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Client Report
FW 11071.1

Final Report

Prevalence and enumeration of *Campylobacter* and *E. coli* on chicken carcasses and portions at retail sale

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Scientific Interpretive Summary

FW11071 *Campylobacter* in retail chicken meat 2015

In 2006, MPI implemented a comprehensive programme of work encompassed in the "*Campylobacter* Risk Management Strategy" to reduce New Zealand's comparatively high burden of notified human campylobacteriosis. A key performance indicator for success of the programme was the reduction of *Campylobacter* contamination on broiler chicken carcasses during processing under the National Microbiological Database (NMD) monitoring programme, which transpired with a one log decrease in the median counts. This was associated with a concurrent 50% reduction in notified human cases.

A question remained, however, as to whether the reduction in carcass contamination immediately after spin-chilling would remain through further processing and the storage and/transport chain to chicken meat products available at retail. Previous surveys showed carcass contamination (2003-2008) of around 81% with median counts around 10^4/carcass (maximum 10^7/carcass), and diced chicken meat up to 89% but only at 10^2/g (2003-2004).

MPI therefore contracted ESR to carry out a comprehensive national microbiological survey of chicken meat at retail (September 2010 to August 2011): whole carcasses and a range of different portions (e.g. skinless breasts and wings); packed at the processor through to the counter-top; a variety of retail packages; three major cities; and all four seasons.

Campylobacter was detected on 79.4% of the 574 samples tested; 79% of whole carcasses and from 61-87% of portions dependent on the product.

While this suggests that there had not been a change in prevalence, the current survey used sampling plans and analytical procedures that detected *Campylobacter* at lower levels than previously. If the results of this survey are, however, adjusted to record as detected only counts greater than 400 cfu/carcass, the limit of detection applicable to a 2007 industry survey, then a decrease in prevalence of **46%** has occurred from 44.8% in 2007 to 24.2% in the 2011 carcass survey.

This estimated reduction exceeds that observed in the NMD with the *Campylobacter* prevalence decreasing from 49.7% in 2007/8 to 37.9% in 2011/12; a **24%** decrease. However, the prevalence of *Campylobacter* on broiler carcasses in the NMD further decreased to 27.3% in 2014/15; a **45%** reduction. Similarly, the one log reduction in median count and two log decrease in maximum count observed in this survey was also observed in the NMD, with the percentage of high counts (>log 3.78) decreasing from 22.4% in 2007/8 to 7.6% in 2011/12 and 3.9% in 2014/15.

This report also suggests that there are seasonal and regional differences in *Campylobacter* contamination of chicken meat at retail.

The data presented in this report confirm that the measures implemented under the *Campylobacter* Risk Management Strategy have resulted in a substantial reduction in the level of consumer exposure to *Campylobacter* through chicken meat at retail. These data can also be used to inform quantitative risk assessments that will strengthen the hypothesis that the measures and consequential reduced exposure to *Campylobacter* through chicken meat have significantly reduced the burden of human campylobacteriosis in New Zealand.

Final Report

Prevalence and enumeration of *Campylobacter* and *E. coli* on chicken carcasses and portions at retail sale

Prepared for the Ministry for Primary Industries
under project MFS10/05 – Microbiological Food Safety,
as part of an overall contract for scientific services

Client report no. FW 11071.1

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March 2015

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We gratefully acknowledge the participation of Compliance Assessors from MAF in the retail sampling of chicken products in Auckland, Palmerston North and Christchurch. We also thank ESR's Public Health laboratory in Christchurch for sample analysis.

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SUMMARY

A survey of raw retail chicken portions and whole chicken carcasses was carried out from September 2010 to August 2011. Each sample was tested for the presence and concentration of *Campylobacter*, and the concentration of *E. coli*. In total 575 samples were collected, with 99 whole birds, and 476 portions sampled. The breakdown of portions was as follows (skin on unless otherwise stated): 52 wings, 71 breasts, 82 thighs, 40 nibbles, 122 skinless boneless breasts, 106 skinless boneless thighs, and 3 portions categorised as "other".

Of the 574 samples tested for the presence of *Campylobacter* 456 (79.4%) were contaminated, with prevalence ranging from 86.8% for skinless and boneless thighs to 61.5% for wings. For whole chicken carcasses the prevalence was 78.8%. Many of the positive samples harboured *Campylobacter* at a concentration less than the limit of detection of the analytical method of quantification (50 CFU/sample for portions and 200 CFU/sample for whole chicken carcasses). Although the prevalence of positive samples was high, the concentrations were therefore generally quite low.

The prevalence of *Campylobacter* in both portion and whole chicken carcass samples differed geographically, with the prevalence of *Campylobacter* in portion samples from Christchurch (71.1%) being lower than for Palmerston North (79.2%) and Auckland (88.5%). The prevalence of *Campylobacter* in portions and whole chicken carcasses also indicated a seasonal variation, with higher prevalence in the summer and autumn, compared to the winter and spring. The highest prevalence of *Campylobacter* in portions was 88.2% in autumn compared to 71.4% in winter.

It is difficult to compare the data from this study to those from previous New Zealand surveys because of differences in the sample size tested (whole birds/portions vs 25g vs 10g subsamples), different sample types (rinses vs subsamples), point of sampling (retail vs during processing) and method (direct plating vs enrichment).

There was no apparent correlation between the concentrations of *Campylobacter* and *E. coli* in the samples.

1 INTRODUCTION

Campylobacteriosis is the leading cause of notifiable bacterial gastroenteritis in New Zealand, comprising 45% of all notifications in 2014[1]. The annual notified campylobacteriosis rates over the period 2008 to 2014 have been stable with notification rates between 151 to 168 cases per 100,000 population (6689-7346 cases), reduced from a national high of 379.3 cases per 100,000 population in 2006 (ESR 2006). Poultry consumption is a significant risk factor for campylobacteriosis in New Zealand (Eberhart-Phillips *et al.* 1997, Ikram *et al.* 1994, Mullner *et al.* 2009, Wilson 2005).

There have been several surveys of New Zealand poultry products for the presence of *Campylobacter*. A survey of 113 raw chicken portions conducted in Christchurch found a prevalence of 56.6% (Hudson *et al.* 1999) in 10g samples, a value consistent with an earlier study which reported a prevalence of 57% (Campbell and Gilbert 1995). In a more recent study of samples collected from 2005-2008, the prevalence in whole carcasses was reported to be 81% for presumptive *Campylobacter* spp. and 74% for *C. jejuni* (French 2008) where 60% of a 200 ml carcass rinse was tested. Concentrations per carcass are reported for three producers with median concentrations approximating 10^4 CFU/carcass, although the maximum numbers recorded exceeded 10^7 (the data are presented as graphs). A survey of 25 g retail minced chicken samples from 2003 to 2004 found 89.1% of samples to be contaminated (Wong *et al.* 2007), although the maximum concentration measured was only 110 MPN/g.

New Zealand's *Campylobacter* Risk Management Strategy has been operating for eight years, starting in late 2006. Since that date, considerable improvement in poultry industry performance has been recorded by the National Microbiological Database (NMD) monitoring system which samples carcasses at the end of the processing line[2]. Industry research in 2007 (Chrystal *et al.* 2008) indicated that 44.8% of 400 ml retail carcass rinse samples were positive for *Campylobacter* with counts ranging from less than 400 CFU to greater than 600,000 CFU per chicken carcass. The level of sensitivity of the methods used by Chrystal *et al*. (2008) was lower than that in the study that follows and that of French (2008). However the effect of the

[1] https://surv.esr.cri.nz/surveillance/annual_surveillance.php (accessed 16 March 2015)
[2] *Campylobacter* Risk Management Strategy 3rd Quarter report 2009

Strategy on consumer retail chicken, especially the proportion of *Campylobacter* contamination on retail chicken carcasses and portions since 2007, is uncertain.

This survey was intended to determine the prevalence and concentration of the pathogen *Campylobacter* and the concentration of *E. coli* as an indicator of hygiene on a range of chicken portions and whole birds available for retail sale in New Zealand during 2010-2011. At the time of the survey both organisms were tested for as part of the NMD monitoring for poultry. However, in 2013, *E. coli* testing for poultry was removed from the NMD monitoring requirements.

2 MATERIALS AND METHODS

2.1 Sampling Parameters

Based on an agreed protocol with MAF, whole chicken carcasses and chicken pieces were obtained by MAF officers from supermarkets, butchery chains and independent butchers, across Auckland, Palmerston North and Christchurch. Portions consisted of whole wings, breasts with skin on, breasts with skin off, boneless thighs with skin off, thighs with skin on. The samples came from a range of packaging types/situations including leakproof, non-leakproof / packed by processor, packed at butchery and packed at display counter. A total of 575 samples comprising 99 whole birds and 476 portions was sampled and tested. For a few samples not all analyses were carried out. Seasonal variation was accounted for by sampling four times a year (4 quarters). In each quarter, 24 samples at a time were targeted to be sent on two occasions from each city. Pre-set dataloggers were included in all containers to log the temperature during freight to ESRs Public Health Laboratory (PHL) in Christchurch.

2.2 Sample Receipt

The temperatures of samples were checked on receipt by either downloading the data from the dataloggers, or by manually measuring five different positions within each container using a calibrated thermometer and a mean temperature was recorded. Frozen samples (below -1.5ºC) were to be rejected, as were samples with temperatures >10°C, but no samples were rejected for these reasons. Samples were tested within 24 h of sampling.

2.3 Laboratory Analysis

2.3.1 Sample preparation

Samples were processed using the two minute rinse method (NMD procedure). All sample bags were swabbed with 70% alcohol before opening and sampling. Chilled Buffered Peptone Water (BPW; Fort Richard Laboratories Ltd, Auckland, New Zealand) was used to rinse the chicken samples.

For whole birds, the carcass was transferred aseptically from the retail bag to a large rinse bag containing 400 ml of BPW. For each portion, 100 ml of BPW was added to a smaller stomacher bag. When chicken portions were presented on a tray containing more than one portion, the

exterior film was swabbed with 70% alcohol before opening a hole in the film and aseptically removing one portion to a stomacher bag for rinsing. Samples were then rinsed for 2 min with agitation before decanting the rinsate into a whirlpak bag (Fort Atkinson, Wisconsin, USA) for further analysis. These rinsates represent a 10^0 dilution for enumeration calculations.

2.3.2 *Campylobacter* spp. presence / absence

Half of the volume of rinsate was enriched using double strength (DS) Exeter broth (200 ml rinsate for a chicken carcass to 200 ml DS Exeter broth; 50 ml rinsate from a portion to 50 ml DS Exeter broth). These enrichment broths were then incubated under microaerophilic conditions (10% CO_2) at 37ºC for at least 4 h, then at 42ºC for a further 48 h.

Following incubation, the enrichments were streaked onto plates of Campylobacter Blood Free Selective Medium with selective supplements (mCCDA, Fort Richard) for single colony isolation. These plates were incubated at 42ºC for 48 h under 10% CO_2. Up to five colonies were then picked from each plate for confirmation by oxidase testing and PCR (Wong *et al.* 2004).

2.3.3 *Campylobacter* enumeration

1 in 10 serial dilutions of the rinsates were made. Two ml of the initial rinsate (10^0 dilution) was spread over 6 mCCDA plates, and 0.1 ml of the 10^0 and 0.1 ml of 10^{-1} dilutions were also spread onto mCCDA plates in duplicate. The mCCDA plates were incubated as above for 48 h before counting all typical *Campylobacter* colonies. Five colonies per sample were picked for confirmation by oxidase testing and PCR.

2.3.4 PCR confirmation of *Campylobacter* spp.

A triplex PCR test was performed on pooled colonies from each positive sample. The PCR reaction amplifies the acyltransferase gene (*lpxA*) exclusive to *C. jejuni*, the lipoprotein component of a protein-binding transport system involved in iron acquisition (*ceuE*) in *C. coli*, and the 23S rRNA gene common to thermotolerant *Campylobacter* spp. Detailed methodology is described by Wong *et al* (2004).

2.3.5 *E. coli* enumeration

Rinsates were enumerated for *E. coli* using 3M™ Petrifilm™ *E. coli* plates (St. Paul, MN, USA). One ml volumes of the 10^0 and 10^{-1} rinsate dilutions were plated onto duplicate *E. coli* petrifilms before incubating at 35ºC for 24 h aerobically. All blue colonies were counted as *E. coli* according to the NMD protocol.

2.3.6 Presentation of the data

Data are presented as the prevalence (% positive) for *Campylobacter* and as bar graphs of concentrations for both organisms. The graphs accommodate negative samples (for *Campylobacter*) and positive samples by presence/absence testing with concentrations beneath the level of detection of the quantification method. Tables of prevalence with confidence intervals for the population prevalence (Zar 1999) are given. For a few samples there were incomplete data and these specific data were excluded from calculations/graphs. The actual number of samples tested in each analysis is shown in the tables.

Quantitative data are presented in the units "CFU/sample". While sample weights were recorded, a unit of CFU/g was not considered to be valid for these samples, as the contamination is confined to the surface and the surface area to volume ratio is different for different poultry portions. For modelling purposes it is likely that servings would correlate to individual portions and so it may be more convenient to consider the number per portion/carcass from this perspective.

3 RESULTS

3.1 Survey numbers

A total of 575 chicken samples was collected, with 99 whole birds, and 476 portions sampled. The breakdown of portions was as follows (skin on unless otherwise stated): 52 wings, 71 breasts, 82 thighs, 40 nibbles (the bottom two joints of the wing, Roy Biggs, Pers. Comm.), 122 skinless boneless breasts, 106 skinless boneless thighs, and 3 portions categorised as "other".

Sampling locations included outlets in Auckland, Palmerston North and Christchurch with eight sampling times each (total 24 sampling times). Sampling times were spread seasonally, with six sets of samples taken in the Spring of 2010 (September to November), five over the Summer months (December 2010 to February 2011), seven during Autumn (March to May 2011) and six during the Winter months of June to August 2011. The discrepancy in the Summer and Autumn sampling resulted from the Christchurch earthquake on February 22nd 2011. A sampling scheduled in Auckland on the 23rd February was postponed until April, meaning that only five sampling times were taken in that season, not six. The sampling locations were also spread seasonally, with two samplings per location, per season, with the exception that Auckland was only sampled once during the Summer months, but three times during the Autumn.

3.2 Analysis by portion type

3.2.1 Prevalence and concentration of *Campylobacter*

Table 1 shows the prevalence of *Campylobacter* spp. in each of the various poultry samples tested. There were three isolates of *Campylobacter* spp. from breast samples that were not *C. jejuni* or *C. coli*. The prevalence of *Campylobacter* spp. was in the range from 61.5% - 86.7% across the product types. The lowest prevalence was associated with wing portions and the highest with nibbles and skinned, boneless breast and thigh portions. The total prevalence of *C. jejuni* in all samples was 73.3% and the *C. coli* prevalence was 13.4%. Overall, 456/574 (79.4%) samples were *Campylobacter* positive.

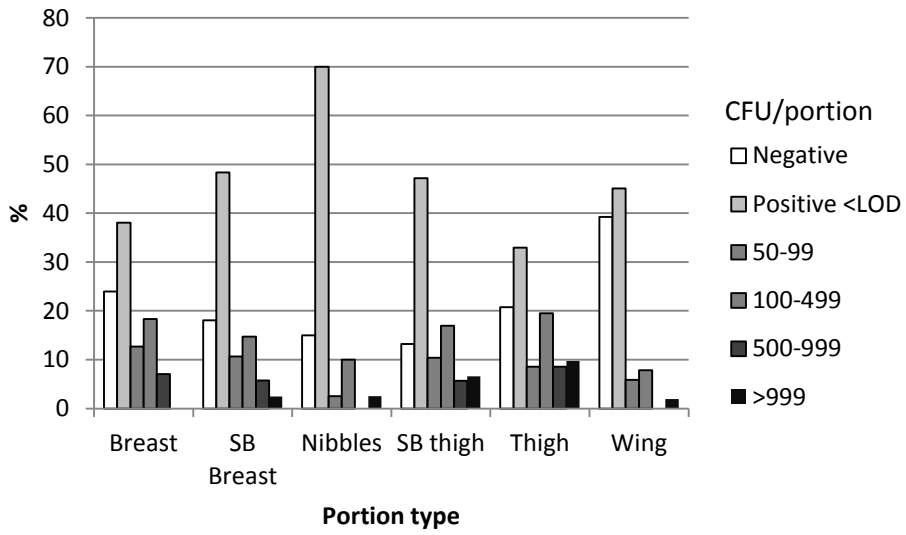
Table 1. Prevalence and 95% confidence intervals of *Campylobacter* in retail poultry samples according to portion type

Poultry sample (number tested)	No. (**% positive**, CI) *C. jejuni* only	No. (**% positive**, CI) *C. coli* only	No. (**% positive**, CI) *C. jejuni* and *C. coli*	No. (**% positive**, CI) non *C. jejuni* or *C. coli* campylobacters	No. (**% positive**, CI) total *C. jejuni*	No. (**% positive**, CI) total *C. coli*	No. (**% positive,** CI) total *Campylobacter*
Breast (71)	48 (**67.6**, 55.5-78.2)	0 (**0.0**, 0.0-5.1)	5 (**7.0**, 2.3-15.7)	1 (**1.4**, 0-7.6)	53 (**74.6**, 62.9-84.2)	5 (**7.0**, 2.3-15.7)	54 (**76.1**, 64.5-85.4)
SB Breast (121*)	76 (**62.8**, 53.6-71.4)	8 (**6.6**, 2.9-12.6)	13 (**10.7**, 5.8-17.7)	2 (**1.7**, 0.2-2.5)	89 (**73.6**, 64.8-81.2)	21 (**17.4**, 11.1-25.3)	99 (**81.8**, 73.8-88.2)
Nibbles (40)	31 (**77.5**, 61.5-89.2)	2 (**5.0**, 0.60-16.9)	1 (**2.5**, 0.1-13.2)	0 (**0.0**, 0.0-8.8)	32 (**80.0**, 64.4-90.9)	3 (**7.5**, 1.6-20.4)	34 (**85.0**, 70.2-94.3)
Thigh (82)	55 (**67.1**, 55.8-77.1)	5 (**6.0**, 2.0-13.5)	4 (**4.8**, 1.3-11.9)	0 (**0.0**, 0.0-4.3)	59 (**72.0**, 60.9-81.3)	9 (**10.8**, 5.1-19.6)	64 (**78.0**, 67.5-86.4)
SB thigh (106)	71 (**66.7**, 56.8-75.8)	10 (**9.4**, 4.6-16.7)	11 (**10.4**, 5.3-17.8)	0 (**0.0**, 0.0-3.4)	82 (**77.4**, 68.2-84.9)	21 (**19.8**, 12.7-28.7)	92 (**86.7**, 78.6-92.5)
Wing (52)	30 (**57.7**, 43.2-71.3)	0 (**0.0**, 0.0-6.8)	2 (**3.8**, 0.5-13.2)	0 (**0.0**, 0.0-6.8)	32 (**61.5**, 47.0-74.7)	2 (**3.8**, 0.5-13.2)	32 (**61.5**, 47.0-74.7)
Other (3)	3 (**100.0**, 29.2-100.0)	0 (**0, 0**-70.8)	0 (**0, 0**-70.8)	0 (**0, 0**-70.8)	3 (**100.0**, 29.2-100)	0 (**0, 0**-70.8)	3 (**100.0**, 29.2-100.0)
Whole (99)	62 (**62.6**, 52.3-72.1)	7 (**7.1**, 2.9-14.2)	9 (**9.2**, 4.3-16.7)	0 (**0.0**, 0.0-3.7)	71 (**71.7**, 61.8-80.3)	16 (**16.3**, 9.6-25.2)	78 (**78.8**, 69.4-86.4)
All (574*)	376 (**65.5**, 61.5-69.4)	32 (**5.6**, 3.8-7.8)	45 (**7.8**, 5.8-10.3)	3 (**0.5**, 0.1-1.5)	421 (**73.3**, 69.5-76.9)	77 (**13.4**, 10.7-16.5)	456 (**79.4**,75.9-82.7)

SB = skinless and boneless, * A presence/absence test for *Campylobacter* was not performed on a single SB Breast sample.

The distribution of *Campylobacter* concentrations (CFU/portion) according to each of the poultry portion sample types is shown in Figure 1, while the distribution for whole chicken carcasses is shown in Figure 2. As expected counts of *Campylobacter* were higher on whole birds than on portions. The modal concentration in each case was in the "positive < LOD" (positive by a presence/absence test but below the limit of detection of quantification; 50 CFU/portion for portions, 200 CFU/carcass for whole chicken carcasses) range. This is notable in the nibbles distribution where 70% of the samples were contaminated but at a concentration <LOD. For thighs, 9.8% of the counts were equal or greater than 1,000 CFU/portion, and the maximum recorded was 2.1 x 10^4 CFU/portion. The maximum for a whole chicken was 6.6 x 10^5 CFU/carcass.

Figure 1. Concentrations of *Campylobacter* in retail poultry portions.

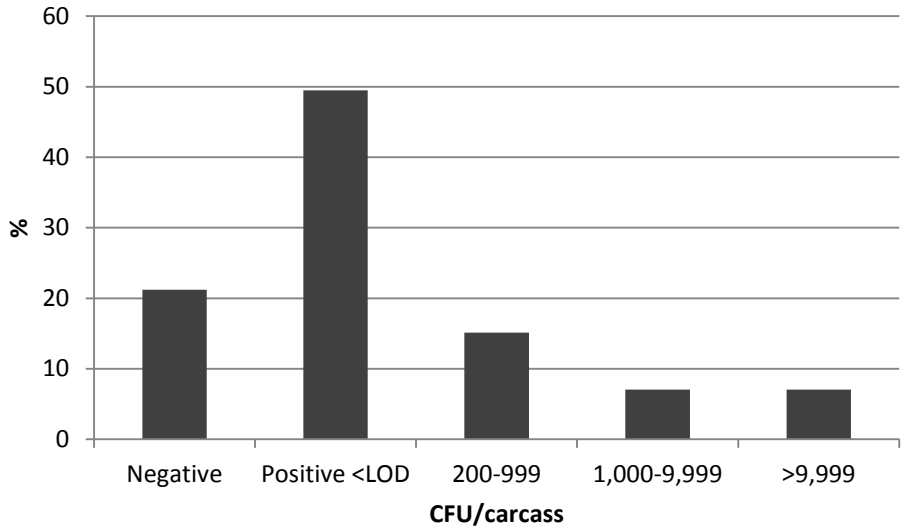


SB = skinless and boneless, LOD = limit of detection (50 CFU/portion)

3.2.2 Concentration of *E. coli*

Since a sensitive method for the detection of *E. coli* was not used (i.e. no enrichment), data for the prevalence of *E. coli* are not presented. A graph was produced to compare the data between poultry portion types and whole chicken carcasses. For the category "other", only one portion contained a value above the LOD and this was 300 CFU/portion. This datum was not plotted as it reduced the resolution between the other samples.

Figure 2. Concentrations of *Campylobacter* in whole chicken carcasses



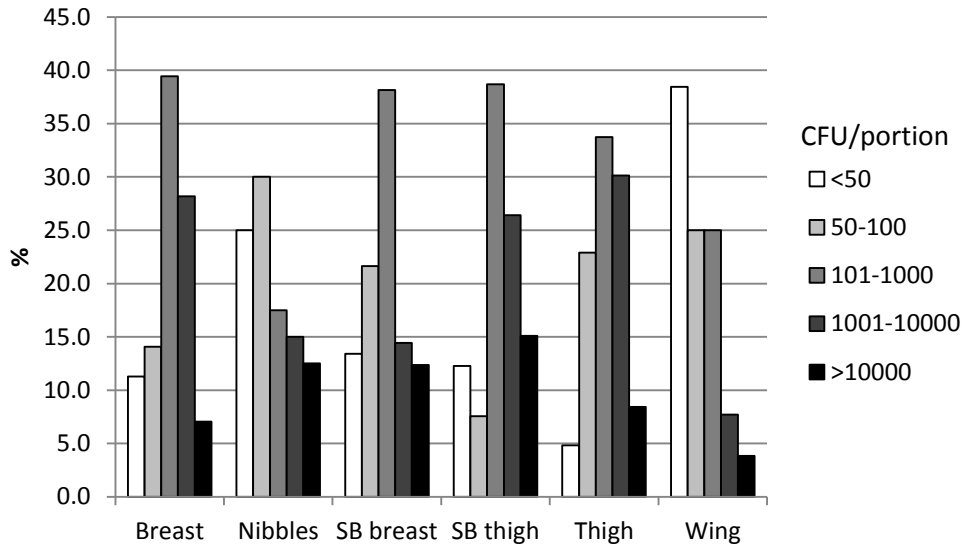
LOD = limit of detection (200 CFU/chicken)

There are some differences apparent among the distributions (Figure 3). For breast, SB breast, SB thigh and thigh, the number most frequently obtained was in the 101-1000 range. However, for nibbles most samples were at 50-100 and for wings there was an equal proportion in both of these ranges. For whole birds the distribution was shifted to higher values, with numbers most frequently occurring in the 1001-10,000 range. This would be expected because the sample size is much larger than for individual poultry parts.

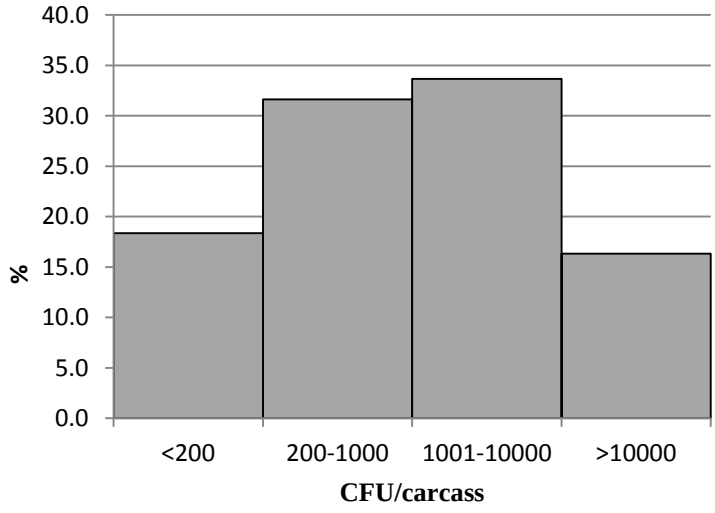
The maximum concentrations (CFU/portion or carcass) for the various samples were; breast 3.1×10^5, nibbles 2.3×10^6, skinless and boneless breast 2.7×10^5 (one sample too numerous to count:TNTC), skinless and boneless thigh 1.9×10^5 (one sample TNTC), thigh 3.8×10^5, wing 2.0×10^5 and whole carcasses 1.2×10^6.

Figure 3. Concentrations of *E. coli* in retail poultry samples

A. Portions (50 CFU/portion is the limit of detection)



B. Whole chicken carcasses (200 CFU/carcass is the limit of detection)

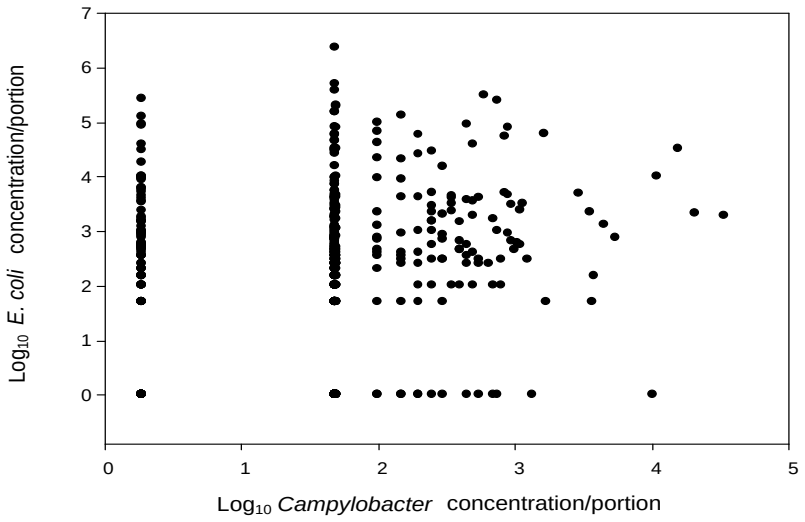


3.2.3 Correlation of *Campylobacter* and *E. coli* concentrations

Samples which did not contain *Campylobacter* spp. had *E. coli* counts ranging from the LOD to concentrations in excess of 10^5/sample (Figures 4 and 5). Negative samples were arbitrarily scored as 0.3 (1.99 $\log_{10}$) for *Campylobacter* or 0 (1 $\log_{10}$) for *E. coli*. Many values are plotted at the LOD or as negative samples in Figures 4 and 5. The large proportion of positive samples

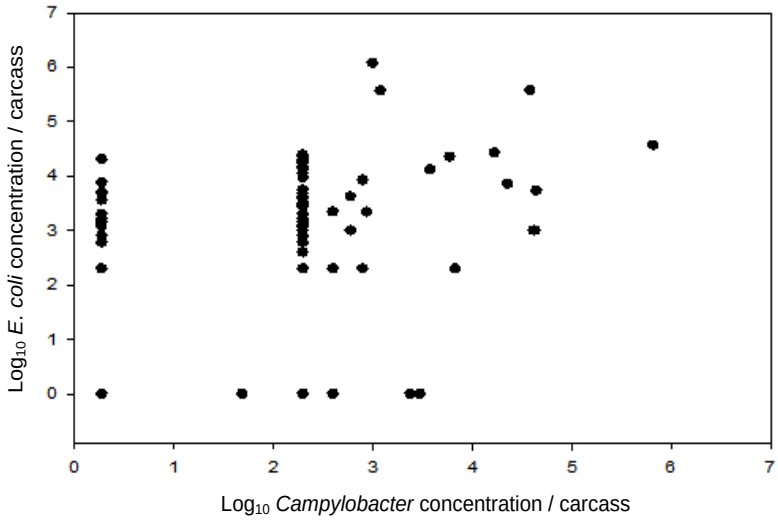
with counts beneath the level of detection precluded statistical tests of correlation. The graphs do not suggest a positive or negative correlation between the two bacterial concentrations.

Figure 4. Plot of the concentrations of *E. coli* against *Campylobacter* for poultry portions



Vertical columns and horizontal rows of points towards the bottom left of the plot represent samples which were < LOD or at the LOD of the methods used.

Figure 5. Plot of the concentrations of *E. coli* against *Campylobacter* for whole chicken carcasses



Vertical columns and horizontal rows of points towards the bottom left of the plot represent samples which were < LOD or at the LOD of the methods used.

3.3 Analysis by geography

3.3.1 Prevalence and concentration of *Campylobacter*

Table 2 shows the prevalence obtained for portions and whole chicken carcasses bought in Auckland, Palmerston North and Christchurch. The prevalence of the various taxa in samples were generally lower in Christchurch than in Auckland. The total *Campylobacter* spp. prevalence rates in portions were 71.1% in Christchurch and 88.5% in Auckland.

The maximum concentrations of *Campylobacter* in portions were; 3.4 x 10^4 CFU/portion (Auckland), 2.1 x 10^4 CFU/portion (Palmerston North) and 1.6 x 10^4 CFU/portion (Christchurch). Figure 6 shows the distribution of concentrations of *Campylobacter* on portions purchased in the three cities.

The distributions for Palmerston North and Auckland are very similar, but the distribution for Christchurch reflects a greater proportion of negative samples.

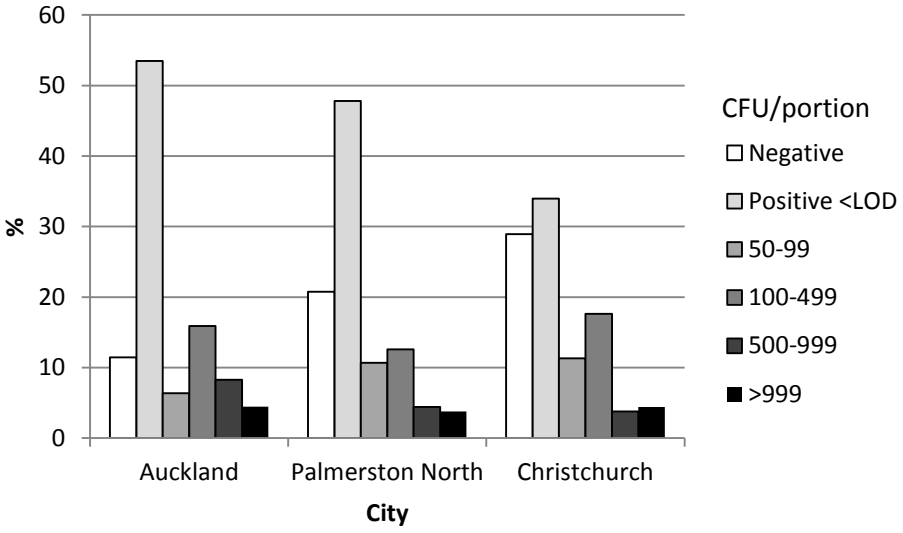
For whole chicken carcasses there was a larger difference in the maximum concentrations measured compared to those obtained for portions; Auckland 6.6 x 10^5 CFU/carcass, Palmerston North 4.2 x 10^4 CFU/chicken carcass and Christchurch 4.6 x 10^4 CFU/carcass. Figure 7 shows the counts for whole chicken carcasses from the three cities.

Table 2. Prevalence and 95% confidence intervals of *Campylobacter* in retail poultry samples according to geographical source

Sample source (n tested)	No. (% **positive**, CI) *C. jejuni* only	No. (% **positive**, CI) *C. coli* only	No. (% **positive**, CI) *C. jejuni* and *C. coli*	No. (% **positive**, CI) non *C. jejuni* or *C. coli* campylobacters	No. (% **positive**, CI) total *C. jejuni*	No. (% **positive**, CI) total *C. coli*	No. (% **positive,** CI) total *Campylobacter*
Portions							
Auckland (157)	108 (**68.8**, 60.9-75.9)	11 (**7.0**, 3.5-12.2)	20 (**12.7**, 8.0-19.0)	0 (**0.0**, 0.0-2.3)	128 (**81.5**, 74.6-87.3)	31 (**19.7**, 13.8-26.8)	139 (**88.5**, 82.5-93.1)
Palmerston North (159)	106 (**66.7**, 58.8-73.9)	6 (**3.8**, 1.4-8.0)	11 (**6.9**, 3.5-12.0)	3 (**1.9**, 0.4-5.4)	117 (**73.6**, 66.0-80.3)	17 (**10.7**, 6.4-16.6)	126 (**79.2**,72.1-85.3)
Christchurch (159)	100 (**62.9**, 54.9-70.4)	8 (**5.0**, 2.2-9.6)	5 (**3.1**, 1.0-7.1)	0 (**0.0**, 0.0-2.3)	105 (**66.0**, 58.1-73.4)	13 (**8.1**, 4.4-13.5)	113 (**71.1**, 63.4-78.0)
Whole Chicken carcasses							
Auckland (34)	25 (**73.5**, 55.6-87.1)	2 (**5.9**, 0.7-19.7)	4 (**11.8**, 3.3-27.5)	0 (**0.0**, 0.0-10.3)	29 (**85.3**, 68.9-95.0)	6 (**17.6**, 6.8-34.5)	31 (**91.2**, 76.3-98.1)
Palmerston North (32)	17 (**53.1**, 34.7-70.9)	1 (**3.1**, 0.1-16.2)	4 (**12.5**, 3.5-29.0)	0 (**0.0**, 0.0-10.9)	21 (**65.6**, 46.8-81.4)	5 (**15.6**, 5.3-32.8)	22 (**68.8**, 50.0-83.9)
Christchurch (33)	20 (**60.6**, 42.1-77.1)	4 (**12.5**, 3.5-29.0)	1 (**3.1**, 0.1-16.2)	0 (**0.0**, 0.0-10.9)	21 (**63.6**, 45.1-79.6)	5 (**15.6**, 5.3-32.8)	25 (**75.8**, 57.7-88.9)

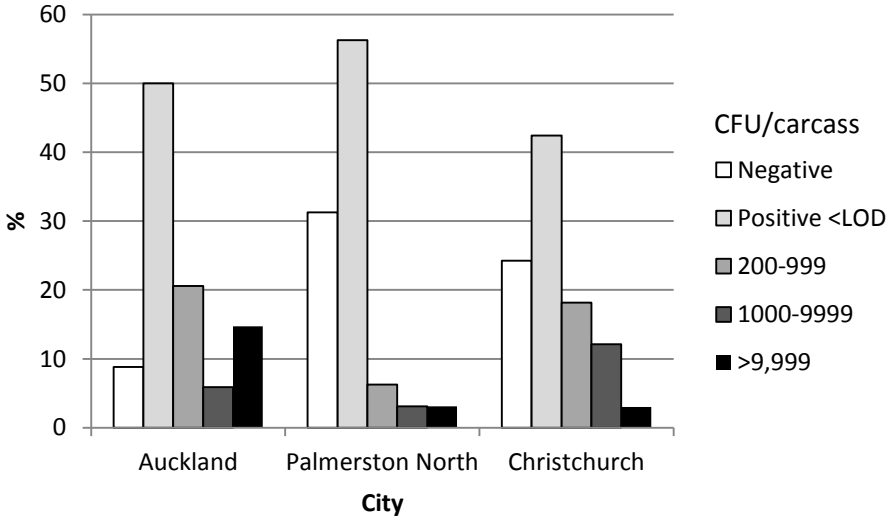
SB = skinless and boneless

Figure 6. Distribution of *Campylobacter* counts in portions from different cities in New Zealand



LOD = limit of detection (50 CFU/portion)

Figure 7. Distribution of *Campylobacter* counts in whole chicken carcasses from different cities in New Zealand

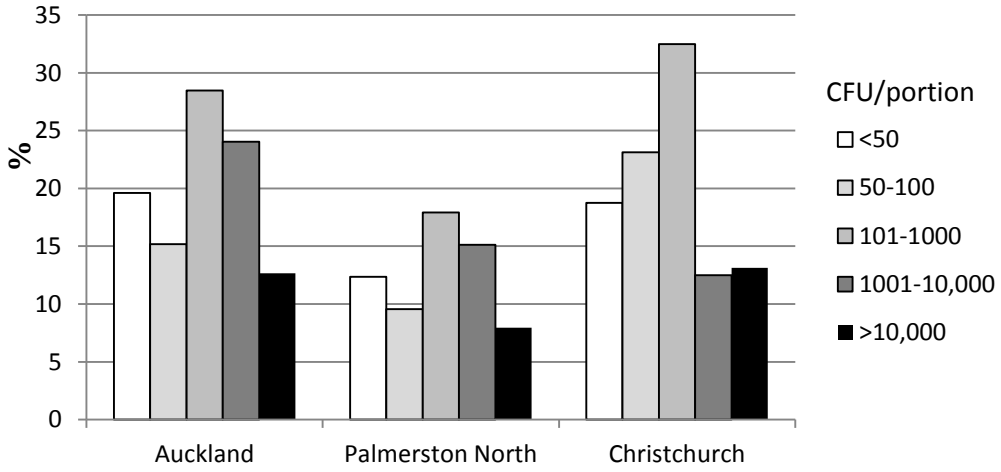


LOD = limit of detection (200 CFU/carcass)

3.3.2 Concentration of *E. coli*

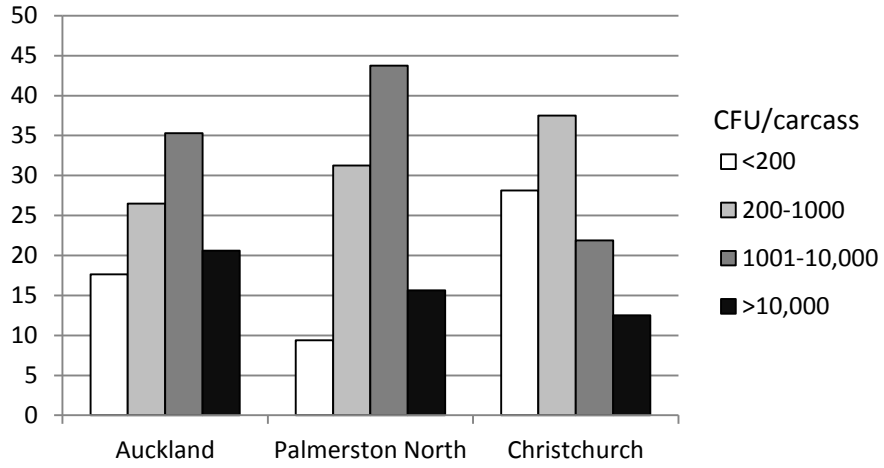
The distributions for concentrations of *E. coli* in portions did not match those for *Campylobacter*, for example the Christchurch samples had a relatively high proportion of samples with *E. coli* counts in the >10,000 CFU/portion range (Figure 8). Differences in distributions on whole chicken carcasses were minor, but the distribution for the Christchurch samples was skewed to the lower counts (Figure 9).

Figure 8. Distribution of *E. coli* counts in portions from different cities in New Zealand



50 CFU/portion is the limit of detection

Figure 9. Distribution of *E. coli* counts in whole chicken carcasses from different cities in New Zealand



200 CFU/portion is the limit of detection

3.4 Analysis by season

3.4.1 Prevalence and concentration of *Campylobacter*

Table 3 shows the prevalence obtained for portions and whole chicken carcasses bought during the four seasons of the year. The prevalence in portions containing *C. jejuni* only was lower in the Southern hemisphere Spring (56.7%) and Winter (57.1%) than in the Autumn (79.4%). For samples containing both *C. jejuni* and *C. coli* the prevalence was higher in the Spring (15.8%) than either the Autumn (2.5%) or Winter (2.2%). The prevalence of total *C. jejuni* was lower in the Winter (59.7%) than either the Autumn (81.6%) or Summer (81.0%). The total *Campylobacter* spp. prevalence rates were 71.4% in Winter and 88.2% in Autumn.

For whole chicken carcasses, the highest prevalence of carcasses positive for *Campylobacter jejuni* or *Campylobacter coli* was also observed in Summer (81.8%) and Autumn (90.0%).

The maximum concentrations for portions were; 2.1 x 10^4 CFU/portion (Spring), 1.6 x 10^4 CFU/portion (Summer), 1.1 x 10^3 CFU/portion (Autumn) and 3.4 x 10^4 CFU/portion (Winter). Figure 10 shows the distribution of concentrations of *Campylobacter* on portions purchased in the four seasons. In all cases the most counts fell into the positive <LOD portion range, but the Winter samples had a higher proportion of negative samples than the others.

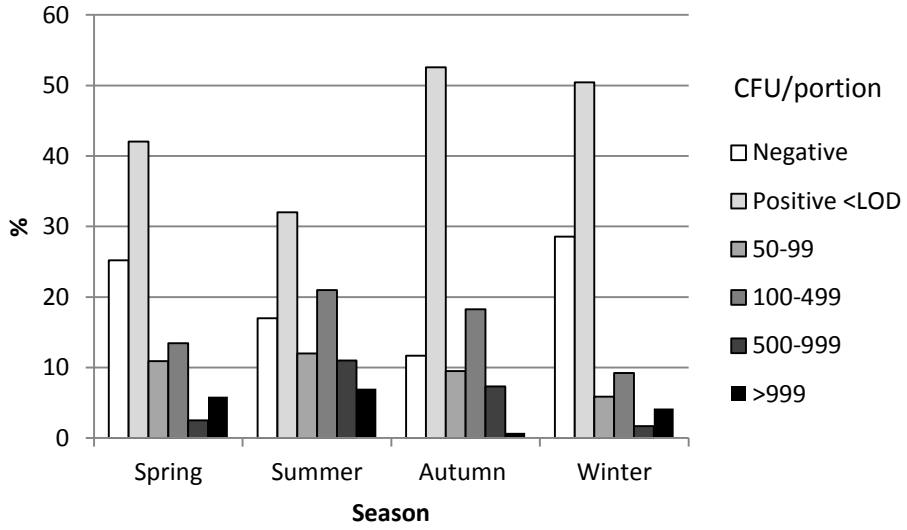
For whole chicken carcasses the Winter samples had the bird with the highest concentration (6.6 x 10^5 CFU/chicken carcass). The highest concentrations were similar for samples tested in the other seasons (Spring 4.6 x 10^4 CFU/chicken carcass, Summer 2.3 x 10^4 CFU/chicken carcass and Autumn 4.3 x 10^4 CFU/chicken carcass). Comparison of the distribution of concentrations (Figure 11) shows the lower prevalence was again reflected in the Winter samples.

Table 3. Prevalence and 95% confidence intervals of *Campylobacter* in retail poultry samples according to season

Sample source (n tested)	No. (**% positive**, CI) *C. jejuni* only	No. (**% positive**, CI) *C. coli* only	No. (**% positive**, CI) *C. jejuni* and *C. coli*	No. (**% positive**, CI) non *C. jejuni* or *C. coli* campylobacters	No. (**% positive**, CI) total *C. jejuni*	No. (**% positive**, CI) total *C. coli*	No. (**% positive,** CI) total *Campylobacter*
Portions							
Spring (120)	68 (**56.7**, 47.3-65.7)	3 (**2.5**, 0.5-7.1)	19 (**15.8**, 9.8-23.6)	0 (**0.0**, 0.0-3.0)	87 (**72.5**, 63.6-80.3)	22 (**18.3**, 11.9-26.4)	90 (**75.0**, 66.3-82.5)
Summer (100)	70 (**70.0**, 60.0-78.8)	2 (**2.0**, 0.2-7.0)	11 (**10.9**, 5.6-18.7)	0 (**0.0**, 0.0-3.6)	81 (**81.0**, 71.9-88.2)	13 (**12.9**, 7.0-21.0)	83 (**83.0**, 74.2-89.8)
Autumn (136)	108 (**79.4**, 71.6-85.9)	9 (**6.6**, 3.1-12.2)	3 (**2.2**, 0.5-6.3)	0 (**0.0**, 0.0-2.7)	111 (**81.6**, 74.1-87.7)	12 (**8.8**, 4.6-14.9)	120 (**88.2**, 81.6-93.1)
Winter (119)	68 (**57.1**, 47.7-66.2)	11 (**9.2**, 4.7-15.9)	3 (**2.5**, 0.5-7.2)	3 (**2.5**; 0.5,7.2)	71 (**59.7**, 50.3-68.6)	14 (**11.8**, 6.6-19.0)	85 (**71.4**, 62.4-79.3)
Whole Birds							
Spring (24)	13 (**54.2**, 32.8-74.4)	1 (**4.2**, 0.1-21.1)	4 (**16.7**, 4.7-37.4)	0 (**0.0**, 0.0-14.2)	17 (**70.8**, 48.9-87.4)	5 (**20.8**, 7.1-42.2)	18 (**75.0**, 52.3-90.2)
Summer (22)	13 (**59.1**, 36.4-79.3)	2 (**9.5**, 1.2-30.4)	3 (**14.3**, 3.0-36.3)	0 (**0.0**, 0.0-16.1)	16 (**72.7**, 49.8-89.3)	5 (**23.8**, 8.2-47.2)	18 (**81.8**, 59.7-94.8)
Autumn (30)	24 (**80.0**, 61.4-92.3)	2 (**6.7**, 0.8-22.1)	1 (**3.3**, 0.1-17.2)	0 (**0.0**, 0.0-11.6)	25 (**83.3**, 65.3-94.4)	3 (**10.0**, 2.1-26.5)	27 (**90.0**, 73.5-97.9)
Winter (25)	14 (**56.0**, 34.9-75.6)	2 (**8.0**, 1.0-26.0)	1 (**4.0**, 0.1-20.4)	0 (**0.0**, 0.0-13.7)	15 (**60.0**, 38.7-78.9)	3 (**12.0**, 2.5-31.2)	17 (**68.0**, 46.5-85.1)

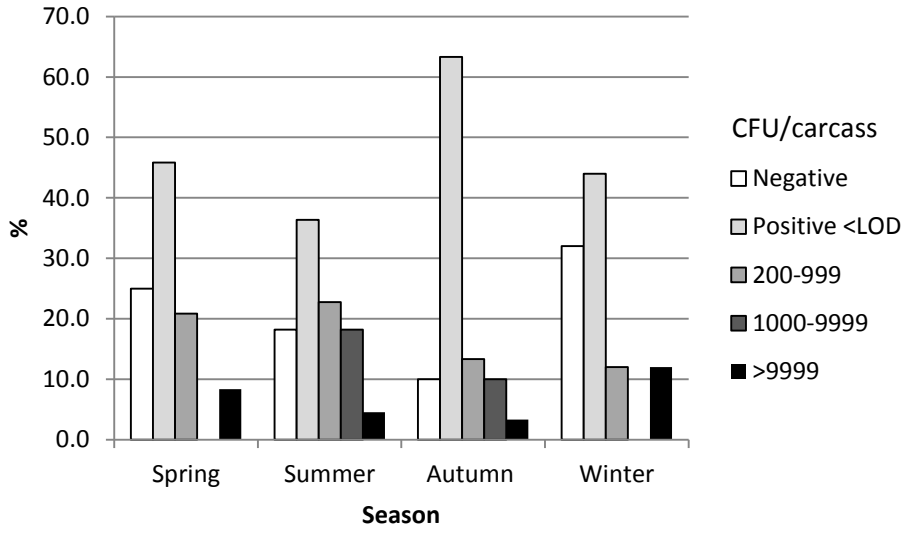
SB = skinless and boneless

Figure 10. Distribution of *Campylobacter* counts in portions sampled in different seasons



LOD = limit of detection (50 CFU/portion)

Figure 11. Distribution of *Campylobacter* counts in whole chicken carcasses sampled in different seasons

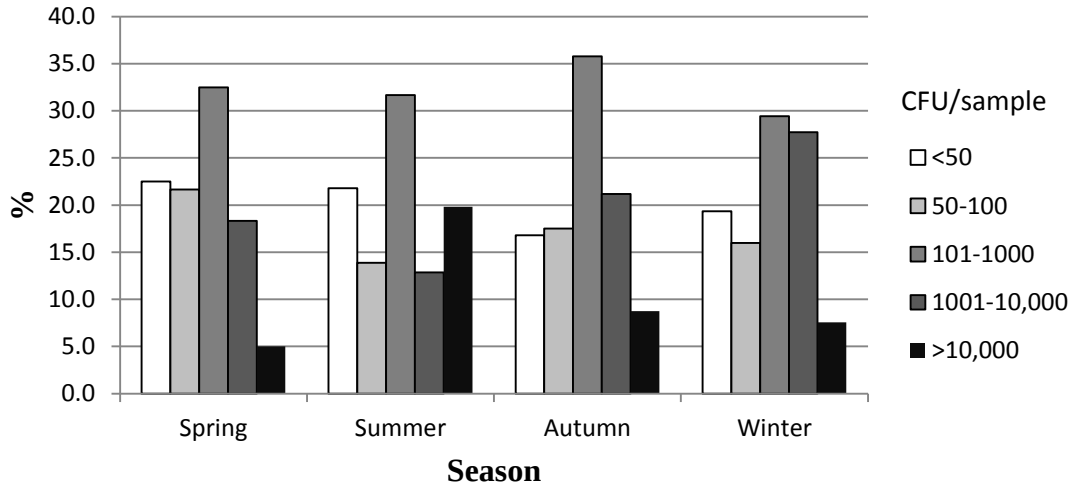


LOD = limit of detection (200 CFU/carcass)

3.4.2 Concentration of *E. coli*

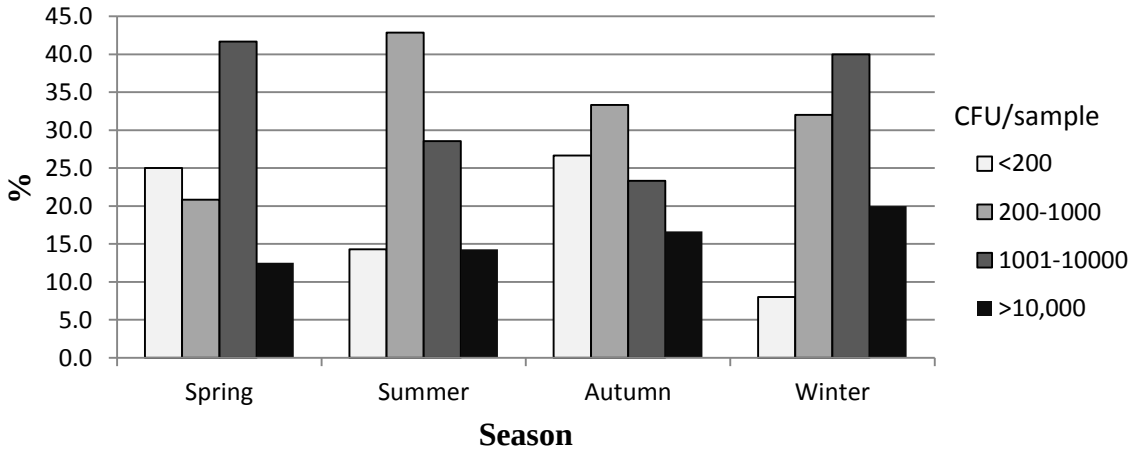
The distributions for concentrations of *E. coli* did not match well those for *Campylobacter*, for example the Winter samples had a relatively high proportion of samples with counts in 1001-10,000 bin (Figure 12). Differences in distributions on whole chicken carcasses were quite different between seasons, with Spring and Winter having the highest proportion of counts in the 1001-10,000 range, while for Summer and Autumn the highest proportion was in the 200-1000 range (Figure 13).

Figure 12. Distribution of *E. coli* counts in portions sampled in different seasons



50 CFU/portion is the limit of detection

Figure 13. Distribution of *E. coli* counts in whole chicken carcasses sampled in different seasons



200 CFU/portion is the limit of detection

4 DISCUSSION

The finding of 78.8% for the prevalence of *Campylobacter* in whole chicken carcasses available at retail in three cities across New Zealand (Table 1) is comparable to the prevalence found in chickens in a previous survey in the Manawatu (French 2008). An industry study also published in 2008 (Chrystal *et al.* 2008), testing samples collected in October and November 2007, reported a prevalence of 44.8% on poultry carcasses, which is appreciably lower than the prevalence reported in this report. The differences in prevalence between the studies may be explained by the observation that the industry study had a detection limit of 400 CFU/carcass and regarded any carcass with <400 CFU/sample as negative; this is inferred from the materials and methods in the paper. In the present ESR study, enrichment was used and this should be a more sensitive means of detecting the presence of *Campylobacter* spp. However, when the concentration data for carcasses are considered as positive if ≥ 400 CFU/carcass then the prevalence for the current survey is 24.2% (24/99 samples "positive"), a prevalence over 20% lower than that of Chrystal *et al*. (2008).

The median concentration of *Campylobacter* on positive carcasses in the 2008 industry study was 3.5 $\log_{10}$ CFU/carcass, which is higher than that reported here where the median was 2.3 $\log_{10}$CFU/carcass, which corresponds to carcasses which were positive but which gave counts beneath the level of detection during enumeration. If only samples for which a count was obtained are analysed then the median in the current study was still only 2.94 $\log_{10}$CFU/chicken carcass. The maximum concentration in the industry study of 6.8 $\log_{10}$CFU/carcass was ten times higher than the maximum reported here (5.8 $\log_{10}$ CFU/carcass). The two studies used the same volume of diluent to rinse the birds, but the current ESR study plated double the volume of the rinsate.

For portions, a previous New Zealand survey found a *Campylobacter* prevalence of only 56.6% (Hudson *et al.* 1999). This may be because the earlier study tested 10g portions of flesh while the current study tested whole portion rinsates and so is likely to be more sensitive. A previous report on the location of *Campylobacter* on carcasses at the processing plant (Paulin and Wong 2008) identified the wing as a site likely to be contaminated by *Campylobacter*, but in the present study the wing represented the portion with the lowest prevalence, and significantly less than the prevalence on skinless and boneless thigh. However, the study of Paulin and Wong (2008) tested portions prior to rinsing and spin chilling. In a study of post spin chiller samples

(Paulin 2011) carcasses from two processors where split into portions which where rinsed in buffered peptone water. Of the leg portions, 82% (24/29) of rinsates contained *Campylobacter* at less than the limit of detection (150 CFU/portion rinsate), while 52% (15/29) of wing portion rinsates contained *Campylobacter* at less than the limit of detection (75 CFU/portion rinsate). The highest concentration in either sample type from either processor was 4.37 x 10^3 CFU/portion.

Comparisons with the previous ESR studies of minced or diced chicken (Wong *et al.* 2007, Wong and Hudson 2011) are inappropriate because of the different nature of the two sample types (mincing chicken will essentially dilute any contamination, increasing prevalence and decreasing concentration). The 2007 study was carried out primarily to increase the probability of isolating *Campylobacter* for subsequent typing, while the 2009 study was undertaken to determine any changes in the intervening period.

When considering geographical location of the retail outlet, the prevalence of *Campylobacter* spp. was higher in portions purchased in Auckland than it was in Christchurch. This may be a reflection of different practices at the poultry processing plants supplying these two areas, a consequence of higher turnover, or a reflection of a higher *Campylobacter* load on pre-slaughter birds in Auckland. Such a difference in prevalence between cities was not noted in a previous report on minced and diced chicken, although a smaller number of samples was tested at each centre in the previous study (Wong and Hudson 2011). The proportion of portions containing >100 CFU/portion *Campylobacter* was similar for each city sampled (28.7% in Auckland, 20.7% in Palmerston North and 25.8% in Christchurch). It should be noted that Auckland was oversampled in the Autumn (section 3.1), the season with the highest prevalence of *Campylobacter*. For chicken carcasses, the proportion of samples containing *Campylobacter* at >200 CFU/chicken carcasses was lower (12.5%) in Palmerston North than Auckland (41.2%) or Christchurch (33.3%).

The prevalence of *Campylobacter* spp. in portions was lower in the Winter than the Autumn, although Auckland was oversampled in the Autumn, as previously mentioned. The proportion of samples containing *Campylobacter* at >100 CFU/portion was also lower in the Winter samples (15.1%) compared to Spring (21.8%), Summer (39.0%) and Autumn (26.3%). Chicken carcasses sampled in the Summer also had a higher proportion of samples containing

>200 CFU/chicken carcass (45.5%) than in the other months; Spring 29.2%, Autumn 26.7% and Winter 24.0%. This seasonal pattern is consistent with the incidence of reported human disease whereby the Winter months usually are the lowest in terms of incidence. No seasonal pattern for prevalence was found in a previous New Zealand study of minced and diced chicken (Wong *et al.* 2007).

An absence of correlation between indicators (including *E. coli*) and pathogens (including *Campylobacter*) has also been shown in Swedish (Lindblad *et al.* 2006) and Australian (Pointon *et al.* 2008) surveys of whole chicken carcasses, as well as in a previous New Zealand survey of mechanically separated poultry meat (Wong *et al.* 2011). In contrast, a US study found the mean concentration of *Campylobacter* in chicken carcass rinses to be higher in samples containing >1.1 $\log_{10}$ CFU *E. coli*/ml than in samples containing lower concentrations of *E. coli*, although this was not statistically significant (Altekruse *et al.* 2009).

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