DOMESTIC FOOD PRACTICES IN NEW ZEALAND

QUANTIFYING THE REDUCTION OF *CAMPYLOBACTER JEJUNI* ON SKIN-ON CHICKEN BREASTS FROZEN AND STORED FOR UP TO 10 WEEKS AT -12°C

FINAL REPORT

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by

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Client Report
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SUMMARY

This project was initiated to quantify the reduction of two *Campylobacter jejuni* strains, ST 3609 (formerly ST u48) and ST 474, on skin-on chicken breast portions frozen to a defined internal temperature of -12°C followed by frozen storage at -12°C for up to 73 days. Significant but variable reductions in *C. jejuni* numbers were observed over time for both strains, although *C. jejuni* was still detectable on samples after 73 days of storage. Overall, mean *C. jejuni* populations declined by approximately 1.2 to 2.2 log_{10} cfu over a 1 week storage period, while reductions of 3.3 and 4.1 log_{10} cfu were achieved after 34 and 73 days of frozen storage, respectively. These mean reductions were of similar magnitude to those determined previously under both simulated domestic and commercial conditions, and suggest that the longer the frozen storage period, the better the pathogen reduction achieved. While freezing has a significant impact on the reduction of *C. jejuni* on poultry, the reductions obtained in this work are likely to be the maximum possible given that *C. jejuni* survives better on muscle than on chicken skin (Ritz *et al*., 2007). The variability in pathogen reductions also demonstrates the potential inconsistency of freezing and thawing in relation to processor, consumer and pathogen factors. This information will be valuable in supporting risk management initiatives by the New Zealand Food Safety Authority to control *C. jejuni* through the food chain, and contribute additional data to ongoing pathogen risk model developments in this area.

1 INTRODUCTION

Campylobacteriosis has been the most frequently reported bacterially-mediated gastrointestinal illness in New Zealand for a number of years. In response to this public health situation, several projects commissioned by the New Zealand Food Safety Authority were undertaken to examine sources and routes of contamination, and to quantify intervention steps suitable for the reduction of *C. jejuni*, particularly on poultry. These included the effects of freezing under both domestic and commercial conditions:

- McIntyre, L., Bayne, G., Gilbert, S. & Lake, R. (2007). Domestic Food Practices in New Zealand: Freezer Survey. ESR Client Report FW0735.
- McIntyre, L. (2008a). Domestic Food Practices in New Zealand: Quantifying the reduction of *Campylobacter jejuni* on skin-on chicken breasts frozen and stored for up to 10 weeks in a domestic freezer. ESR Client Report FW0776.
- McIntyre, L. (2008b). Domestic Food Practices in New Zealand: Quantifying the reduction of *Campylobacter jejuni* on skin-on chicken breasts commercially frozen and stored for up to 10 weeks in a domestic freezer. ESR Client Report FW0799.

The laboratory-based evaluations of *C. jejuni* reduction under simulated domestic (McIntyre, 2008a) and commercial (McIntyre, 2008b) freezing conditions reported above both demonstrated significant pathogen reductions over time. Commercial freezing and short term storage (up to 28 days) at -18 to -21°C produced significantly greater mean *C. jejuni* reductions compared to domestic freezing over the same time period, but similar maximum mean reductions of *ca.* 3.5 $\log_{10}$ cfu were achieved by both processes after approximately six weeks of frozen storage. In both studies, pathogen reductions were highly variable despite the use of controlled experimental conditions, a phenomenon which has also been shown by other researchers.

In New Zealand, if poultry is to be frozen, it is a regulatory requirement to freeze it to a maximum of -12°C. This project was therefore commissioned to evaluate the reduction of *C. jejuni* under this mandated temperature. Further experiments, based on those previously conducted, were therefore designed to quantify the reduction of *C. jejuni* poultry strains ST 474 and ST 3609 on skin-on chicken breast portions frozen under conditions mimicking commercial freezing to achieve an internal temperature of -12°C.

2 MATERIALS AND METHODS

2.1 Cultures

Campylobacter jejuni strains p145a and p110b [hereafter referred to as sequence type (ST) 3609 (formerly u48) and 474 respectively] were obtained from the culture collection of Professor Nigel French, Massey University. Both were isolated from whole chicken carcasses purchased in the Manawatu region and stored long term at -80°C. Strains were streaked onto Columbia Blood Agar (CBA) and incubated in a CO_2 incubator at 37°C for 48 hours prior to sub-culture in modified Exeter broth (Wong *et al*., 2004). Prior to experimentation, 48 hour old cultures previously incubated as described in modified Exeter broth were enumerated by serial dilution (using sterile 0.1% peptone water) and spread plating on modified charcoal cephoperazone desoxycholate agar (mCCDA; Oxoid). All plates were incubated at 37°C for 48 hours in a MACS VA5000 microaerophilic work station (81% N_2:3% O_2:6% H_2:10% CO_2 gas mix) to determine total counts. Enumeration was conducted twice and counts obtained were used to calculate the extent of dilution necessary to achieve the appropriate inoculum level.

2.2 Irradiation of chicken samples

Individual skin-on chicken breast samples of similar size and weight (mean 150 g with a standard deviation of 14 g) were individually packaged in Whirlpak bags. Samples were then couriered in chilly bins to Schering-Plough Animal Health, Wellington and irradiated in bulk at 25kGy to provide *Campylobacter*-free samples for this work. Samples were returned by courier in chilly bins re-packed with ice packs and stored in a walk-in cooler prior to use.

2.3 Sample inoculation, freezing and temperature monitoring

Skins of irradiated chicken portions were surface-inoculated under contained laboratory conditions at ESR using 0.1 mL volumes of diluted suspensions of individual *C. jejuni* strains to achieve a level of ~10^5 cfu per portion. Twelve samples per strain were prepared for each sampling point excluding days 0, 34 and 73 where six samples per strain were prepared. Inoculated samples and uninoculated data logger control samples (see below) were split equally across 12 cardboard boxes under chilled conditions prior to transfer to an on-site 20 ft commercial refrigerated container (hired from Kiwi Box Ltd., Christchurch) operating at -14°C. The boxes were placed on the floor in the first half of the container in a 3 row configuration (Figure 1).

Samples were frozen for a pre-determined period of 9 hours at -14°C (data now shown) then the air temperature of the container was adjusted to -12°C to ensure that samples did not drop below an internal temperature of -14°C once fully frozen. Samples were held for a further 24 h prior to the commencement of sampling to mimic industry practice. Day 34 and day 73 samples were removed at the end of short-term storage (seven sampling days) and stored in the freezer compartment of a domestic bottom-loading fridge-freezer operating at -12°C until required.

To determine sample internal temperatures during freezing, i-button data loggers programmed to record every 6 minutes were placed into plastic bags and inserted into uninoculated chicken portions. One portion was then placed into the centre of each box. Loggers were removed from temperature control samples after Day 7 sampling. Air temperatures were also recorded throughout freezing and frozen storage using individual data loggers taped to the inside of box lids and placed in the freezer compartment of the domestic fridge-freezer. Short-term storage was monitored every 6 minutes, while longer term storage under domestic conditions was evaluated every 30 minutes. Temperature data were downloaded using OneWire software and exported to Microsoft Excel for analysis.

Figure 1: Distribution of boxes in freezer container [boxes 1-4 (top), 5-8 (middle) and 9-12 (bottom) from left to right].

2.4 Microbiological analysis

2.4.1 Verification of irradiation

A small number of irradiated chicken portions were microbiologically tested for *Campylobacter* and other microbes to confirm the effectiveness of irradiation treatment. A presence-absence Exeter broth enrichment and mCCDA plating method was used for the detection of *Campylobacter*. The rinse method employed for enumeration (see section 2.4.2) was used to determine the presence of general microbial contamination using CBA.

2.4.2 Bacterial enumeration

At daily intervals up to seven days of storage, one sample per strain was removed from a random location within each box and thawed prior to enumeration. To facilitate testing during normal working hours, samples were removed at 5 pm and thawed either overnight for approx. 17 hours in a 20°C incubator (to mimic room temperature conditions) or in a walk-in cooler (4°C) for 41 hours. Thawed samples were then rinsed with 30 mL of 0.1% peptone water and the weight of recovered rinse water (including sample drip) was recorded. A fixed volume of rinse (dependent on total recovered rinse volume) was then removed and centrifuged at 1600 *x g* for 5 minutes (Heraeus Labofuge GL) to concentrate cells prior to plating. The pellet was re-suspended in 5 mL of sterile 0.1% peptone water, diluted as required, and plated onto both mCCDA and CBA to determine *C. jejuni* counts. For samples stored short-term, 0.1 mL volumes were spread plated in duplicate while subsequent testing of samples stored for longer periods employed 1 mL volumes spread over 3 plates. All plates were incubated at 37°C for 48 hours in a CO_2 incubator prior to counting. Reduction data were calculated by subtracting day 1 to 73 plate count data from the day 0 mean counts recovered from inoculated control portions (unfrozen). Reductions were then graphed using GraphPad Prism® version 5.0 software.

2.4.3 *C. jejuni* confirmation

The identities of randomly selected presumptive *C. jejuni* colonies isolated from enumeration plates for Day 0, 7 and 73 samples were confirmed by PCR using the method published by Wong *et al*. (2004). Atypical or non-*Campylobacter* colony morphologies were noted and investigated as required.

2.5 Statistical analysis

One-way ANOVA and Tukey-Kramer multiple comparisons tests were used to investigate the significance of differences in *C. jejuni* $\log_{10}$ counts between days using SAS System V.9.1. A two-sample (unequal variance) t-test was employed to compare mCCDA and CBA counts per sampling day using Microsoft Excel. Results below detectable limits were given absolute values in order to include them in analyses. A p-value of <0.05 was taken to be statistically significant in all cases.

3 RESULTS AND DISCUSSION

3.1 Sample and air temperature profiles during freezing and frozen storage

Internal temperature profiles for 12 skin-on chicken portions frozen and stored over an eight day period in a commercial container were determined using data loggers. The freezing process employed (-14°C for 9 hr followed by -12°C) ultimately achieved internal sample temperatures ranging from -12.0 to -13.5°C, with mean air temperatures of -11.4°C to -13.1°C (Table 1). The domestic freezer used for longer term storage (34 and 73 days) operated at a mean air temperature of -12.2°C.

To determine whether box location within the container created any variability in freezing of samples, the times required for control portions in each box to reach an internal temperature of -12°C were calculated and summarised in Table 1. A fixed temperature range (from 0°C to -12°C) was chosen to facilitate comparisons. The majority of times required ranged from 11.6 h to 18.8 h, with shorter timeframes more commonly associated with boxes located closer to the fan unit (1-4) and longer timeframes for boxes located further away (9-12). Nevertheless, the freezing process was reasonably consistent across the area of the container with the possible exception of the portion in box 9 which took 18.8 h to reach a temperature of -11.5°C (within the range observed for other samples) but took a further 18.4 h to drop to -12°C. This is probably due to uneven cooling as a result of differences in air flow within the container as previously alluded to above, which resulted in slightly warmer air temperatures in boxes 9 to 12 positioned furthest away from the container fan unit (Table 1).

Table 1: Summary of times and temperatures associated with freezing and short-term frozen storage of chicken portions over an eight day time period

Logger location:	Final internal temp. (°C)	Time (hrs) required to reduce portion temp. from 0 to -12°C	Mean air temp. (°C)
Box 1	-12.5	15.8	-12.9
Box 2	-13.5	11.6	-12.6
Box 3	-12.0	18.8	-13.1
Box 4	-13.0	13.7	-12.9
Box 5	-13.0	15.7	-12.1
Box 6	-12.5	17.1	-11.9
Box 7	-12.5	15.9	-12.2
Box 8	-13.0	16.0	-12.6
Box 9	-12.0	37.2	-12.0
Box 10	-12.5	17.4	-11.6
Box 11	-12.5	18.2	-11.4
Box 12	-13.0	17.9	-11.4

3.2 Decline in *C. jejuni* numbers following freezing and frozen storage

Surface inoculation of skin-on chicken breast portions with diluted suspensions of *C. jejuni* ST 3609 and ST 474 resulted in mean starting levels of 5.99 and 5.03 $\log_{10}$ cfu respectively prior to freezing. The almost 1 log_{10} difference in cell concentration between strains was unexpected based on data from preliminary experiments, and is likely to be related to the variable nature of the pathogen under *in vitro* conditions (Ritz *et al*., 2007). Nevertheless, both strains were at or above the target inoculum level of 5.0 log_{10} cfu per portion thus sensitivity of enumeration was not compromised by this difference.

C. jejuni was enumerated using a rinse method followed by plating using both a selective (mCCDA) and non-selective (CBA) agar medium to determine whether recovery was influenced by the selective pressure of the medium. Counts obtained using CBA (Appendix 2) were consistently higher but not significantly different ($p>0.05$) to those obtained using mCCDA (Appendix 3) on each sampling day (with the exception of day 5). These findings are in general agreement with previous recovery data (McIntyre, 2008a) and with published literature (Moorhead and Dykes, 2002). Given the high degree of handling and manipulation of samples during enumeration, some contamination of rinses occurred; the remainder of this report therefore discusses *C. jejuni* reductions calculated from data obtained using mCCDA (Appendix 3). Multiplex PCR testing confirmed that randomly selected colonies from day 0, 7 and 73 mCCDA enumeration plates were *C. jejuni* (Appendix 1).

Freezing and frozen storage at -12°C significantly reduced *C. jejuni* populations on skin-on chicken breast portions on average by 1.2 to 2.2 log_{10} cfu after 1 to 7 days, and 3.3 and 4.1 log_{10} cfu after 34 and 73 days, respectively (Figure 2; Appendix 4).

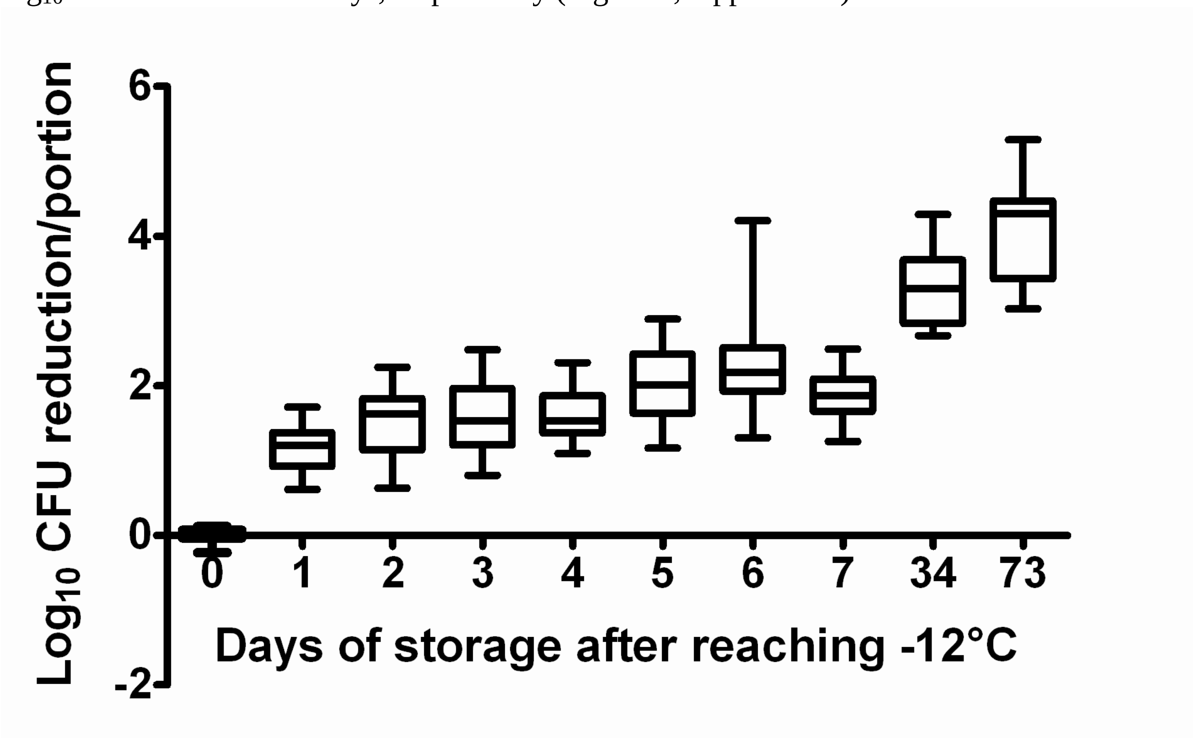


Figure 2 Overall reductions in *C. jejuni* after freezing, frozen storage (-12°C) and thawing (plating on mCCDA)

The pathogen reductions observed in this study are in general agreement with previous reports detailing reductions under domestic (-18°C) and commercial (-30°C) processing conditions (McIntyre, 2008a; McIntyre, 2008b) and published data previously reviewed (Sandberg *et al*., 2003; Zhao *et al*., 2003; Bhaduri & Cottrell, 2004; Georgsson *et al*., 2006; El-Shibiny *et al*., 2007; Ritz *et al*., 2007), but are likely to be the maximum reductions possible given that *C. jejuni* survives better on muscle than on chicken skin (Ritz *et al*., 2007). However, it should be noted that freezing and subsequent storage did not completely eliminate either of the *C. jejuni* strains, with individual levels of survival ranging from 1.2 to 2.6 log_{10} cfu for ST 3609 and 1.6 to 2.0 log_{10} cfu for ST 474 after 73 days of frozen storage.

Minimum and maximum reductions of 0.62 and >5.3 log_{10} cfu per portion were observed for ST 474 (day 1) and ST 3609 (day 73), respectively. This maximum reduction for ST 3609 is higher than observed in the previous domestic and commercial freezing studies, but the initial inoculum level used in this study was 1 log unit higher than anticipated. This suggests that higher initial contamination levels may potentially yield larger reductions during long-term frozen storage than previously determined. However, it is unknown whether this would also be the case for ST 474. The range of reductions obtained at each individual sampling time point varied from 1.1 log_{10} cfu (at day 1) to 2.9 log_{10} cfu (at day 6). It should however be noted that the maximum reduction estimated for day 6 samples (4.2 log_{10} cfu) was at least 1 log unit higher than any other individual reduction at the same time point. Nevertheless, this variability in reductions is important as it further illustrates the difficulties associated with both data comparisons and the development of predictive models for freezing and thawing of *Campylobacter* (as previously reported by Ritz *et al*., 2007).

The effect of freezing and frozen storage on reductions of the two individual *C. jejuni* strains is further detailed in Table 2. Mean reductions were consistently larger for ST 3609, but due to the difference in inoculum levels used and the variability in counts, it is impossible to determine whether this represents a possible difference in the susceptibility of the isolates to temperature stresses associated with freezing and/or thawing.

Table 2: Mean reduction of *C. jejuni* strains ST 3609 and ST 474 after freezing, frozen storage (-12°C) and thawing (plating on mCCDA)

Storage time (days)	ST 3609		ST 474	
	Reduction	SD	Reduction	SD
0	0.00	0.12	0.00	0.06
1	1.39	0.20	0.94	0.28
2	1.82	0.24	1.22	0.47
3	1.87	0.39	1.36	0.40
4	1.81	0.35	1.50	0.27
5	2.29	0.31	1.72	0.41
6	2.39	0.64	>2.07	>0.54
7	1.95	0.27	1.82	0.30
34	>3.45	>0.77	>3.22	>0.20
73	>4.35	>0.70	>3.75	>0.63

Data were further examined on the basis of thawing temperature to determine whether any differences in survival were evident (Table 3). Both strains tended to show larger reductions when samples were thawed at 4°C (versus 20°C), and at every sampling time point, the largest mean reductions in counts were observed for strain ST 3609 on chicken thawed at 4°C. As before, differences in inoculum levels and the large variability in counts preclude any further data comparisons in this regard. For each of the four strain and thawing temperature combinations investigated, Day 34 and 73 counts were statistically different to Day 1 to 7 counts (Appendix 4). However, there were no statistical differences between counts obtained on sequential days within the first week of frozen storage. This is in agreement with previous trends observed under both domestic and commercial conditions (McIntyre, 2008a; McIntyre, 2008b).

Table 3: Mean reduction of *C. jejuni* strains ST 3609 and ST 474 after freezing, frozen storage (-12°C) and thawing at either 4 or 20°C (plating on mCCDA)

Storage time (days)	ST 3609 20°C thaw/17 h		ST 3609 4°C thaw/41 h		ST 474 20°C thaw/17 h		ST 474 4°C thaw/41 h	
	Reduction	SD	Reduction	SD	Reduction	SD	Reduction	SD
0	0.00	0.12	0.00	0.12	0.00	0.06	0.00	0.06
1	1.25	0.09	1.53	0.19	0.79	0.13	1.09	0.32
2	1.77	0.23	1.87	0.26	1.39	0.37	1.06	0.52
3	1.55	0.21	2.18	0.23	1.10	0.16	1.62	0.41
4	1.76	0.43	1.86	0.29	1.39	0.11	1.61	0.35
5	2.18	0.28	2.41	0.32	1.59	0.32	1.84	0.48
6	2.24	0.28	2.54	0.88	1.82	0.43	>2.32	>0.55
7	1.90	0.28	2.00	0.27	1.80	0.18	1.85	0.41
34	2.77	0.13	>4.12	>0.28	>3.29	>0.01	>3.15	>0.29
73	4.19	0.45	>4.51	>0.96	>3.47	>0.70	>4.03	>0.52

Although *C. jejuni* has been demonstrated to survive better at 4°C than at higher temperatures (Blankenship & Craven, 1982; Thurnston Solow *et al*., 2003), this does not take into account the sequential impact of combined temperature stresses as is the situation with freezing and thawing, or the potential differences in survival between strains (On *et al*., 2006). In research published by Bhaduri and Cotttrell (2004), pre-refrigeration for 3 and 7 days prior to freezing resulted in larger reductions in *C. jejuni* populations on chicken skin than no or 1 day of pre-refrigeration (a thawing temperature was not defined). Likewise, the extended duration of thawing at 4°C (compared to 20°C) in this study may also affect the survival of *C. jejuni* following freezing. By extension, the apparently poorer survival of ST 3609 following refrigerated thawing suggests that the strain may also survive less well under longer term refrigerated conditions typically employed for display and storage of poultry in supermarkets and in the home. Larger decreases in survival over time may thus explain (in part) the observation that poultry strain ST 3609 has yet to be isolated from human cases of campylobacteriosis.

While freezing has a significant impact on the levels of *C. jejuni* on poultry, the results of this work and the previous domestic and commercial freezing projects undertaken clearly show that, even under controlled conditions, freezing is an inconsistent process subject to variability. Important factors affecting the impact of freezing include the types of chicken being processed (skin-on versus skin-off for example), different processor practices and different consumer habits regarding duration of storage of frozen foods and thawing temperature/duration. The effect of freezing and thawing on different *Campylobacter* strains remains unclear and testing of additional poultry strains under more controlled (*in vitro*) conditions would be required to confirm this.

4 CONCLUSIONS

The results of this study demonstrate that freezing poultry to an internal temperature of -12°C followed by frozen storage at -12°C for up to 73 days significantly reduces, but does not entirely eliminate, *C. jejuni* on skin-on chicken breast portions. Larger reductions following longer periods of frozen storage are consistent with data obtained previously under simulated commercial and domestic frozen storage conditions.

5 REFERENCES

Bhaduri, S. & Cottrell, B. (2004). Survival of Cold-Stressed *Campylobacter jejuni* on Ground Chicken and Chicken Skin during Frozen Storage. Applied and Environmental Microbiology, 70:7103-7109.

El-Shibiny, A., Connerton, P.L. & Connerton, I.F. (2007). Survival of *C. coli* and *C. jejuni* strains at low temperatures (4°C and -20°C). Poster presented at the 14th International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms (CHRO) 2007, Rotterdam, The Netherlands, 2-5 September.

Georgsson, F., Þorkelsson, Á.E., Geirsdóttir, Reiersen, J. & Stern, N.J. (2006). The influence of freezing and duration of storage on *Campylobacter* and indicator bacteria in broiler carcasses. Food Microbiology, 23:677-683.

McIntyre, L. (2008a). Quantifying the reduction of *Campylobacter jejuni* on skin-on chicken breasts frozen and stored for up to 10 weeks in a domestic freezer. Client Report FW0776, Christchurch Science Centre: ESR.

McIntyre, L. (2008b). Quantifying the reduction of Campylobacter jejuni on skin-on chicken breasts commercially frozen and stored for up to 10 weeks in a domestic freezer. Client Report FW0799. Christchurch Science Centre: ESR.

McIntyre, L., Bayne, G., Gilbert, S. & Lake, R. (2007). Domestic Food Practices in New Zealand – Freezer Survey. Client Report FW0735. Christchurch Science Centre: ESR.

Moorhead, S.M. & Dykes, G.A. (2002). Survival of *Campylobacter jejuni* on beef trimmings during freezing and frozen storage. Letters in Applied Microbiology, 34:72-76.

Ritz, M., Nauta, M.J., Teunis, P.F.M., van Leusden, F., Federighi, M. & Havelaar, A.H. (2007). Modelling of *Campylobacter* survival in frozen chicken meat. Journal of Applied Microbiology, 103:594-600.

Sandberg, M., Hofshagen, M., Østensvik, Ø., Skjerve, E. & Innocent, G. (2005). Survival of *Campylobacter* on frozen broiler carcasses as a function of time. Journal of Food Protection, 68:1600-1605.

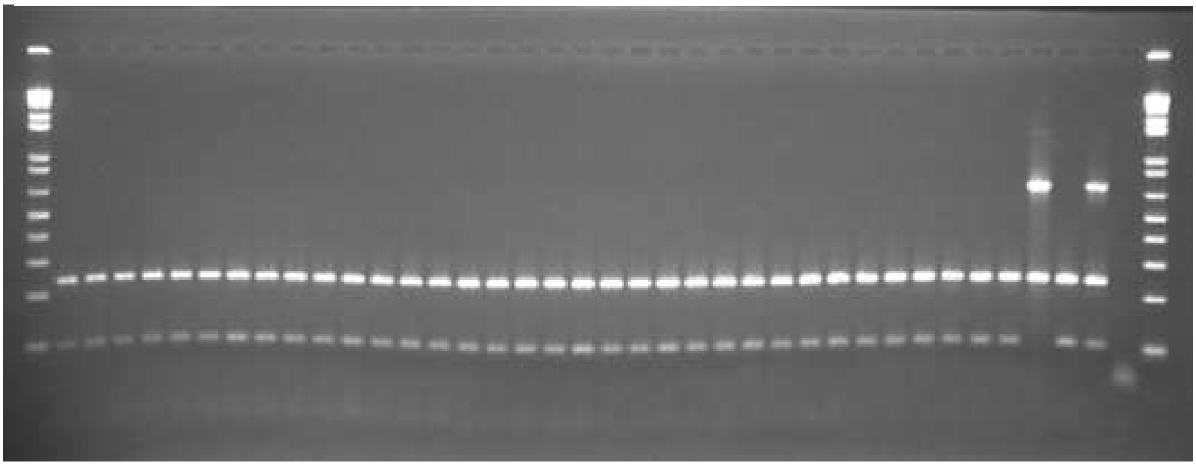
Wong, T. L., Devane, M., Hudson, J. A., Scholes, P., Savill, M. and Klena, J. (2004). Validation of a PCR method for *Campylobacter* detection on poultry packs. British Food Journal, 106:642-650.

Zhao, T., Ezeike, G.O.I., Doyle, M.P., Hung, Y-C. & Howell, R.S. (2003). Reduction of *Campylobacter jejuni* on Poultry by Low-Temperature Treatment. Journal of Food Protection, 66:652-655.

APPENDIX 1: PCR CONFIRMATION OF PRESUMPTIVE COLONIES AS *C. JEJUNI*

Lane No.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40



Gel electrophoresis of multiplex PCR products. Lanes 1 & 40: molecular weight marker (1 kb Plus DNA); Lanes 2-3: ST 3609 culture; Lanes 4-5: ST 474 culture; Lanes 6-10: ST 3609 day 0; Lanes 11-15: ST 474 day 0; Lanes 16-20: ST 3609 day 7; Lanes 21-25: ST 474 day 7; Lanes 26-30: ST 3609 day 73; Lanes 31-35: ST 474 day 73; Lane 36: *C. coli* control; Lane 37: *C. jejuni* control; Lane 38: PCR +ve; Lane 39: PCR –ve.

APPENDIX 2: RAW DATA (CBA) FOR *C. JEJUNI* ST 3609

Day	Thaw	log_{10} cfu	Reduction	Day	Thaw	log_{10} cfu	Reduction
0	NA	5.716003	0.22	0	NA	5.716003	0.22
0	NA	5.942008	0.00	0	NA	5.942008	0.00
0	NA	5.854306	0.09	0	NA	5.854306	0.09
0	NA	6.021189	-0.08	0	NA	6.021189	-0.08
0	NA	6.058805	-0.12	0	NA	6.058805	-0.12
0	NA	6.027350	-0.09	0	NA	6.027350	-0.09
1	20	4.865565	1.07	1	4	4.752048	1.19
1	20	5.058426	0.88	1	4	4.524180	1.42
1	20	4.892095	1.05	1	4	4.553074	1.39
1	20	4.886491	1.05	1	4	4.774111	1.17
1	20	3.977724	1.96	1	4	4.905796	1.03
1	20	4.721811	1.22	1	4	4.579784	1.36
2	20	4.462398	1.48	2	4	3.845098	2.09
2	20	4.123852	1.82	2	4	4.525045	1.41
2	20	NR	NR	2	4	4.000000	1.94
2	20	NR	NR	2	4	4.176091	1.76
2	20	4.537819	1.40	2	4	4.379406	1.56
2	20	NR	NR	2	4	4.230449	1.71
3	20	4.469822	1.47	3	4	3.788875	2.15
3	20	4.557641	1.38	3	4	4.222080	1.72
3	20	4.314101	1.63	3	4	3.290035	2.65
3	20	NR	NR	3	4	4.077247	1.86
3	20	4.632795	1.31	3	4	3.791457	2.15
3	20	4.770852	1.17	3	4	3.517636	2.42
4	20	4.341544	1.60	4	4	4.646077	1.29
4	20	3.764425	2.18	4	4	4.342423	1.60
4	20	4.699741	1.24	4	4	4.117271	1.82
4	20	4.703291	1.24	4	4	3.841985	2.10
4	20	4.519390	1.42	4	4	4.380211	1.56
4	20	3.728354	2.21	4	4	4.579784	1.36
5	20	3.722451	2.22	5	4	3.301030	2.64
5	20	4.434569	1.51	5	4	4.236089	1.70
5	20	3.833643	2.11	5	4	3.361728	2.58
5	20	4.062289	1.88	5	4	4.352183	1.59
5	20	NR	NR	5	4	3.689111	2.25
5	20	3.685344	2.25	5	4	3.598426	2.34
6	20	3.698970	2.24	6	4	4.642355	1.30
6	20	3.809784	2.13	6	4	4.054358	1.89
6	20	3.840942	2.10	6	4	3.672098	2.27
6	20	4.025761	1.91	6	4	4.255273	1.68
6	20	NR	NR	6	4	2.380211	3.56
6	20	4.081027	1.86	6	4	3.672098	2.27
7	20	4.093032	1.85	7	4	4.112270	1.83
7	20	3.860859	2.08	7	4	4.208173	1.73
7	20	NR	NR	7	4	4.125481	1.81
7	20	4.767156	1.17	7	4	3.991226	1.95
7	20	4.656524	1.28	7	4	3.951823	1.99
7	20	NR	NR	7	4	4.612784	1.33
34	20	NR	NR	34	4	2.311754	3.63
34	20	3.867271	2.07	34	4	1.698970	4.24
34	20	3.744554	2.20	34	4	2.823637	3.12
73	20	2.087150	3.85	73	4	1.602060	4.34
73	20	2.240549	3.70	73	4	0.698970	5.24
73	20	1.000000	4.94	73	4	2.352183	3.59

APPENDIX 2 CONT.: RAW DATA (CBA) FOR *C. JEJUNI* ST 474

Day	Thaw	log_{10} cfu	Reduction	Day	Thaw	log_{10} cfu	Reduction
0	NA	5.060698	-0.08	0	NA	5.060698	-0.08
0	NA	4.875061	0.10	0	NA	4.875061	0.10
0	NA	5.113943	-0.13	0	NA	5.113943	-0.13
0	NA	4.954243	0.03	0	NA	4.954243	0.03
0	NA	5.008600	-0.03	0	NA	5.008600	-0.03
0	NA	4.883661	0.10	0	NA	4.883661	0.10
1	20	4.322219	0.66	1	4	4.045757	0.93
1	20	4.500602	0.48	1	4	4.150927	0.83
1	20	4.271842	0.71	1	4	3.916161	1.06
1	20	4.386796	0.59	1	4	4.293240	0.69
1	20	4.882082	0.10	1	4	4.017033	0.96
1	20	4.236369	0.74	1	4	4.443002	0.54
2	20	3.977724	1.00	2	4	3.346787	1.63
2	20	3.845098	1.13	2	4	3.397940	1.58
2	20	4.161368	0.82	2	4	4.413486	0.57
2	20	4.301030	0.68	2	4	4.505150	0.47
2	20	4.021189	0.96	2	4	4.388180	0.59
2	20	3.397940	1.58	2	4	4.338014	0.64
3	20	4.000000	0.98	3	4	3.829304	1.15
3	20	4.097681	0.88	3	4	3.816241	1.16
3	20	3.832509	1.15	3	4	3.361728	1.62
3	20	4.243038	0.74	3	4	2.768391	2.21
3	20	3.929419	1.05	3	4	3.454845	1.53
3	20	4.066947	0.91	3	4	3.476316	1.50
4	20	3.804139	1.18	4	4	3.371068	1.61
4	20	3.653213	1.33	4	4	3.638489	1.34
4	20	3.978637	1.00	4	4	3.991226	0.99
4	20	3.537119	1.44	4	4	3.968483	1.01
4	20	3.623249	1.36	4	4	3.434569	1.55
4	20	3.703291	1.28	4	4	3.146128	1.83
5	20	4.856528	0.12	5	4	2.957448	2.02
5	20	4.079181	0.90	5	4	3.690196	1.29
5	20	3.977724	1.00	5	4	3.651278	1.33
5	20	3.567941	1.41	5	4	3.414973	1.57
5	20	3.792392	1.19	5	4	4.070038	0.91
5	20	3.704722	1.28	5	4	4.000000	0.98
6	20	3.329059	1.65	6	4	2.071063	2.91
6	20	3.550364	1.43	6	4	3.716003	1.26
6	20	3.097681	1.88	6	4	2.812913	2.17
6	20	3.134699	1.85	6	4	2.221849	2.76
6	20	3.967059	1.01	6	4	3.161368	1.82
6	20	3.807760	1.17	6	4	2.477121	2.50
7	20	3.530057	1.45	7	4	3.462398	1.52
7	20	3.623249	1.36	7	4	3.322219	1.66
7	20	3.778151	1.20	7	4	3.643453	1.34
7	20	3.602060	1.38	7	4	3.352183	1.63
7	20	3.123489	1.86	7	4	3.937016	1.04
7	20	3.291270	1.69	7	4	2.929419	2.05
34	20	2.230449	2.75	34	4	2.009545	2.97
34	20	<1.726999	>3.25	34	4	2.217484	2.76
34	20	1.744727	3.24	34	4	1.709694	3.27
73	20	2.161368	2.82	73	4	1.176091	3.80
73	20	2.079181	2.90	73	4	<0.698970	>4.28
73	20	<0.753328	>4.23	73	4	2.301030	2.68

APPENDIX 3: RAW DATA (MCCDA) FOR *C. JEJUNI* ST 3609

Day	Thaw	log$_{10}$ cfu	Reduction
0	NA	5.872156	0.12
0	NA	5.903090	0.09
0	NA	5.946943	0.04
0	NA	6.217484	-0.23
0	NA	5.991226	0.00
0	NA	6.027350	-0.04
1	20	4.725549	1.26
1	20	4.814839	1.18
1	20	4.863323	1.13
1	20	4.703291	1.29
1	20	4.698970	1.29
1	20	4.616651	1.37
2	20	4.230449	1.76
2	20	3.843025	2.15
2	20	4.290035	1.70
2	20	4.556303	1.43
2	20	4.146128	1.84
2	20	4.267172	1.72
3	20	4.278754	1.71
3	20	4.416825	1.57
3	20	4.167973	1.82
3	20	4.477121	1.51
3	20	4.518221	1.47
3	20	4.767156	1.22
4	20	4.141972	1.85
4	20	3.748188	2.24
4	20	4.568462	1.42
4	20	4.469822	1.52
4	20	4.726999	1.26
4	20	3.694605	2.30
5	20	3.564271	2.43
5	20	4.146954	1.84
5	20	3.725549	2.26
5	20	3.818739	2.17
5	20	4.130334	1.86
5	20	3.491828	2.50
6	20	3.531479	2.46
6	20	3.686040	2.30
6	20	3.810979	2.18
6	20	4.045757	1.94
6	20	3.364184	2.63
6	20	4.059605	1.93
7	20	3.943385	2.05
7	20	3.724276	2.27
7	20	3.851258	2.14
7	20	4.371068	1.62
7	20	4.393283	1.60
7	20	4.227315	1.76
34	20	3.263241	2.73
34	20	3.321529	2.67
34	20	3.075547	2.91
73	20	1.485090	4.50
73	20	2.322219	3.67
73	20	1.602060	4.39

Day	Thaw	log$_{10}$ cfu	Reduction
0	NA	5.872156	0.12
0	NA	5.903090	0.09
0	NA	5.946943	0.04
0	NA	6.217484	-0.23
0	NA	5.991226	0.00
0	NA	6.027350	-0.04
1	4	4.612784	1.38
1	4	4.297396	1.69
1	4	4.271067	1.72
1	4	4.434924	1.56
1	4	4.759668	1.23
1	4	4.380211	1.61
2	4	3.740363	2.25
2	4	4.431364	1.56
2	4	3.977724	2.01
2	4	4.204120	1.79
2	4	4.359203	1.63
2	4	4.000000	1.99
3	4	3.863323	2.13
3	4	4.114092	1.88
3	4	3.596597	2.39
3	4	4.003461	1.99
3	4	3.780077	2.21
3	4	3.509949	2.48
4	4	4.533179	1.46
4	4	4.118926	1.87
4	4	4.002166	1.99
4	4	3.676694	2.31
4	4	4.190332	1.80
4	4	4.278754	1.71
5	4	3.591065	2.40
5	4	3.962211	2.03
5	4	3.096910	2.89
5	4	3.892095	2.10
5	4	3.510396	2.48
5	4	3.425426	2.56
6	4	4.276206	1.71
6	4	3.765917	2.22
6	4	3.462398	2.53
6	4	3.959041	2.03
6	4	1.778151	4.21
6	4	3.439333	2.55
7	4	4.000000	1.99
7	4	4.041393	1.95
7	4	3.926857	2.06
7	4	3.809560	2.18
7	4	3.667453	2.32
7	4	4.469822	1.52
34	4	1.709694	4.28
34	4	<1.698970	>4.29
34	4	2.186815	3.80
73	4	1.176091	4.81
73	4	<0.698970	>5.29
73	4	2.556303	3.43

APPENDIX 3 CONT.: RAW DATA (MCCDA) FOR *C. JEJUNI* ST 474

Day	Thaw	$\log_{10}$ cfu	Reduction
0	NA	5.146128	-0.12
0	NA	4.995635	0.03
0	NA	5.021189	0.01
0	NA	5.023252	0.01
0	NA	5.039414	-0.01
0	NA	4.951823	0.08
1	20	4.096910	0.93
1	20	4.378196	0.65
1	20	4.200486	0.83
1	20	4.258478	0.77
1	20	4.408051	0.62
1	20	4.098067	0.93
2	20	3.698970	1.33
2	20	3.875061	1.15
2	20	3.977724	1.05
2	20	3.000000	2.03
2	20	3.875061	1.15
2	20	3.397940	1.63
3	20	3.929419	1.10
3	20	3.966402	1.06
3	20	3.832509	1.20
3	20	4.217484	0.81
3	20	3.812913	1.22
3	20	3.823909	1.21
4	20	3.769377	1.26
4	20	3.752048	1.28
4	20	3.672406	1.36
4	20	3.550907	1.48
4	20	3.568202	1.46
4	20	3.531479	1.50
5	20	3.408051	1.62
5	20	3.000000	2.03
5	20	3.161368	1.87
5	20	3.509949	1.52
5	20	3.728354	1.30
5	20	3.813359	1.22
6	20	2.982271	2.05
6	20	3.433859	1.60
6	20	2.849897	2.18
6	20	2.657577	2.37
6	20	3.720698	1.31
6	20	3.587087	1.44
7	20	3.176091	1.85
7	20	3.371068	1.66
7	20	3.397940	1.63
7	20	3.350248	1.68
7	20	3.141972	1.89
7	20	2.932248	2.10
34	20	1.753328	3.28
34	20	<1.726999	>3.30
34	20	1.744727	3.29
73	20	1.929419	3.10
73	20	2.000000	3.03
73	20	<0.753328	>4.28

Day	Thaw	$\log_{10}$ cfu	Reduction
0	NA	5.146128	-0.12
0	NA	4.995635	0.03
0	NA	5.021189	0.01
0	NA	5.023252	0.01
0	NA	5.039414	-0.01
0	NA	4.951823	0.08
1	4	3.786120	1.24
1	4	4.058173	0.97
1	4	3.372093	1.66
1	4	4.293240	0.74
1	4	4.040514	0.99
1	4	4.088727	0.94
2	4	3.221849	1.81
2	4	3.397940	1.63
2	4	4.232431	0.80
2	4	4.389166	0.64
2	4	4.357511	0.67
2	4	4.241104	0.79
3	4	3.829304	1.20
3	4	3.770852	1.26
3	4	3.556303	1.47
3	4	2.726999	2.30
3	4	3.278754	1.75
3	4	3.292256	1.74
4	4	3.217484	1.81
4	4	3.484300	1.55
4	4	3.934498	1.10
4	4	3.694605	1.34
4	4	3.158362	1.87
4	4	3.021189	2.01
5	4	2.425969	2.60
5	4	3.036984	1.99
5	4	3.329059	1.70
5	4	3.060698	1.97
5	4	3.860338	1.17
5	4	3.423246	1.61
6	4	<1.770033	>3.26
6	4	3.318063	1.71
6	4	2.977724	2.05
6	4	2.744727	2.29
6	4	3.041393	1.99
6	4	2.397940	2.63
7	4	3.230449	1.80
7	4	3.190332	1.84
7	4	3.352183	1.68
7	4	3.000000	2.03
7	4	3.774517	1.26
7	4	2.544068	2.49
34	4	<1.708515	>3.32
34	4	2.217484	2.81
34	4	<1.709694	>3.32
73	4	<0.698970	>4.33
73	4	<0.698970	>4.33
73	4	1.602060	3.43

APPENDIX 4: STATISTICAL ANALYSIS OF *C. JEJUNI* COUNT DATA

One-way ANOVA (p19) and Tukey-Kramer multiple comparisons tests (p20) were used to investigate the significance of differences in *C. jejuni* log_{10} counts between days for each of the isolate and thawing temperature combinations employed.

ST 3609 & 20°C thaw - log cfu counts by day					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	43.6835537	4.85372819	67.11	<.0001
Error	44	3.18228333	0.07232462		
Corrected Total	53	46.865837			

ST 3609 & 4°C thaw - log cfu counts by day					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	60.6730926	6.74145473	37.08	<.0001
Error	44	7.99951667	0.1818072		
Corrected Total	53	68.6726093			

ST 474 & 20°C thaw - log cfu counts by day					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	40.0952704	4.45503004	54.8	<.0001
Error	44	3.57725	0.08130114		
Corrected Total	53	43.6725204			

ST 474 & 4°C thaw - log cfu counts by day					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	47.2373926	5.24859918	30.67	<.0001
Error	44	7.52935	0.17112159		
Corrected Total	53	54.7667426			

APPENDIX 4 CONT.: STATISTICAL ANALYSIS OF *C. JEJUNI* COUNT DATA

ST 3609 & 20°C thaw - TUKEY Results: Least Squares Means for effect Day, Pr > |t| for H0: LSMean(i)=LSMean(j)

i/j	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 34	Day 73
Day 0		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Day 1			0.0532	0.6621	0.0546	<.0001	<.0001	0.0044	<.0001	<.0001
Day 2				0.9222	1	0.2301	0.0982	0.9956	0.0002	<.0001
Day 3					0.9255	0.0074	0.0022	0.411	<.0001	<.0001
Day 4						0.2255	0.0958	0.9952	0.0002	<.0001
Day 5							1	0.7676	0.0832	<.0001
Day 6								0.5064	0.1726	<.0001
Day 7									0.0016	<.0001
Day 34										<.0001

ST 3609 & 4°C thaw - TUKEY Results: Least Squares Means for effect Day, Pr > |t| for H0: LSMean(i)=LSMean(j)

i/j	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 34	Day 73
Day 0		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Day 1			0.9266	0.2331	0.9434	0.0271	0.0061	0.6587	<.0001	<.0001
Day 2				0.9588	1	0.4804	0.1968	0.9999	<.0001	<.0001
Day 3					0.9451	0.9943	0.8969	0.9992	<.0001	<.0001
Day 4						0.4416	0.1742	0.9998	<.0001	<.0001
Day 5							0.9999	0.8151	<.0001	<.0001
Day 6								0.4804	0.0002	<.0001
Day 7									<.0001	<.0001
Day 34										0.981

ST 474 & 20°C thaw - TUKEY Results: Least Squares Means for effect Day, Pr > |t| for H0: LSMean(i)=LSMean(j)

i/j	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 34	Day 73
Day 0		0.0007	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Day 1			0.0215	0.6733	0.0215	0.0005	<.0001	<.0001	<.0001	<.0001
Day 2				0.7545	1	0.9622	0.2293	0.2963	<.0001	<.0001
Day 3					0.7545	0.1103	0.0025	0.0038	<.0001	<.0001
Day 4						0.9622	0.2293	0.2963	<.0001	<.0001
Day 5							0.9184	0.9561	<.0001	<.0001
Day 6								1	<.0001	<.0001
Day 7									<.0001	<.0001
Day 34										0.9986

ST 474 & 4°C thaw - TUKEY Results: Least Squares Means for effect Day, Pr > |t| for H0: LSMean(i)=LSMean(j)

i/j	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 34	Day 73
Day 0		0.0015	0.0023	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Day 1			1	0.4597	0.4776	0.0794	0.0002	0.0719	<.0001	<.0001
Day 2				0.3742	0.3907	0.0567	0.0001	0.0511	<.0001	<.0001
Day 3					1	0.9949	0.1256	0.9929	0.0002	<.0001
Day 4						0.9936	0.1182	0.9913	0.0002	<.0001
Day 5							0.5924	1	0.002	<.0001
Day 6								0.6201	0.1574	<.0001
Day 7									0.0022	<.0001
Day 34										0.2457