $12^{\circ}\text{C}$  for 0 to 14 days were fitted to the Weibull model using version 5.0 of the Prism software program (GraphPad Software, Inc., San Diego, CA):

$$\Delta(t) = -\left(\frac{t}{\delta}\right)^p$$

where  $\Delta(t)$  is the log change of *C. jejuni* or *C. coli* counts at time  $t$  (days),  $\delta$  is the storage time (days) for the first log reduction, and  $p$  is the shape parameter (8). Six Weibull models were developed: model 1, *C. jejuni* 1065 at  $-20^{\circ}\text{C}$ ; model 2, *C. coli* 947 at  $-20^{\circ}\text{C}$ ; model 3, *C. jejuni* 971 at  $4^{\circ}\text{C}$ ; model 4, *C. coli* 947 at  $4^{\circ}\text{C}$ ; model 5, *C. jejuni* 1065 at  $12^{\circ}\text{C}$ ; and model 6, *C. coli* 947 at  $12^{\circ}\text{C}$ .

Performance of the six Weibull models was evaluated against dependent and independent data using the APZ method (29). This method simultaneously assesses prediction bias and accuracy and in its evaluation of predictive model performance considers that model predictions can err more in the "fail-safe" direction than in the "fail-dangerous" direction when they are used to predict food

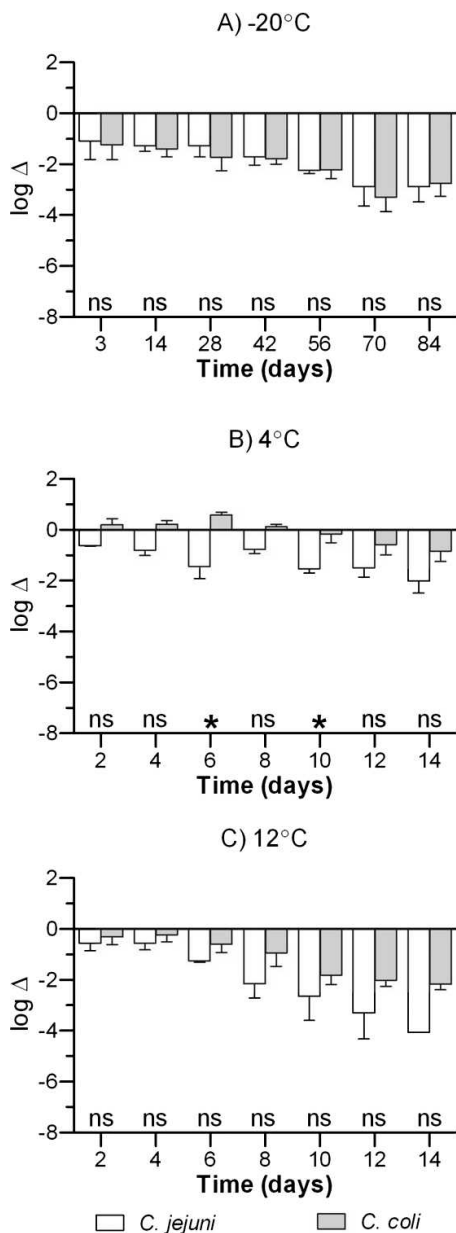


FIGURE 1. Results of the Bonferroni posttest for comparing means within storage times among *Campylobacter jejuni* and *C. coli* strains at three temperatures. ns, no difference in means; \*, difference in means at  $P < 0.05$ .

TABLE 1. Results of two-way analysis of variance for effects of species and time on survival of *Campylobacter* on raw chicken breast meat stored at three temperatures

Temp (°C)	Source of variation	df	Sum of squares	Mean square	F	P value
-20	Interaction	6	0.41	0.06	0.09	0.9961
	Species	1	0.26	0.26	0.37	0.5475
	Time	6	20.25	3.37	4.76	0.0018
	Residual	28	19.82	0.70		
4	Interaction	6	1.53	0.25	1.00	0.4441
	Species	1	13.54	13.54	53.16	<0.0001
	Time	6	7.36	1.22	4.81	0.0020
	Residual	26	6.62	0.25		
12	Interaction	6	2.27	0.37	0.48	0.8153
	Species	1	7.56	7.56	9.59	0.0048
	Time	6	35.13	5.85	7.43	0.0001
	Residual	25	19.7	0.78		

safety. The performance factor for this APZ method is the percentage of residuals (observed minus predicted values) that fall within an APZ from  $-1$  (fail-safe) to  $0.5$  (fail-dangerous) log units. When  $\geq 70\%$  of the residuals are in the APZ, the model is considered to provide acceptable predictions of the test data.

Independent data for other strains of *C. jejuni* and *C. coli* were collected in this study using the same methods as used to collect the dependent data used to develop the Weibull models. Independent data for survival of *C. jejuni* in other media and for other strains and experimental methods were retrieved from ComBase (2) using search criteria that limited the records to the pH range of 5 to 7. No data for *C. coli* were recovered in this search of ComBase.

**Statistical analysis.** Within each temperature, effects of *Campylobacter* species, time, and their interaction on  $\Delta$  were evaluated with a two-way analysis of variance (ANOVA) using the Prism software program. When a significant effect ( $P < 0.05$ ) of species was observed, means among species within storage times were compared using Bonferroni's posttest.

## RESULTS

### Survival of *C. jejuni* and *C. coli* at $-20$ , $4$ , and $12$ °C.

Survival of *Campylobacter* was highest at  $4$ °C, lowest at  $12$ °C, and intermediate at  $-20$ °C (Fig. 1). Storage of inoculated raw chicken breast meat at  $-20$ °C for 84 days resulted in reductions of  $2.88 \pm 0.59$  and  $2.75 \pm 0.51$  log CFU/g for *C. jejuni* and *C. coli*, respectively (Fig. 1A). Storage of inoculated meat at  $4$ °C for 14 days resulted in reductions of  $0.83 \pm 0.41$  and  $2.01 \pm 0.48$  log CFU/g for *C. coli* and *C. jejuni*, respectively (Fig. 1B). The reductions when meat was stored at  $12$ °C for 14 days were  $2.02 \pm 0.25$  and  $4.04 \pm 0$  log CFU/g for *C. coli* and *C. jejuni*, respectively (Fig. 1C). The two-way ANOVA indicated that survival of *C. jejuni* and *C. coli* was similar at  $-20$ °C but different at  $4$  and  $12$ °C (Table 1). Bonferroni's posttest indicated that survival was lower ( $P < 0.05$ ) for *C. jejuni* than *C. coli* at 6 and 10 days of storage at  $4$ °C (Fig. 1B).

**Typing of bacterial strains.** PFGE typing of the inoculated strain and the strains collected at the end of the experiments confirmed that the inoculated strain was the strain recovered at the end of the experiments (data not shown).

TABLE 2. Weibull model parameters for survival of *Campylobacter jejuni* (Cj) or *C. coli* (Cc) on raw chicken breast meat stored at three temperatures

Model	Strain	Temp (°C)	$\delta$ (days)		$p$		95% PI <sup>a</sup>
			Best fit	SE	Best fit	SE	
1	Cj 1065	-20	12.240	5.447	0.546	0.152	3.294
2	Cc 947	-20	8.145	4.136	0.461	0.120	3.168
3	Cj 971	4	5.326	1.217	0.614	0.193	2.080
4	Cc 947	4	14.300	0.915	5.121	3.322	1.954
5	Cj 1065	12	4.753	1.069	1.303	0.346	3.776
6	Cc 947	12	7.605	0.823	1.407	0.312	2.152

<sup>a</sup> PI, prediction interval.

**Survival modeling.** Variations in the survival of *C. jejuni* and *C. coli* among replicate storage trials were observed at all three storage temperatures. The 95% prediction interval, which quantified this variation, ranged from 1.95 log CFU/g for Weibull model 4 to 3.78 log CFU/g for Weibull model 5 (Table 2). The time for a 1-log reduction (i.e.,  $\delta$ ) ranged from 4.75 days for *C. jejuni* 1065 at 12°C to 14.3 days for *C. coli* 947 at 4°C (Fig. 2D and 2E). The Weibull parameter  $p$  was <1 for models 1 through 3, indicating concave upward survival curves, and were >1

for models 4 through 6, indicating concave downward survival curves.

**Survival model performance: comparison with independent data for other species and strains.** Predictions of the Weibull models were compared with independent data collected using the same methods but other species and strains of *Campylobacter* (Table 3). These comparisons confirmed the results of the two-way ANOVA (Table 1) that survival of *C. jejuni* and *C. coli* was similar at -20°C

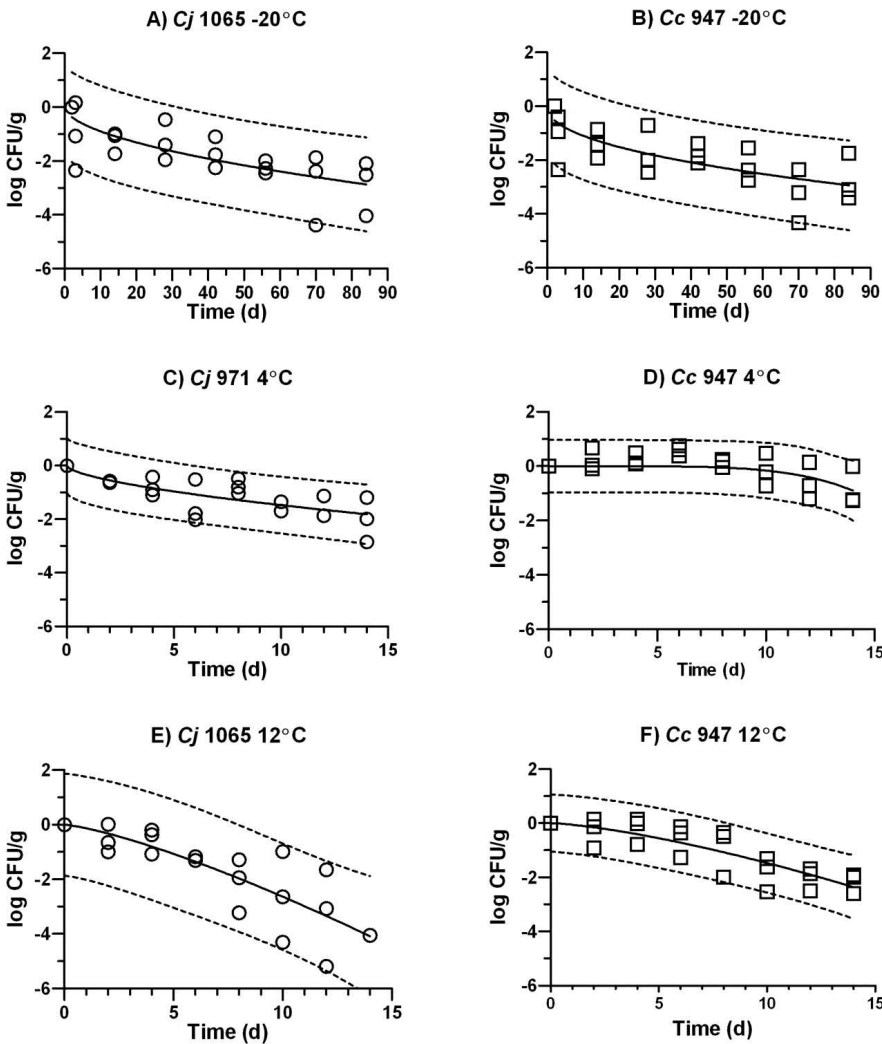


FIGURE 2. Weibull model fits (solid line) to kinetic data for survival of *Campylobacter jejuni* (Cj, ○) or *C. coli* (Cc, □) on broiler breast meat stored at -20°C (A and B), 4°C (C and D), and 12°C (E and F). Dashed lines are the 95% prediction intervals.



TABLE 3. Performance of the Weibull models for predicting survival of *Campylobacter jejuni* (Cj) or *C. coli* (Cc) on raw chicken breast meat stored at three temperatures

Data type	Model	Model strain	Test strain	Temp (°C)	n	APZ (% of residuals) <sup>a</sup>
Dependent	1	Cj 1065	Cj 1065	-20	24	67
	2	Cc 947	Cc 947	-20	24	<b>71</b>
	3	Cj 971	Cj 971	4	22	<b>77</b>
	4	Cc 947	Cc 947	4	24	<b>75</b>
	5	Cj 1065	Cj 1065	12	21	67
	6	Cc 947	Cc 947	12	24	<b>79</b>
Independent	1	Cj 1065	Cc 947	-20	24	<b>75</b>
	2	Cc 947	Cj 1065	-20	24	54
	3	Cj 971	Cc 947	4	24	21
	3	Cj 971	Cc 956	4	8	<b>88</b>
	3	Cj 971	Cj 1065	4	8	13
	4	Cc 947	Cj 971	4	22	64
	4	Cc 947	Cj 1065	4	8	<b>100</b>
	4	Cc 947	Cc 956	4	8	<b>75</b>
	5	Cj 1065	Cc 947	12	24	42
	5	Cj 1065	Cc 956	12	8	50
	5	Cj 1065	Cj 971	12	8	38
	6	Cc 947	Cj 1065	12	21	<b>71</b>
	6	Cc 947	Cc 956	12	8	<b>88</b>
	6	Cc 947	Cj 971	12	8	63

<sup>a</sup> Comparison of dependent and independent data using the acceptable prediction zone (APZ) method. Values are the percentage of residuals in the APZ from -1 (fail-safe) to 0.5 (fail-dangerous) log units. When  $\geq 70\%$  of the residuals were in the APZ, the model was classified as providing acceptable predictions (bold values) of the test data.

but differed at 4 and 12°C. These comparisons also indicated that survival of the two tested strains of *C. jejuni* differed (i.e., APZ includes <70% of residuals) at 4 and 12°C, whereas the survival of the two strains of *C. coli* evaluated was similar (i.e., APZ includes >70% of residuals).

**Survival model performance: comparison with independent data from ComBase.** Weibull model predictions were also compared with archived data from ComBase for survival of *C. jejuni* strains that were collected using other methods (Table 4). Weibull model 1 for *C. jejuni* 1065 survival at -20°C (Fig. 2A) and Weibull model 2 for *C. coli* 947 survival at -20°C (Fig. 2B) provided acceptable predictions for all three sets of survival data for *C. jejuni* on beef stored at -18 or -19°C. Weibull model 3 for *C. jejuni* 971 survival at 4°C (Fig. 2C) provided acceptable predictions for 3 of 10 sets of data for survival of *C. jejuni* in broth, 1 of 8 sets of data for survival of *C. jejuni* in milk, 2 of 6 sets of data for survival of *C. jejuni* on turkey roll, and 2 of 3 sets of data for survival of *C. jejuni* on chicken stored at 4°C. Weibull model 4 for *C. coli* 947 survival at 4°C (Fig. 2D) provided acceptable predictions for 7 of 10 sets of data for survival of *C. jejuni* in broth, 1 of 8 sets of data for survival of *C. jejuni* in milk, 3 of 6 sets of data for survival of *C. jejuni* on turkey roll, and 0 of 3 sets of data for survival of *C. jejuni* on chicken stored at 4°C. Weibull model 5 for *C. jejuni* 1065 survival at 12°C (Fig. 2E) provided acceptable predictions for none of the five sets of data for survival of *C. jejuni* in broth, whereas Weibull model 6 for *C. coli* 947 survival at 12°C (Fig. 2F) provided acceptable predictions for one of the five sets of data for survival of *C.*

*jejuni* in broth at 12°C. These results demonstrate that survival of *C. jejuni* and *C. coli* on raw chicken breast meat differs more often than it is similar to published data for *C. jejuni* survival in other studies.

**Survival model performance: comparison with dependent data.** The Weibull model provided acceptable predictions (APZ  $\geq 70\%$  of residuals) for four of the six sets of dependent data used in model development (Table 3). The Weibull models for *C. jejuni* 1065 at -20°C (i.e., model 1) and 12°C (i.e., model 5) had unacceptable performance for dependent data (APZ = 67% of residuals). Residual plots indicated the absence of systematic prediction bias for all six Weibull models (Fig. 3).

## DISCUSSION

Our first experiments were conducted to collect survival data for *C. jejuni* and *C. coli* inoculated on boneless, skinless breast meat. *C. coli* is the second most prevalent species of *Campylobacter* in retail broiler meat (25, 31, 34); therefore, survival in this food product should not be underestimated. The public health significance of *C. coli* is not completely understood. Although case-case comparison studies suggest important differences in exposures for these two *Campylobacter* species, exposures that carry a risk for infection (e.g., same food contaminated with both pathogens) may be either not identified or underestimated by case-case analysis (19). This report may be one of the first publications of survival data for *C. coli* in broiler meat with native microflora. A search of ComBase returned no archived records for *C. coli*.

TABLE 4. Comparison of Weibull model predictions to data for *Campylobacter jejuni* retrieved from ComBase<sup>a</sup>

Temp (°C)	ComBase record	Medium	$N_0$ (log CFU/g)	pH	$a_w$	Maximum time (days)	$n$	APZ (% of residuals) <sup>b</sup>		Source or reference
								$C_j$	$C_c$	
-19	M118_Cj	Beef (minced)	6.30	6		70	8	<b>100</b>	<b>100</b>	3
-19	M119_Cj	Beef (minced)	6.00	6	0.98	70	8	<b>88</b>	<b>100</b>	3
-18	M15_CJ	Beef (strip loin)	3.60	5.8		40	6	<b>100</b>	<b>100</b>	16
4	B417_14	Broth	7.66	7.1	0.97	13.9	7	<b>86</b>	<b>86</b>	FSA-IFR <sup>c</sup>
4	B417_15	Broth	7.72	6.3	0.97	13.9	6	33	<b>100</b>	FSA-IFR
4	B417_16	Broth	7.81	6.7	0.99	14	5	20	<b>80</b>	FSA-IFR
4	B417_17	Broth	7.62	6.4	0.97	14	7	29	<b>100</b>	FSA-IFR
4	B417_5	Broth	7.59	6.8	0.99	13	5	20	<b>80</b>	FSA-IFR
4	B68_CJ	Broth	6.70	6.9	0.99	14	5	40	<b>80</b>	12
4	B69_CJ	Broth	6.70	6.9	0.99	14	5	<b>80</b>	<b>100</b>	12
4	B70_CJ	Broth	6.70	6.9	0.99	14	5	<b>100</b>	60	12
4	B71_CJ	Broth	6.70	6.9	0.98	14	5	<b>100</b>	60	12
4	B72_CJ	Broth	6.70	6.9	0.97	14	5	60	40	12
4	B73_CJ	Broth	6.70	6.9	0.97	14	5	20	20	12
4	L160_CJ	Milk	7.10	6.7		9	7	29	29	11
4	L161_CJ	Milk	7.30	6.7		14	11	55	36	11
4	L162_CJ	Milk	7.20	6.7		7	5	40	40	11
4	L163_CJ	Milk	7.10	6.7		12	9	33	33	11
4	L164_CJ	Milk	7.30	6.7		14	8	38	38	11
4	L165_CJ	Milk	7.40	6.7		14	8	50	38	11
4	L166_CJ	Milk	7.10	6.7		14	13	46	38	11
4	L167_CJ	Milk	7.00	6.7		14	12	<b>100</b>	<b>75</b>	11
4	M6_CJ	Turkey roll	6.40	6.5	0.99	12	5	40	60	30
4	M7_CJ	Turkey roll	6.50	6.5	0.99	12	5	<b>80</b>	60	30
4	M8_CJ	Turkey roll	6.00	6.5	0.99	12	5	44	<b>100</b>	30
4	M9_CJ	Turkey roll	6.90	6.5	0.99	12	5	33	<b>100</b>	30
4	M10_CJ	Turkey roll	6.20	6.5	0.99	12	5	30	<b>100</b>	30
4	M11_CJ	Turkey roll	6.50	6.5	0.99	12	5	<b>80</b>	20	30
4	M29_CJ	Chicken	6.20	6		14	9	<b>80</b>	60	6
4	M30_CJ	Chicken	6.60	6		14	9	<b>80</b>	60	6
4	M31_CJ	Chicken	7.00	6		12	10	20	20	6
12	B417_1	Broth	8.25	7.3	0.99	12.7	4	50	50	FSA-IFR
12	B417_18	Broth	8.04	6.9	0.97	13.8	7	43	<b>86</b>	FSA-IFR
12	B417_20	Broth	8.23	6.4	0.97	13.8	7	29	43	FSA-IFR
12	B417_23	Broth	8.02	6.6	0.97	12.8	6	17	50	FSA-IFR
12	B417_7	Broth	8.20	5.9	0.99	13.9	6	33	33	FSA-IFR

<sup>a</sup> A combined database for predictive microbiology available at: <http://combase.arserrc.gov/BrowserHome.aspx>.

<sup>b</sup> Values are the percentage of residuals in the acceptable prediction zone (APZ) from -1 (fail-safe) to 0.5 (fail-dangerous) log units. When  $\geq 70\%$  of the residuals were in the APZ, the model was classified as providing acceptable predictions (bold values) of the test data.  $C_j$ , *C. jejuni*;  $C_c$ , *C. coli*.

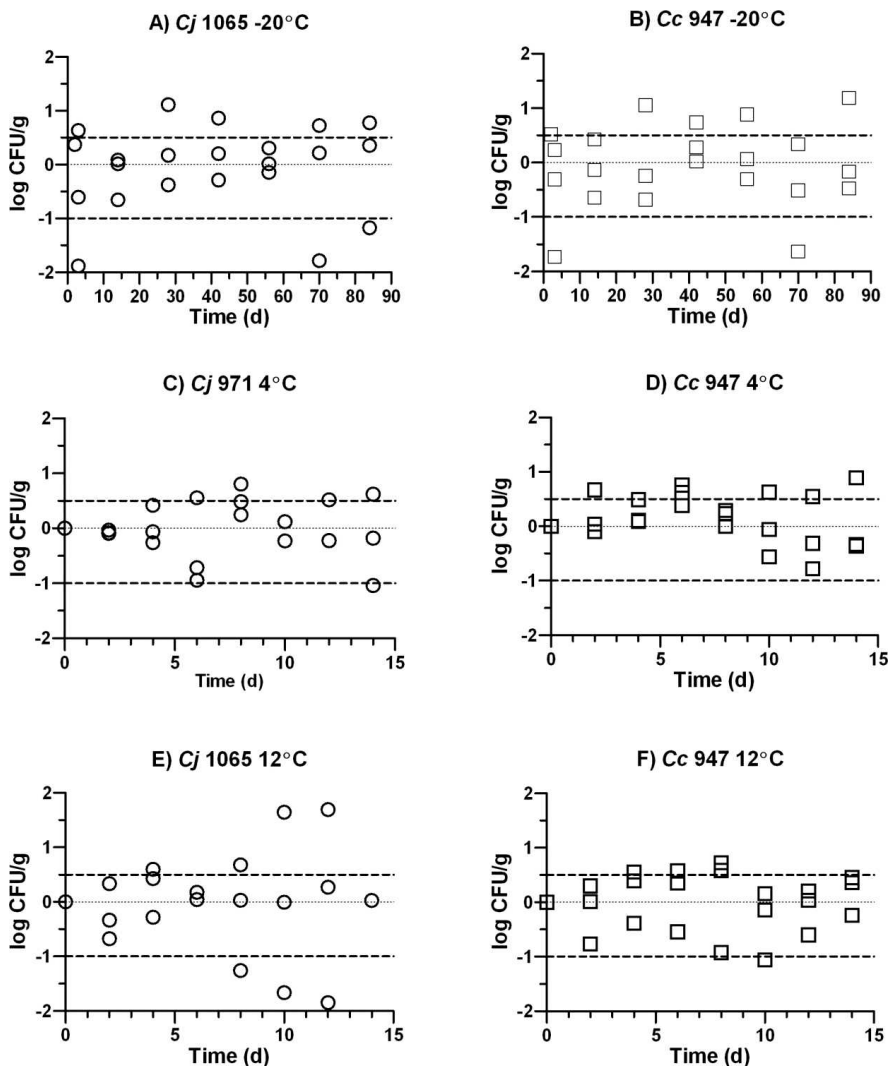
<sup>c</sup> Food Standards Agency, Institute of Food Research, Colney, UK.

Inoculated products were stored at  $-20^\circ\text{C}$  for up to 84 days or at 4 or  $12^\circ\text{C}$  for up to 14 days. We collected the time 0 counts after 24 h of refrigeration of the meat samples. Thus, the number of cells found on the meat before refrigeration was not relevant to our studies. Samples stored at  $-20^\circ\text{C}$  were thawed at room temperature because this is the traditional thawing temperature used in freezing-thawing experiments (41) and because thawing at  $7^\circ\text{C}$  did not provide any advantage for the recovery of *Campylobacter* cells in previous studies (17). Storage of frozen meat at  $4^\circ\text{C}$  increases the cell death of oxidative stress-sensitive populations and has been suggested as a means for reducing *C. jejuni* in broiler carcasses (15). We are not aware of any publication of survival data for *C. jejuni* at  $-20^\circ\text{C}$  for more than 56 days in skinless chicken meat (24) or beef (18), or of any survival

studies in which the inoculated product was held at  $12^\circ\text{C}$  for up to 14 days. Most of the products held at  $12^\circ\text{C}$  were spoiled by the end of the study, which may explain the large variation in survival of *C. jejuni* after 8 days of storage at  $12^\circ\text{C}$ . Therefore, we believe these trials represented the worst-case scenario of temperature-time abuse for these products. Data from these worst-case scenario experiments using a high, nonecological dose of *Campylobacter* cells could be used for comparison purposes and to determine the robustness of models design to predict the survival of *C. jejuni* and *C. coli* based on different cell numbers and temperatures. The data from our studies will be provided to the scientific community by submission to ComBase.

*Campylobacter* spp. in retail broiler meat are usually at low numbers (approximately 0.7 to 0.8 CFU/g of meat);

FIGURE 3. Acceptable prediction zone analysis of the performance of the Weibull models for predicting the kinetic data for survival of *Campylobacter jejuni* (Cj, ○) or *C. coli* (Cc, □) on broiler breast meat stored at  $-20^{\circ}\text{C}$  (A and B),  $4^{\circ}\text{C}$  (C and D), and  $12^{\circ}\text{C}$  (E and F). Dashed lines are the boundaries of the acceptable prediction zone, which is from  $-1$  (fail-safe) to  $0.5$  (fail-dangerous) log units.



therefore, enrichment of the samples is necessary for the isolation of the contaminating strains (31). Because our inoculation resulted in a countable number of *Campylobacter* cells per gram of meat (6 to 7 log CFU), we were confident that the isolates retrieved by direct plating were indeed the inoculated strains. We also used PFGE patterns to confirm the typing profile of the collected isolates.

Survival experiments at  $4^{\circ}\text{C}$  usually do not extend beyond 9 days (14), although a report exists for survival up to 18 days in cooked (autoclaved) meat (43) and 24 days in raw chicken drumsticks (6). We decided to test up to 14 days to extend beyond most of the published survival studies in broiler skin and meat. However, for all practical purposes any survival beyond 8 days is outside the shelf life of commercial broiler meat stored at  $4^{\circ}\text{C}$  (9). In the United States, product dating is not required by federal regulations, but stores and processors voluntarily date packages of chicken or chicken products with a “sell by” date.

Storage of inoculated meat at  $-20^{\circ}\text{C}$  for up to 84 days resulted in a reduction of less than 3 log CFU/g for *C. jejuni* and *C. coli* in meat products, with the most important reduction appearing in the first day and a relatively constant survival up to 44 days (Fig. 1). This decrease in the first 24 h of freezing also appears to be consistent with results from

experiments on chicken meat (27), chicken skin (5, 14, 24, 40), and culture media (7). Storage at  $4^{\circ}\text{C}$  for 14 days resulted in a reduction of less than 1 log CFU/g for *C. coli* and approximately 2.0 log CFU/g for *C. jejuni*. The results from the experiments at  $4^{\circ}\text{C}$  are in agreement with those in a previous report (35), whereas at  $-1.5^{\circ}\text{C}$  (13) there was no major reduction of *Campylobacter* spp. during the shelf life of the product. However, a much higher reduction was seen for *C. jejuni* than for *C. coli* when inoculated meat was stored at  $12^{\circ}\text{C}$ . The trend noticed at  $4^{\circ}\text{C}$ , at which *C. coli* survival was greater than that for *C. jejuni*, was highly amplified at  $12^{\circ}\text{C}$ .

We could not find previous reports comparing the survival rates of *C. jejuni* and *C. coli* in broiler breast meat. *C. jejuni* and *C. coli* have been reported to have similar survival rates on inoculated chicken skin. Both of these *Campylobacter* species exhibited a reduction of more than 3 log CFU when skin was stored at  $4^{\circ}\text{C}$  for 9 days (14) and a reduction of 2 log CFU or more when skin was stored at  $-20^{\circ}\text{C}$  for 7 to 9 days (5, 14, 24). However, the survival rate of *C. jejuni* on retail broiler meat appears to be different than that on chicken skin, with higher survival in raw meat during the shelf life of the product (6, 35). Although several publications have documented survival of *C. jejuni* on

chicken skin, few have included survival of *C. jejuni* in retail broiler meat, and no studies have addressed the survival of *C. coli*. The difference in the food matrix (skin versus meat) is important because the use of survival data from chicken skin may result in underestimation of *Campylobacter* survival in raw meat.

An objective of our study was to model the survival of these pathogens as a function of time at three storage temperatures. The survival kinetics of *Campylobacter* has been reported as highly variable among replicate experiments with autoclaved cooked chicken breast meat (43). This biological variation complicates curve fitting to determine survival kinetics. Thus, rather than try to fit the survival data to individual survival curves, we replicated the experiment and then combined the replicate data to facilitate curve fitting and comparisons among independent variables, such as species of *Campylobacter*.

The Weibull model provided acceptable predictions for survival of *C. jejuni* and *C. coli* on raw chicken breast meat in this study (20). These survival models developed for storage at  $-20$ ,  $4$ , and  $12^{\circ}\text{C}$  can be powerful tools for predicting the survival of *C. jejuni* and *C. coli* as log CFU per gram of chicken breast meat for all times that fall within the total duration of the study, with the exceptions of *C. jejuni* on chicken breast meat stored at  $-20$  or  $12^{\circ}\text{C}$ . After additional data are collected, these models should be modified before they can be recommended for use to predict the safety of retail broiler meat because they did not meet the criterion of acceptable performance, i.e.,  $\geq 70\%$  of residuals in an APZ from  $-1$  (fail-safe) to  $0.5$  (fail-dangerous) log units.

Comparison of predictive microbiology model predictions with independent data published in the scientific literature is complicated by the fact that the test data are often collected using other methods, media, and strains. A model's predictions cannot necessarily be extended to these different media and strains, as in the present study. A comparison of our Weibull model predictions with the independent data archived in ComBase for *C. jejuni* indicated that the survival of *C. jejuni* and *C. coli* in this study differed from the survival of other strains of *C. jejuni* in studies with broth, milk, turkey roll, and chicken. Thus, the development of models for *C. jejuni* and *C. coli* on raw chicken breast meat in this study was justified because current data archived in ComBase do not closely agree with the survival data obtained in the present study.

In this study, evidence was obtained that strain differences affected the survival of *C. jejuni*, not *C. coli*, on raw chicken breast meat. The Weibull model for *C. jejuni* 971 survival at  $4^{\circ}\text{C}$  did not provide acceptable predictions (APZ = 13% of residuals) of *C. jejuni* 1065 survival at  $4^{\circ}\text{C}$ , and the Weibull model for *C. jejuni* 1065 survival at  $12^{\circ}\text{C}$  did not provide acceptable predictions (APZ = 38% of residuals) of *C. jejuni* 971 survival at  $12^{\circ}\text{C}$ . In contrast, the Weibull models for *C. coli* 947 at  $4$  and  $12^{\circ}\text{C}$  provided acceptable predictions (APZ  $\geq 70\%$  of residuals) of *C. coli* 956 survival at  $4$  and  $12^{\circ}\text{C}$ . More research is needed to further and better define the effect of strain variation on

survival kinetics of *C. jejuni* and *C. coli* on raw chicken breast meat obtained at retail.

According to epidemiological data, a failure by the consumer to properly prepare or handle contaminated food accounts for a significant proportion of the reported foodborne diseases (36). Presently, commercial broiler processing facilities do not apply control measures that completely guarantee the elimination of these human pathogens (30). Therefore, the consumer is responsible for using proper food handling techniques. Although suggestions are given for storing meat at lower temperatures, recent data show that the actual storage temperature in household refrigerators may range from  $1$  to  $12^{\circ}\text{C}$  and that approximately 25% of domestic refrigerators may have temperatures exceeding  $10^{\circ}\text{C}$  (23). Consequently, studies of the survival of *C. jejuni* and *C. coli* at different temperatures become even more important for understanding the public health impact of these pathogens.

In summary, data and modeling results indicated that the kinetics of survival were affected by storage temperature and species of *Campylobacter*, and survival on broiler meat differed from that in published studies. Survival of *C. jejuni* and *C. coli* was similar at  $-20^{\circ}\text{C}$ , but at  $4$  and  $12^{\circ}\text{C}$ , *C. coli* had higher survival rates than did *C. jejuni*. Although the survival of *C. coli* and *C. jejuni* may be similar at freezing temperatures, the survival at refrigeration temperatures may be different. Therefore, more survival studies should be carried out with retail broiler meat to provide more accurate data for risk assessment studies aimed at improving the microbiological safety of this important food commodity.

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