



Campylobacter spp. in uncooked retail chicken meats

MAF Technical Paper No: 2011/56

Prepared for the New Zealand Food Safety Authority under project
MFSO/08/02 (revised) – Science Programme Reporting,
as part of an overall contract for scientific services.
by Dr Teck Lok Wong and Dr John Andrew Hudson

ISBN: 978-0-478-38450-5 (online)
ISSN: 2230-2794 (online)

April 2011



Ministry of Agriculture and Forestry
Te Manatū Ahuwhenua, Ngāherehere



Disclaimer

This report or document (“the Report”) is given by the Institute of Environmental Science and Research Limited (“ESR”) solely for the benefit of the New Zealand Food Safety Authority (“NZFSA”) (now part of the Ministry of Agriculture and Forestry (MAF), Public Health Services Providers and other Third Party Beneficiaries as defined in the Contract between ESR and the NZFSA, and is strictly subject to the conditions laid out in that Contract. Neither ESR nor any of its employees makes any warranty, express or implied, or assumes any legal liability or responsibility for use of the Report or its contents by any other person or organisation.

Requests for further copies should be directed to:

Publication Adviser
MAF Information Bureau
P O Box 2526
WELLINGTON

Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the MAF website at
www.foodsafety.govt.nz/elibrary

© Crown Copyright, 2011 - Ministry of Agriculture and Forestry

Acknowledgments

We gratefully acknowledge the participation of Food Act Officers from Public Health Units in the retail sampling of chicken products in Auckland, Hamilton, Wellington, Christchurch and Dunedin. We thank the technical assistants in ESR’s Public Health Laboratory in Christchurch for sample analysis. We would also like to thank Dr Beverley Horn for reviewing the data analysis and Dr Hilary Michie for editing the report. The project was funded by the New Zealand Food Safety Authority.

Client Report
FW10009

***Campylobacter* spp. in uncooked retail chicken meats**

Dr Stephen On
Food Safety Programme Leader

Dr Teck Lok Wong
Project Leader

Dr Susan Paulin
Peer Reviewer

TABLE OF CONTENTS

SUMMARY	III
1 INTRODUCTION.....	1
2 MATERIALS AND METHODS	3
2.1 Sample collection.....	3
2.2 Analytical methodology	3
3 RESULTS AND DISCUSSION	5
3.1 Prevalence	5
3.2 Quantitative data	5
3.3 Comparison of the 2009 survey with the 2003–2004 survey.....	9
3.3.1 <i>Comparison of qualitative data</i>	9
3.3.2 <i>Comparison of quantitative data</i>	11
3.3.3 <i>Comparison of enumeration methods in the two surveys</i>	14
3.4 Comparison with International Data	15
4 CONCLUSIONS	17
5 REFERENCES.....	18
APPENDIX 1 <i>Campylobacter jejuni</i> isolated and enumerated from chicken meat samples from Auckland.....	20
APPENDIX 2. <i>Campylobacter jejuni</i> isolated and enumerated from chicken meat samples from Christchurch	21
APPENDIX 3. <i>Campylobacter jejuni</i> isolated and enumerated from chicken meat samples from Dunedin.....	22
APPENDIX 4. <i>Campylobacter jejuni</i> isolated and enumerated from chicken meat samples from Hamilton	23
APPENDIX 5. <i>Campylobacter jejuni</i> isolated and enumerated from chicken meat samples from Wellington	24

INDEX OF TABLES

Table 1. Prevalence of <i>Campylobacter jejuni</i> in 25 g chicken samples.....	5
Table 2. Distribution of <i>Campylobacter jejuni</i> counts in positive chicken samples purchased from five centres in NZ.....	7
Table 3. Prevalence of <i>Campylobacter jejuni</i> and <i>C. coli</i> in uncooked retail chicken meats from the 2003-2004 survey	10
Table 4. Prevalence of <i>Campylobacter</i> spp. chicken samples obtained from the same period in both poultry surveys.....	11
Table 5. Counts of <i>Campylobacter jejuni</i> in uncooked retail chicken meats from the 2003-2004 survey	12

INDEX OF FIGURES

Figure 1. Distribution of counts for <i>Campylobacter</i> spp. in all positive chicken meat samples including counts at $<1.0 \text{ Log}_{10} \text{ CFU g}^{-1}$	6
Figure 2. Distribution of <i>Campylobacter jejuni</i> counts in chicken meat samples from the five centres.	8
Figure 3. Distribution of counts in <i>Campylobacter jejuni</i> -positive samples of chicken meat (from the 2003–2004 data set).	13
Figure 4. Comparison of the 2003/04 data set in samples of chicken meat positive for <i>Campylobacter</i> spp.with the 2009 data set.....	14

SUMMARY

One hundred and seventy-five samples of diced or minced retail chicken meat were tested for the prevalence and concentration of *Campylobacter* spp. to measure the impact of introducing the mandatory *Campylobacter* performance target (CPT) to primary broiler chicken processing on *Campylobacter* spp. levels in retail uncooked chicken meats. Samples were obtained from retail outlets in Auckland, Hamilton, Wellington, Christchurch and Dunedin using the protocol of the 2003–2004 survey. Data generated on the prevalence and concentration of *Campylobacter* spp. in the chicken meat samples were compared with those from the survey conducted in 2003–2004.

The results from the 2009 survey showed a range of *Campylobacter* prevalence values for each city, from 51.4% in Christchurch to 88.6% in Hamilton. The prevalence for *Campylobacter* spp. of 69.7% (95% CI: 62.3–76.4) was found in the current survey, while the equivalent data for the 2003–2004 survey showed a prevalence of 89.1% (95% CI: 84.4–92.8). These data demonstrate a significant reduction ($P = 0.001$, chi-square test) in *Campylobacter* spp. prevalence between the two surveys. When seasonality and duration of sampling were taken into account, a significant reduction of 16.4% ($P = 0.002$, Z test for two proportions), from 86.1% to 69.7%, in *Campylobacter* spp. prevalence was evident in the chicken products in the five-to-six year period between surveys.

In addition, a decrease in the distribution of concentration data in *Campylobacter*-positive samples was also measured in the 2009 survey, particularly the percentage of counts in the higher ranges ($>1.0 \text{ Log}_{10} \text{ CFU g}^{-1}$, $P=0.037$, chi square test). Further, despite of changing the enumeration method from an MPN method used in the 2003-2004 study to a spread plate method used in this 2009 study, the inclusion of a presence/absence test in a 25 g sample accounted for the presence of *Campylobacter* spp. in positive samples that contained counts of $<1.0 \text{ Log}_{10} \text{ CFU g}^{-1}$ by the spread plate method.

The study shows that following the introduction of the CPT in 2008 to primary broiler chicken processing, there was a significant reduction in the prevalence and concentration of *Campylobacter* spp. in positive samples of retail minced or diced chicken meats in NZ in 2009.

1 INTRODUCTION

Campylobacteriosis is the leading cause of notifiable bacterial gastrointestinal disease in New Zealand (Anonymous 2008). In 2006, the notified campylobacteriosis rate was 379.3 cases per 100,000 population, this reduced to 302.2 cases per 100,000 population in 2007 (Anonymous 2007). In 2008, there was a significant decrease in the campylobacteriosis rate ($P < 0.05$) to 156.8 cases per 100,000 population (Anonymous 2008). The six-monthly rate to December 2009 tracked at 166.2 cases per 100,000 population (Anonymous 2009).

Poultry consumption is a significant risk factor for campylobacteriosis in New Zealand (Ikram *et al.* 1994; Eberhardt-Phillips *et al.* 1997; Wilson 2007). In a survey of retail meats in 2003–2004, poultry meats were identified as having the highest prevalence of *Campylobacter* spp. (89.1%) amongst meat from four species (chicken, beef, lamb/mutton and pork) (Wong *et al.* 2007). A recent source-attribution study in the Manawatu region of New Zealand identified poultry as the source of an estimated 80% of human campylobacteriosis (Mullner *et al.* 2009). This attribution to poultry was supported by another study in 2007 in Auckland and Christchurch that showed >60% of *Campylobacter jejuni* isolates belonging to certain sequence types (STs) in retail chickens, based on multi-locus sequence typing (MLST), were also isolated from human campylobacteriosis cases (Wong *et al.* 2008a).

The reasons for the significant decrease in the campylobacteriosis rate during 2007 and 2008 have yet to be fully understood. However, it coincided with the time period in 2007 when poultry processors were either fine-tuning existing, or introducing new, interventions in primary poultry processing in preparation for the regulatory introduction of a mandatory *Campylobacter* Performance Target (CPT) in 2008. This was driven by the New Zealand Food Safety Authority (NZFSA) which has put systems in place to reduce the level of foodborne illness in New Zealand – this requires a robust understanding of the exposure to pathogens, in this case *Campylobacter*. A 50% reduction in foodborne cases of campylobacteriosis over five years is a key organisational measure (NZFSA 2008).

Since the mandatory CPT was introduced in April 2008, *Campylobacter* spp. counts on carcass rinsates at the end of primary processing have fallen (<http://www.nzfsa.govt.nz/foodborne-illness/campylobacter/strategy/campylobacter-strategy-2010-13.pdf>). In a second study on MLST genotyping of *Campylobacter* spp. isolates from human cases of campylobacteriosis and retail poultry in Christchurch conducted from February to April 2008, *Campylobacter* ST genotypes isolated from human cases were more diverse than the chicken isolates while rarefaction analysis of poultry isolates obtained in the 2007 (Wong *et al.* 2008a) and 2008 study (Wong *et al.* 2008b) showed a similar level of diversity of the poultry ST genotypes. Proportional similarity estimates of *C. jejuni* STs isolated from human cases and retail chickens in both studies showed that human isolates in 2008 were less similar to all poultry isolates compared with data for 2007.

This short survey was commissioned by NZFSA to re-estimate levels of *Campylobacter* spp. in retail uncooked chicken meats, and was undertaken between April and June 2009. The survey was initiated to measure the impact of introducing the CPT in 2008 to primary broiler chicken processing, and in view of the downward trend in *Campylobacter* spp. counts from carcass rinsates at the end of primary processing recorded in the NMD programme¹. In this report, we present data from this survey and compare the new data with those from the survey conducted in 2003–2004.

¹ Web site: <http://www.nzfsa.govt.nz/animalproducts/publications/manualsguides/nmd/natprofiles/index.htm>
(Note: Accessibility to this website is restricted to NZFSA Laboratory Approval Scheme Administrator and approved NMD registered members.)

2 MATERIALS AND METHODS

2.1 Sample collection

One hundred and seventy-five uncooked, chilled retail chicken samples that were minced, diced or cut into strips, were purchased from April–July 2009. Samples were purchased fortnightly from retail outlets in the five main cities in the North and South Islands of New Zealand – Auckland, Hamilton, Wellington, Christchurch and Dunedin. On each sampling occasion, five samples were purchased from different retail outlets within each city.

Retail outlets included butcher shops where samples were purchased across the counter, and supermarkets where the samples were selected from areas selling raw meat. Butchery samples were either taken by hand through an inverted plastic bag, or scooped up with a utensil from bulk display trays into plastic bags and then taped closed. Supermarket samples were pre-packed in plastic-wrapped polystyrene trays and were selected from open display refrigerated cabinets. At the time of purchase, samples were double-bagged to prevent surface cross-contamination.

A minimum sample amount weighing at least 300 g was purchased and held in an insulated container with frozen cooling packs to keep samples chilled at $<8^{\circ}\text{C}$ during overnight transport to the ESR laboratory in Christchurch. All samples were received within 24 h of purchase, held at 4°C in the laboratory and tested within 2 h of receipt.

2.2 Analytical methodology

The outside surfaces of the bags or meat trays were sanitised by wiping with 70% alcohol. Contents were sampled aseptically through an excised window on the plastic wrap, or after cutting off the taped portion of bags with scissors. Leftover samples were securely taped, bagged and kept at 4°C until tests were completed.

In the 2003–2004 study, samples were prepared and tested using the same procedure as described here except that a most probable number (MPN) method was used to estimate *Campylobacter* spp. concentrations. In this study, a spread plate method was used for the enumeration. To capture the lower ranges of counts, a presence/absence test in a 25 g

sample was included. The sensitivity of the presence/absence test is theoretically one CFU per 25 g or 0.04 CFU g⁻¹.

Samples were tested for the presence of *Campylobacter* spp. in 25 g of meat by enrichment in 225 mL of m-Exeter broth (Wong *et al.* 2004), and incubated at 37°C in a microaerobic environment of 10% enriched CO₂ in accordance to Fraser *et al.* (1992) for 4 h, followed by a further microaerobic incubation at 42°C for another 44 h. The enrichment broths were streaked onto modified charcoal cefoperazone desoxycholate agar (mCCDA) plates and incubated at 42°C for 48 h microaerobically. Plates were examined for the presence of presumptive *Campylobacter* spp. colonies at 48 h. When growth of typical colonies was observed on a plate after 48 h, up to five colonies were selected and streaked onto sheep blood agar plates and incubated at 42°C microaerobically for 48 h. DNA was extracted from each colony and identified by a validated multiplex polymerase chain reaction (PCR) method (Wong *et al.* 2004).

Concurrently, a spread-plating method was used to enumerate *Campylobacter* spp. A 1:10 dilution of the meat sample was prepared by homogenising 10 g of sample in 90 mL of Maximum Recovery Medium (Oxoid, Basingstoke, Hampshire, UK) for 2 min in a stomacher (Bagmixer, Interscience, St Nom, France). The homogenate was further diluted to a 10⁻² dilution. One mL of a 10⁻¹ dilution was spread evenly over three mCCDA plates. In addition, 0.1 mL samples from each dilution (10⁻¹ and 10⁻²) were inoculated and spread onto mCCDA plates (in duplicate) and incubated microaerobically for 48 h as described before. Presumptive *Campylobacter* spp. colonies showing the typical morphology were counted. Up to five colonies were selected and re-cultured on sheep blood agar plates before identification by PCR. The sensitivity of the PCR in broth culture is down to an equivalent of 57 cells of *C. jejuni* and 69 cells of *C. coli* per PCR (Wong *et al.* 2004).

3 RESULTS AND DISCUSSION

ESR has provided the NZFSA with a spreadsheet containing the raw data for this survey (dated 23/07/2009). Summaries of these data for each city are appended in Appendices 1–5. This spreadsheet was used in the analysis that follows.

All *Campylobacter* spp. were identified as *C. jejuni* by PCR (Wong *et al.* 2004).

3.1 Prevalence

The *Campylobacter* isolation data (Table 1) show a range of mean prevalence values, from 51.4% in Christchurch to 88.6% in Hamilton. The prevalence of *Campylobacter* spp. in the retail chicken meats was 69.7% (95% CI: 62.3–76.4).

Table 1. Prevalence of *Campylobacter jejuni* in 25 g chicken samples.

Area sampled	n	No. (%) Positive	95% CI
Auckland	35	26 (74.3)	56.7–87.5
Christchurch	35	18 (51.4)	34.0–68.6
Dunedin	35	20 (57.1)	39.4–73.7
Hamilton	35	31 (88.6)	73.3–96.8
Wellington	35	27 (77.1)	59.9–89.6
Total	175	122* (69.7)	62.3–76.4

*Only *C. jejuni* were identified from positive samples.

3.2 Quantitative data

The distribution of counts for the 122 positive samples is shown in [Figure 1](#). In the positive samples, 86.9% of the counts were below the limit of detection of the spread plate method, that is $<1.0 \text{ Log}_{10} \text{ CFU g}^{-1}$. The presence of *C. jejuni* in these positive samples was confirmed from the enrichment culture of 25 g of sample (range of count from 0.04 to 9 CFU g⁻¹). Of the 16 samples where counts were over $1.0 \text{ Log}_{10} \text{ CFU g}^{-1}$, 13 had low counts of between $1.0 \text{ Log}_{10} \text{ CFU g}^{-1}$ and $1.5 \text{ Log}_{10} \text{ CFU g}^{-1}$. The other three samples contained *C. jejuni* counts of between $1.5 \text{ Log}_{10} \text{ CFU g}^{-1}$ to $2.5 \text{ Log}_{10} \text{ CFU g}^{-1}$ (Fig. 1).

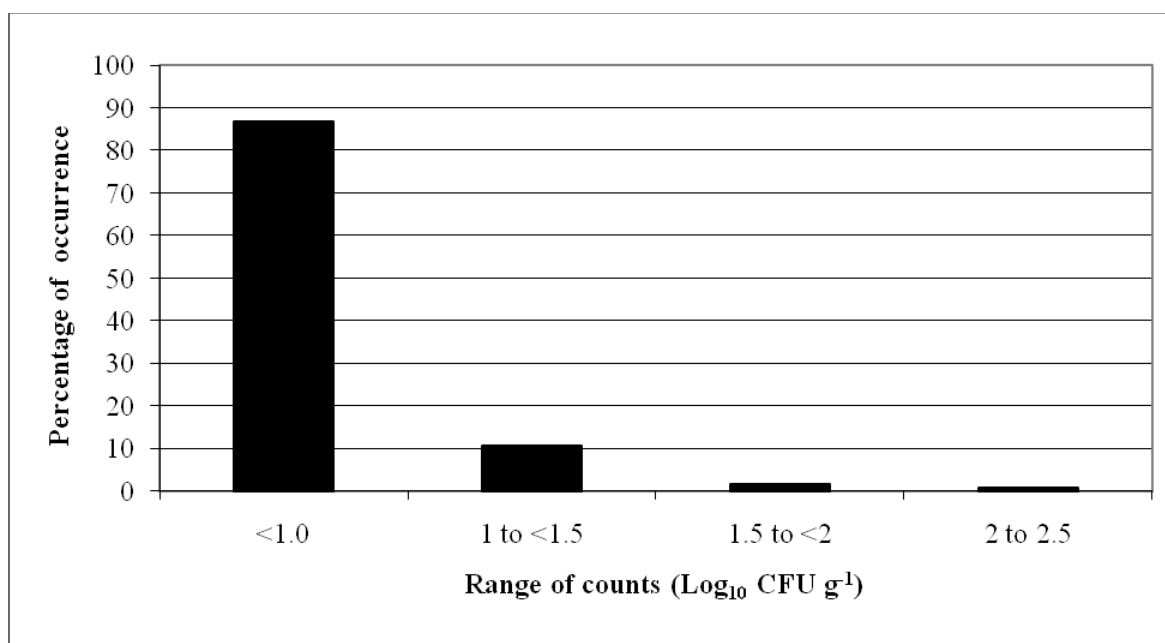


Figure 1. Distribution of counts for *Campylobacter* spp. in all positive chicken meat samples including counts at $<1.0 \text{ Log}_{10} \text{CFU g}^{-1}$.

A further breakdown of the *Campylobacter* counts enumerated from the chicken samples from each centre is presented in Table 2. Fifty-three samples (30.3%) were negative for *Campylobacter* spp. and 60% of these samples were purchased from retail outlets in Christchurch and Dunedin. One hundred and six samples were positive for *C. jejuni* from the 25 g enrichment cultures.

Of the 16 samples that had *Campylobacter* counts of between $1.0\text{--}2.1 \text{ Log}_{10} \text{CFU g}^{-1}$, three came from Christchurch and Dunedin with counts of $1.0 \text{ Log}_{10} \text{CFU g}^{-1}$, while the remaining 13 samples (80%) came from the North Island centres (Table 2). For an easier visual comparison of the *Campylobacter* counts from the five centres, the information was converted to percentage values and is presented in Figure 2.

Table 2. Distribution of *Campylobacter jejuni* counts in positive chicken samples purchased from five centres in NZ.

Area	No. of samples positive for <i>C. jejuni</i> per 25g enrichment (%) (N = 175)	No. of samples containing the following <i>C. jejuni</i> counts in positive samples (Log ₁₀ CFU g ⁻¹)						No. of samples negative for <i>C. jejuni</i> per 25 g enrichment
		<1.0	1.0	1.3	1.7	1.9	2.1	
Auckland	26	19	6	1				9
Christchurch	18	16	2					17
Dunedin	20	19	1					15
Hamilton	31	29		1	1			4
Wellington	27	23	2			1	1	8
Total	122	106	11	2	1	1	1	53
Percentage	69.7%	86.9	9.0	1.6	0.8	0.8	0.8	30.3%

Note: <1.0 Log₁₀ CFU g⁻¹ signifies that no colonies were observed on the spread plates when 1 mL of a 1/10 dilution of the homogenate was spread on three mCCDA plates (count that is below the limit of detection of the spread plate method), but *Campylobacter* was isolated from the 25 g enrichment culture.

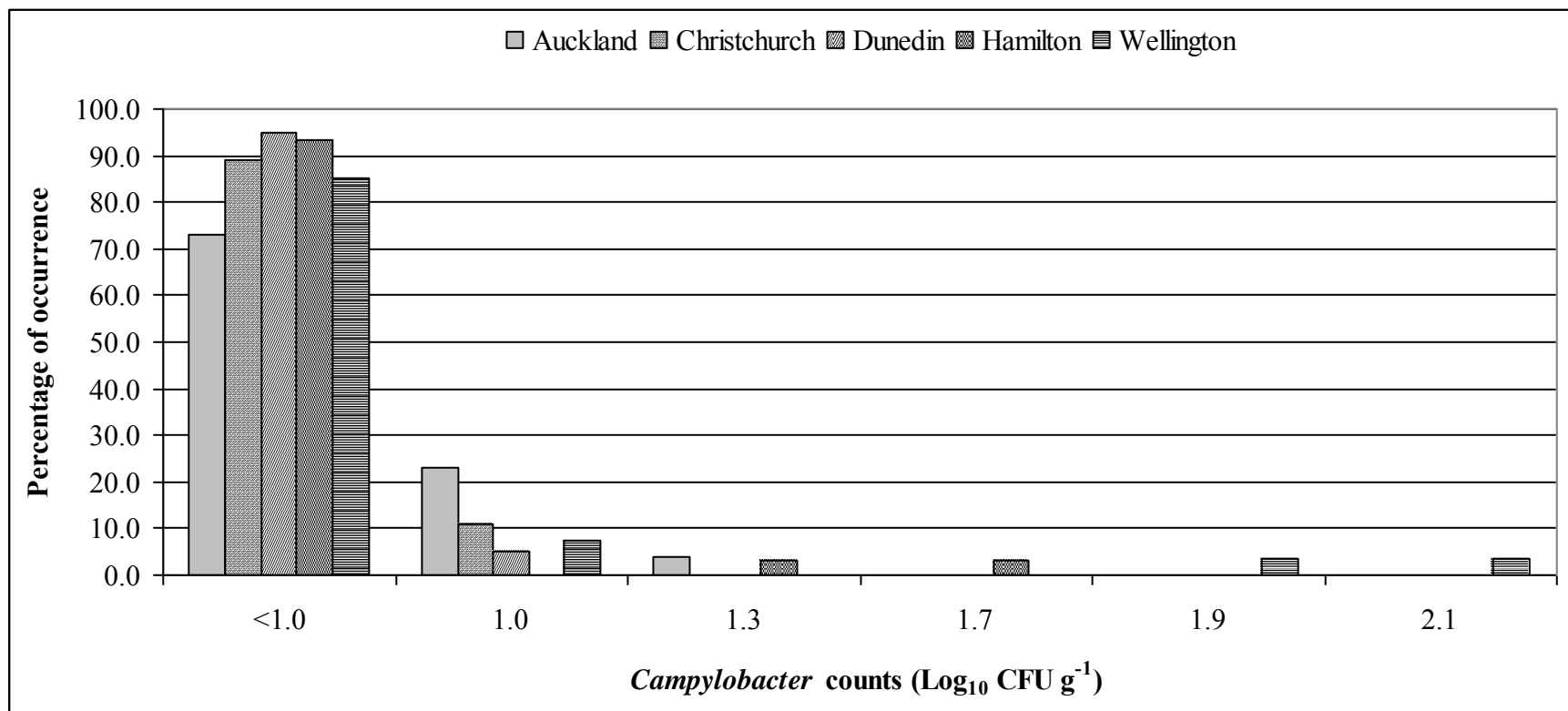


Figure 2. Distribution of *Campylobacter jejuni* counts in chicken meat samples from the five centres.

3.3 Comparison of the 2009 survey with the 2003–2004 survey

3.3.1 Comparison of qualitative data

In the 2003–2004 survey, 1011 uncooked retail meat samples were tested for *Campylobacter* spp. Chicken was one of the four meat types tested and data are available for a total of 230 chicken samples (minced, diced or cut into strips). Following a thorough interrogation of the 2003–2004 data, an amended summary of quantitative data for chicken samples is now presented in Table 3. Of these, 198 tested positive for *C. jejuni*, six for *C. jejuni* and *C. coli*, and one for *C. coli*. The total prevalence was 89.1% for the presence of either species, and *C. jejuni* was the predominant species present in 88.7% of the samples.

The current survey (Table 1) showed an overall prevalence of 69.7% (95% CI: 62.3–76.4%) for *Campylobacter* spp. (where all representative isolates were identified as *C. jejuni*), while the original 2003–2004 survey showed an overall prevalence of 89.1% (95% CI: 84.44–92.8) for *Campylobacter* spp. (Table 3). The large number of samples tested in both surveys produced narrow 95% CIs. These data demonstrate a significant reduction ($P = 0.001$, chi-square test) in *Campylobacter* spp. prevalence in minced or diced uncooked retail chicken meats between the two surveys.

Table 3. Prevalence of *Campylobacter jejuni* and *C. coli* in uncooked retail chicken meats from the 2003-2004 survey

Meat type	No. of samples tested	Samples positive for:							
		<i>C. jejuni</i>		<i>C. jejuni</i> and <i>C. coli</i>		<i>C. coli</i>		Total positive samples	
		No.	% (95% CI*)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
Chicken	230	198	86.1(80.9-90.3)	6	2.6(1.0-5.6)	1	0.4(0.0-2.4)	205	89.1(84.4-92.8)

*CI, confidence interval.

When sampling periods were compared for the two surveys, the 230 samples obtained in the 2003–2004 survey were collected over a 10-month period (August to June) while in the 2009 survey, the 175 samples were collected over three months (April to July). To account for factors such as seasonality and duration of sampling, the results from samples collected in April to June in 2004 were compared with samples taken in April to early July in 2009. Seventy-two samples from the 2003–2004 survey were compared with 175 samples in this survey (Table 4). A significant reduction ($P = 0.002$, Z test for two proportions) in *Campylobacter* spp. prevalence was evident. It can be concluded that a 16.4% (95% CI: 5.9% - 26.8%) reduction in *Campylobacter* spp. prevalence occurred in uncooked chicken products in the five-to-six year period between surveys.

Table 4. Prevalence of *Campylobacter* spp. chicken samples obtained from the same period in both poultry surveys.

Survey year	No. of samples	No. of positive samples	Prevalence (%)	95% CI
2004 (April to June)	72	62*	86.1	75.9–93.1
2009 (April to early July)	175	122 [#]	69.7	62.3–76.4

*Positive samples from 2004 include one sample contaminated with *C. coli* only while the other 61 samples were contaminated with *C. jejuni*.

[#]Only *C. jejuni* was isolated from contaminated samples in 2009.

3.3.2. Comparison of quantitative data

In addition to the significant drop in prevalence, quantitative data from the two surveys were compared to determine whether the introduction of CPT in 2008 to primary broiler chicken processing has an impact on the concentrations of *Campylobacter* spp. in retail chicken meats in 2009.

The quantitative data generated from the 2003–2004 survey were based on the use of the MPN method and the results were reported as MPN g⁻¹. This data has been amended following a thorough interrogation of the original data (Table 5). While the prevalence remains unchanged (89.1%), slight changes to the binning of quantitative data were made (Table 5).

Table 5. Counts of *Campylobacter jejuni* in uncooked retail chicken meats from the 2003-2004 survey

Meat type	Total no. of positive samples	No. of samples containing <i>C. jejuni</i> counts ($\text{Log}_{10} \text{MPN g}^{-1}$) of:				
		<-0.5	-0.5 to <1.0	0 to <1.0	1.0 to <2.0	2.0 to 2.5
Chicken	204*	79	56	50	17	2 [#]

*Out of the 6 samples co-contaminated with *C. coli*, one sample counted $-0.5 \text{ Log}_{10} \text{MPN g}^{-1}$ and 5 samples counted $<-0.5 \text{ Log}_{10} \text{MPN g}^{-1}$.

[#]One sample was estimated to contain $>1.04 \text{ Log}_{10} \text{MPN g}^{-1}$ (1.04 to $>2.04 \text{ Log}_{10} \text{MPN g}^{-1}$). Since there was no end point in this MPN result, it was placed in this range.

Seventy-nine of the 204 *C. jejuni*-positive samples (38.7%) were contaminated at the $<-0.5 \text{ Log}_{10} \text{ MPN g}^{-1}$ level while 90.7% of samples had counts of $\leq 1.0 \text{ Log}_{10} \text{ MPN g}^{-1}$. Nineteen samples yielded $>1.0 \text{ Log}_{10} \text{ MPN g}^{-1}$, and the highest MPN count obtained was $2.04 \text{ Log}_{10} \text{ MPN g}^{-1}$ (Figure 3).

The samples with a recorded count of $<-0.5 \text{ Log}_{10} \text{ MPN g}^{-1}$ represent the samples which tested positive for *Campylobacter* spp. in the presence/absence test of a 25 g sample but had contamination below the limit of detection of the MPN method. In these *Campylobacter*-positive samples, the minimum concentration of *C. jejuni* present would have been one CFU in a 25 g sample, or $-1.4 \text{ Log}_{10} \text{ CFU g}^{-1}$ (0.04 CFU g^{-1}).

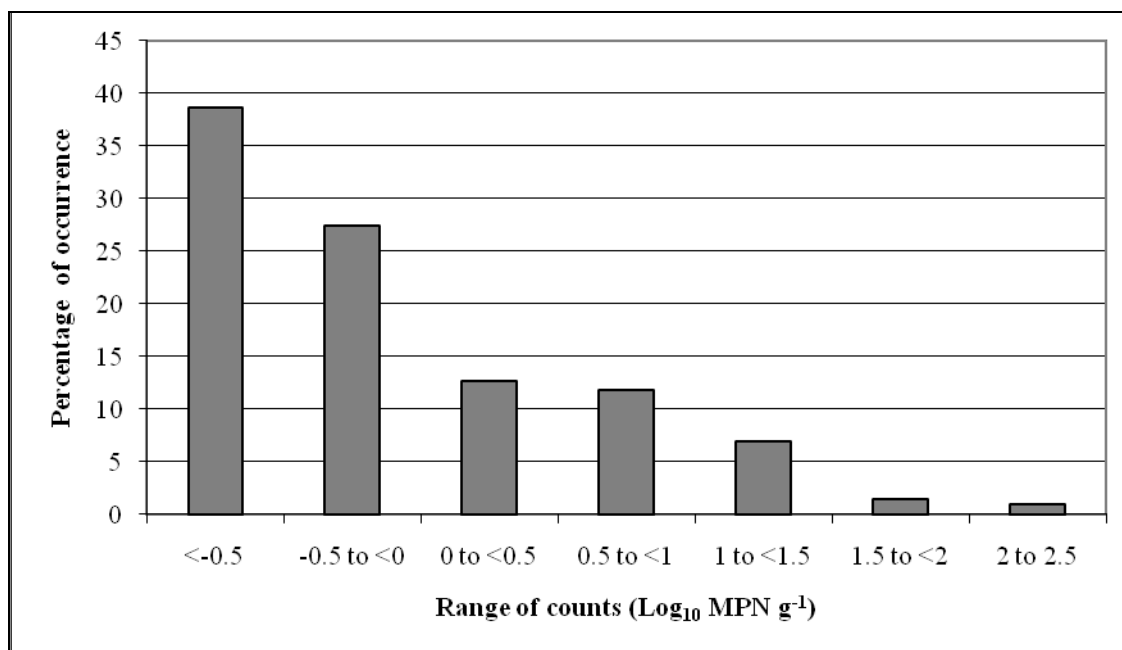


Figure 3. Distribution of counts in *Campylobacter jejuni*-positive samples of chicken meat (from the 2003–2004 data set).

To compare the two sets of quantitative data, all samples that were tested negative by the presence/absence test per 25 g, and those that contained counts that ranged from -1.4 to $1.0 \text{ Log CFU or MPN g}^{-1}$ and $>1.0 \text{ Log CFU or MPN g}^{-1}$, were reviewed. The distribution of counts in the two sets of data for all samples is illustrated in Figure 4.

The comparison showed that between the two sampling periods, the number of samples where *Campylobacter* spp. were not isolated per 25 g of chicken meats, was higher (30.3%) in 2009 compared to 11.3% for 2003/04 period. This improvement in quantitative data is also noticeable in the distribution of counts between the -1.4 to 1.0 Log₁₀ CFU or MPN g⁻¹ range, and particularly significant (P=0.037, chi-square test) for samples that were contaminated with higher levels of *Campylobacter* spp. exceeding 1.0 Log₁₀ CFU or MPN g⁻¹ (Figure 4).

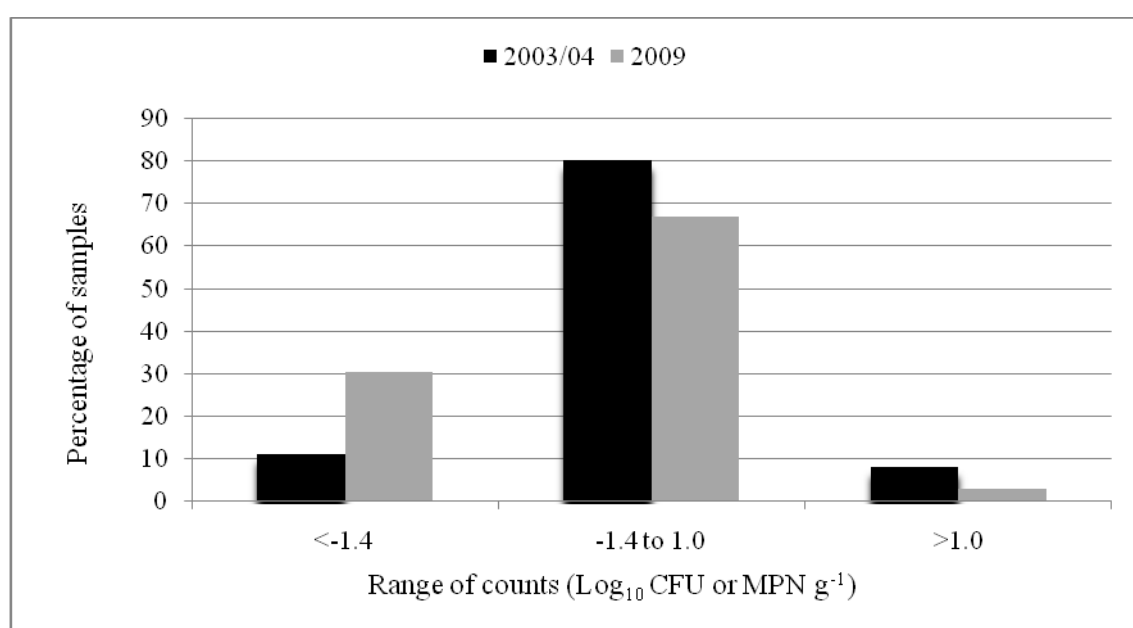


Figure 4. Comparison of the 2003/04 data set in samples of chicken meat positive for *Campylobacter* spp. with the 2009 data set.

■ Data (in Log₁₀ MPN g⁻¹) from the 2003–2004 survey;

■ Data (in Log₁₀ CFU g⁻¹) from the 2009 survey.

Samples expressed in the <-1.4 Log₁₀ CFU or MPN g⁻¹ range are those that were negative for *Campylobacter* spp. in the presence/absence test per 25 g.

3.3.3. Comparison of enumeration methods in the two surveys

Some attention has been given to comparing MPN and surface plating methods for the enumeration of *Campylobacter* spp. in chicken. The direct plating method was considered superior in one study because it is less tedious to perform (Scherer *et al.* 2006). However, they tested chicken skin and leg rinses which contained high concentrations of *Campylobacter* spp. While the correlation between the MPN and the plating data was very

good ($r = 0.9$), it is clear that the sensitivity of the MPN method was greater than the direct plating method if a 1 mL volume of rinsate or sample homogenate was spread over three plates for enumeration. The MPN method would capture the distribution of the lower counts as there is a higher proportion of *Campylobacter* spp. counts that are below $1.0 \text{ Log}_{10} \text{ CFU g}^{-1}$. However, this sensitivity issue would be negated if a presence/absence test on 25 g of sample was also performed, which was the case in both the 2003–2004 and 2009 surveys. Including a presence/absence test in a 25 g sample provided the same sensitivity of 0.04 g^{-1} ($-1.4 \text{ Log}_{10} \text{ CFU or MPN g}^{-1}$) in both studies.

3.4 Comparison with International Data

Campylobacter cells are located on the exterior surfaces of a poultry carcass, which means mincing or dicing the chicken meat will dilute the bacteria as they mix with the sterile internal tissue and possibly, meat from uninfected birds. Comparisons of the current data with *Campylobacter* spp. concentrations derived from the surfaces of chicken portions or whole birds therefore cannot be made. This limits comparisons of the survey data to a few studies only.

The United States Department of Agriculture Food Safety Inspection Service (USDA FSIS) published MPN data for 118 *Campylobacter*-positive minced chicken samples (USDA FSIS 1996). The mean concentration was $0.68 \text{ Log}_{10} \text{ MPN g}^{-1}$ with a standard error of 0.04. This figure lies just below the $1.0\text{--}1.5 \text{ Log}_{10} \text{ MPN g}^{-1}$ or $\text{Log}_{10} \text{ CFU g}^{-1}$ range of counts for both surveys (Figure 5). No further analysis of the American quantitative data was presented. In the same survey, the prevalence of *C. jejuni* or *C. coli* was reported as 59.8% (4.1% standard error) was based on an enrichment of a 25 g sample. This is around 10% lower than the prevalence reported here.

In a more recent survey of Belgian poultry products, a *Campylobacter* prevalence of 42.4% (42/99 samples) was reported for minced chicken (Habib *et al.* 2008). However the prevalence of *Campylobacter* in minced chicken samples recorded in Belgium was based on a 10 g sample compared to the 25 g used in this NZ study. The median plate count was approximately $1.5 \text{ Log}_{10} \text{ CFU g}^{-1}$ (data read from graph), which is at the higher end of the

distribution observed in this survey. Presence/absence testing was performed on 10 g samples.

In an investigation into recovery methods, *C. jejuni* was isolated from five out of 16 (31.3%) Japanese “chopped” chicken samples (Fukushima *et al.* 2007). The five positive samples contained 2.38, 2.30, 1.18, 0.36 and 0.36 Log₁₀ MPN g⁻¹. These data were based on sample weights of 25 g prepared in a 10-fold diluted slurry. No presence/absence testing per 25 g was undertaken.

In the 2009 NZ survey, the mean count was estimated at 0.89 Log₁₀ CFU g⁻¹ (based on a mean calculation from all positive samples and where each of the 106 samples that recorded a count of <10 CFU g⁻¹ but positive in a 25 g sample enrichment, is assigned a mean count of 5 CFU g⁻¹). This data is difficult to compare with the USDA data where the mean concentration was 0.68 Log₁₀ MPN g⁻¹ but the method of calculation was not given. However, as both the surveys have similar methodology sensitivity (presence/absence testing were performed on 25 g samples), the prevalence of *Campylobacter* spp. in NZ raw chicken meats in the 2009 survey was therefore higher than in the US. In the Belgian and Japanese studies, the sensitivity of the methods was lower hence reflected in the lower prevalences.

4 CONCLUSIONS

Campylobacter spp. prevalence in uncooked retail chicken meats (minced, diced or cut in strips) reduced significantly ($P < 0.001$) between the two surveys. When the sampling period and duration of sampling were taken into account, the reduction in prevalence (16%) of *Campylobacter* spp. between the two surveys remained significant ($P = 0.002$). In addition, a decrease in the distribution of concentration data in *Campylobacter*-positive samples was also measured in the 2009 survey. The percentage of counts in the higher ranges in the 2009 survey ($> 1.0 \text{ Log}_{10} \text{ CFU g}^{-1}$) is lower than in 2003/04 survey. Further, despite of changing the enumeration method from an MPN method used in the 2003-2004 study to a spread plate method used in this 2009 study, the inclusion of a presence/absence test in a 25 g sample accounted for the presence of *Campylobacter* spp. in positive samples that contained counts of $< 1.0 \text{ Log}_{10} \text{ CFU g}^{-1}$ by the spread plate method.

It can be concluded that a significant reduction in *Campylobacter* spp. prevalence and concentration in positive samples of minced or diced chicken meats were measured in 2009 compared to 2003/04.

5 REFERENCES

- Anonymous (2007). Annual New Zealand Notifiable Disease Report 2007. Available at: http://www.surv.esr.cri.nz/PDF_surveillance/AnnualRpt/AnnualSurv/2007AnnualSurvTables.pdf (last accessed on the 19 February 2010).
- Anonymous (2008). Annual New Zealand Notifiable Disease Report 2008. Available at: http://www.surv.esr.cri.nz/PDF_surveillance/AnnualRpt/AnnualSurv/2008AnnualSurvRptTables.pdf (last accessed on the 19 February 2010).
- Anonymous (2009). ESR Monthly Surveillance Report, December 2009. Available at: http://www.surv.esr.cri.nz/PDF_surveillance/MthSurvRpt/2009/200912DecRpt.pdf (last accessed on the 19 February 2010).
- Eberhardt-Phillips, J., Walker, N., Garrett, N., Bell, D., Sinclair D., Rainger, W., and Bates, M. (1997). Campylobacteriosis in New Zealand: results of a case-control study. *Journal of Epidemiology and Community Health* 51, 686-691.
- Fraser, A.D. E., Chandan, V., Yamazaki, H., Brooks, B.W. and Garcia, M.M. (1992). Simple and economical culture of *Campylobacter jejuni* and *Campylobacter coli* in CO₂ in moist air. *International Journal of Food Microbiology* 15, 377-382.
- Fukushima, H., Katsube, K., Hata, Y., Kishi, R and Fujiwara, S. (2007). Rapid separation and concentration of food-borne pathogens in food samples prior to quantification by viable-cell counting and real-time PCR. *Applied and Environmental Microbiology* 73, 92-100.
- Habib, I., Sampers, I., Uyttendaele, M., Berkvens, D. and De Zutter, L. (2008) Baseline data from a Belgium-wide survey of *Campylobacter* species contamination of chicken meat preparations and considerations for a reliable monitoring programme. *Applied and Environmental Microbiology* 74, 5483-5489.
- Ikram, R., Chambers, S., Mitchell, P., Brieseman, M. A., and Ikram, O.H. (1994). A case control study to determine risk factors for *Campylobacter* infection in Christchurch in the summer of 1992-3. *New Zealand Medical Journal* 107, 430-432.
- Mullner, P., Jones, G., Noble, A., Spencer, S.E.F., Hathaway, S. and French, N.P. (2009). Source attribution of food borne zoonoses in New Zealand: A modified Hald model. *Risk Analysis* 29(7), 970-984.
- NZFSA (2008). *Campylobacter* risk management strategy 2008-2011. Available at: <http://www.nzfsa.govt.nz/foodborne-illness/campylobacter/strategy/Campylobacter>

_risk_management_strategy_2008-2011.pdf (last accessed on the 19 February 2010)

- Scherer, K., Bartelt, E., Sommerfeld, C. and Hildebrandt, G. (2006). Comparison of different sampling techniques and enumeration methods for the isolation and quantification of *Campylobacter* spp. in raw retail chicken legs. *International Journal of Food Microbiology* 108, 115-119.
- United States Department of Agriculture Food Safety Inspection Service (1996). Nationwide raw ground chicken microbiological survey. Available at: www.fsis.usda.gov/OPHS/baseline/rwgrchck.pdf (last accessed on 23 February 2010).
- Wilson, N. (2005). A systematic review of the aetiology of human campylobacteriosis in New Zealand. A report to the Food Safety Authority of New Zealand, June 2005. Available at: <http://www.nzfsa.govt.nz/science/research-projects/campy-aetiol/campy-aetiol.pdf> (last accessed on 23 February 2010).
- 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.
- Wong, T.L., Hollis, L., Cornelius, A., Nicol, C., Cook, R.C. and Hudson, J.A. (2007). Prevalence, numbers, and subtypes of *Campylobacter jejuni* and *Campylobacter coli* in uncooked retail meat samples. *Journal of Food Protection* 70, 566-573.
- Wong, T.L., French, N.P., Carter, P. and Mullner, P. (2008a). Comparison of human and poultry *Campylobacter jejuni* isolates using multi-locus sequence typing in Auckland and Christchurch with those isolated from the Mananwatu. Client report FW0712 to the New Zealand Food Safety Authority, Wellington, New Zealand.
- Wong, T.L., French, N.P. and Carter, P. (2008b). MLST genotyping of *Campylobacter* isolated from human cases and retail poultry in Canterbury following Sanova intervention at a poultry processing plant. Client report FW0872 to the New Zealand Food Safety Authority, Wellington, New Zealand.

APPENDIX 1 *Campylobacter jejuni* isolated and enumerated from chicken meat samples from Auckland

AUCKLAND					
Type of chicken meat	Type of packaging	Date tested	Count CFU/g	Presence/absence (25 g)	PCR results
Minced	Tray	7/04/2009	<10	P	<i>C. jejuni</i>
Minced	Loose	7/04/2009	<10	A	-
Diced	Tray	7/04/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	7/04/2009	<10	A	-
Diced	Tray	7/04/2009	<10	A	-
Diced	Loose	21/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	21/04/2009	10	P	<i>C. jejuni</i>
Diced	Tray	21/04/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	21/04/2009	10	P	<i>C. jejuni</i>
Diced	Tray	21/04/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	5/05/2009	10	P	<i>C. jejuni</i>
Diced	Tray	5/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	5/05/2009	<10	A	-
Diced	Tray	5/05/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	5/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	5/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	5/06/2009	10	P	<i>C. jejuni</i>
Diced	Tray	5/06/2009	20	P	<i>C. jejuni</i>
Minced	Tray	5/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	5/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	12/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	12/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	12/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	12/06/2009	<10	A	-
Diced	Tray	12/06/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	17/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	17/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	17/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	17/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	17/06/2009	<10	A	-
Diced	Tray	4/07/2009	<10	A	-
Diced	Tray	4/07/2009	<10	A	-
Diced	Tray	4/07/2009	10	P	<i>C. jejuni</i>
Diced	Tray	4/07/2009	10	P	<i>C. jejuni</i>
Diced	Tray	4/07/2009	<10	A	-

APPENDIX 2. *Campylobacter jejuni* isolated and enumerated from chicken meat samples from Christchurch

CHRISTCHURCH					
Type of chicken meat	Type of packaging	Date tested	Count CFU/g	Presence/absence (25 g)	PCR results
Minced	Tray	8/04/2009	<10	A	-
Minced	Tray	8/04/2009	<10	A	-
Minced	Tray	8/04/2009	<10	A	-
Minced	Loose	8/04/2009	<10	A	-
Diced	Tray	8/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	22/04/2009	<10	A	-
Diced	Tray	22/04/2009	<10	A	-
Diced	Tray	22/04/2009	<10	A	-
Minced	Tray	22/04/2009	<10	A	-
Diced	Tray	22/04/2009	<10	A	-
Minced	Tray	5/05/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	5/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	5/05/2009	<10	A	-
Minced	Tray	5/05/2009	<10	A	-
Minced	Tray	5/05/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	27/05/2009	<10	A	-
Minced	Tray	27/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	27/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	27/05/2009	10	P	<i>C. jejuni</i>
Minced	Tray	27/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	4/06/2009	10	P	<i>C. jejuni</i>
Diced	Tray	4/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	4/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	4/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	4/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	27/06/2009	<10	A	-
Diced	Tray	27/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	27/06/2009	<10	A	-
Minced	Tray	27/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	27/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	10/07/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	10/07/2009	<10	A	-
Minced	Tray	10/07/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	10/07/2009	<10	A	-
Minced	Tray	10/07/2009	<10	A	-

APPENDIX 3. *Campylobacter jejuni* isolated and enumerated from chicken meat samples from Dunedin

DUNEDIN					
Type of chicken meat	Type of packaging	Date tested	Count CFU/g	Presence/absence (25 g)	PCR results
Diced	Tray	7/04/2009	<10	A	-
Diced	Loose	7/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	7/04/2009	<10	A	-
Minced	Tray	7/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	7/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	21/04/2009	<10	A	-
Minced	Tray	21/04/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	21/04/2009	<10	A	-
Diced	Loose	21/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	21/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	7/05/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	7/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	7/05/2009	<10	A	-
Diced	Loose	7/05/2009	<10	A	-
Diced	Tray	7/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	20/05/2009	<10	A	-
Diced	Loose	20/05/2009	<10	A	-
Diced	Tray	20/05/2009	10	P	<i>C. jejuni</i>
Minced	Tray	20/05/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	20/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	13/06/2009	<10	A	-
Minced	Tray	13/06/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	13/06/2009	<10	A	-
Diced	Tray	13/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	13/06/2009	<10	A	-
Diced	Tray	19/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	19/06/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	19/06/2009	<10	A	-
Diced	Tray	19/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	19/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	4/07/2009	<10	A	-
Minced	Tray	4/07/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	4/07/2009	<10	A	-
Diced	Tray	4/07/2009	<10	A	-
Minced	Tray	4/07/2009	<10	P	<i>C. jejuni</i>

APPENDIX 4. *Campylobacter jejuni* isolated and enumerated from chicken meat samples from Hamilton

HAMILTON					
Type of chicken meat	Type of packaging	Date tested	Count CFU/g	Presence/absence (25 g)	PCR results
Diced	Tray	8/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	8/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	8/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	8/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	8/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	22/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	22/04/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	22/04/2009	<10	A	-
Diced	Tray	22/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	22/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	12/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	12/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	12/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	12/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	12/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	22/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	22/05/2009	50	P	<i>C. jejuni</i>
Diced	Tray	22/05/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	22/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	22/05/2009	<10	A	-
Diced	Tray	4/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	4/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	4/06/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	4/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	4/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	20/06/2009	20	P	<i>C. jejuni</i>
Diced	Tray	20/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	20/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	20/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	20/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	3/07/2009	<10	A	-
Diced	Tray	3/07/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	3/07/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	3/07/2009	<10	A	-
Diced	Tray	3/07/2009	<10	P	<i>C. jejuni</i>

APPENDIX 5. *Campylobacter jejuni* isolated and enumerated from chicken meat samples from Wellington

WELLINGTON					
Type of chicken meat	Type of packaging	Date tested	Count CFU/g	Presence/absence (25 g)	PCR results
Minced	Tray	7/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	7/04/2009	<10	A	-
Diced	Tray	7/04/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	7/04/2009	<10	A	-
Diced	Tray	7/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	21/04/2009	<10	A	-
Minced	Tray	21/04/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	21/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	21/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	21/04/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	13/05/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	13/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	13/05/2009	10	P	<i>C. jejuni</i>
Diced	Loose	13/05/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	13/05/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	21/05/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	21/05/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	21/05/2009	<10	A	-
Minced	Tray	21/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	21/05/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	12/06/2009	<10	A	-
Diced	Loose	12/06/2009	<10	P	<i>C. jejuni</i>
Minced	Loose	12/06/2009	<10	A	-
Minced	Tray	12/06/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	12/06/2009	140	P	<i>C. jejuni</i>
Diced	Tray	26/06/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	26/06/2009	<10	A	-
Minced	Tray	26/06/2009	<10	A	-
Minced	Tray	26/06/2009	10	P	<i>C. jejuni</i>
Minced	Tray	26/06/2009	80	P	<i>C. jejuni</i>
Minced	Tray	11/07/2009	<10	P	<i>C. jejuni</i>
Diced	Loose	11/07/2009	<10	P	<i>C. jejuni</i>
Minced	Loose	11/07/2009	<10	P	<i>C. jejuni</i>
Minced	Tray	11/07/2009	<10	P	<i>C. jejuni</i>
Diced	Tray	11/07/2009	<10	P	<i>C. jejuni</i>