QUANTIFICATION OF *CAMPYLOBACTER* FROM INTERNAL AND EXTERNAL CARCASS RINSES.

FINAL REPORT

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FINAL REPORT

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SUMMARY

Campylobacteriosis is a leading cause of foodborne disease worldwide, and is the most frequently reported notifiable gastrointestinal illness in New Zealand comprising 65.9% of all notifiable disease cases in 2007 (ESR Annual Report, 2007). Poultry, and poultry products, represent an important risk factor for campylobacteriosis in humans. Bacteria can be transferred onto poultry via fluid and faeces from the gastro-intestinal tract of infected birds either prior to, or during processing itself. Bacterial persistence on carcasses can therefore provide a major source of *Campylobacter*, and can create a risk for consumers if undercooked poultry is eaten, or if contaminated chicken is not adequately stored or handled. A recent study comparing 'source attribution' models for human campylobacteriosis in New Zealand concluded that chicken accounted for between 55 and 71% of human cases (depending on the model used) (French *et al*., 2008).

This study was conducted in order to quantify the distribution of *Campylobacter* on various sites of the poultry carcass, and to determine whether any differences existed in the relative distribution of *Campylobacter* on chicken between two New Zealand poultry processors (defined as Processor A and Processor B). Samples taken throughout the trial included 'selected sites' (cavity, neck, vent, skin remaining on the carcass after portion removal) and 'portions' (parts of the chicken commonly sold as separate pieces including thighs, drumsticks, breasts, wings) (Tables 1 and 4). Samples were taken throughout the trial at a position prior to the employment of any major intervention strategies therefore, the results may not be fully representative of the final retail product.

A pilot study was conducted initially, by sampling 4 birds per poultry slaughterhouse. Results suggested that the cavity represented the site where the highest *Campylobacter* counts were found. Furthermore, following three consecutive rinses of this area, there was very little difference in the bacterial recovery between the first and last rinse. Of the individual portions selected for sampling (Table 1), the wings had the highest proportion of *Campylobacter* on birds from both poultry processors. Interestingly, from Processor A, the left hand portions consistently had higher *Campylobacter* counts than the right hand portions.

The complete trial consisted of rinsates taken from at least eight sample sites per bird and of 61 birds in total (this included the 'pilot' birds from which 15 rinsates per bird were sampled and the 'main' trial from which 8 rinsates per bird were sampled). In terms of comparisons between the poultry processors, the neck skin was the only site where the relative proportion of total *Campylobacter* was statistically different. When considering individual sample sites, the main trial (all birds except the pilot birds) results were consistent with the pilot studies in that the cavity and the wings represented potential 'hot spots' for *Campylobacter*.

Results from this study will be used by the New Zealand Food Safety Authority (NZFSA) to assist in approaches to control *Campylobacter* through the food chain, and will contribute to ongoing pathogen risk model developments in this area. Furthermore, data obtained from this study will help inform poultry processors regarding the impact of some dressing procedures.

1. INTRODUCTION

Campylobacteriosis is a leading cause of foodborne disease worldwide, and is the most frequently reported notifiable gastrointestinal illness in New Zealand with 12, 776 cases reported during 2007 (a rate of 302.2 per 100, 000 population). While this is significantly lower than the 2006 rate of 379.3 per 100 000 population (15, 873 cases), campylobacteriosis continues to remain a major human health issue in New Zealand, comprising 65.9% of all notifiable disease cases in 2007 (ESR Annual Report, 2007).

Campylobacter spp. are commonly found in the intestinal flora of a large number of wild and domesticated animals and birds, including chickens. Poultry, and poultry products, represent an important risk factor for campylobacteriosis in humans as the bacteria can be transferred onto poultry via fluid and faeces from the gastro-intestinal tract of infected birds either prior to, or during poultry processing itself. Bacterial persistence on carcasses can therefore provide a major source of *Campylobacter*, and can potentially create a risk for consumers if undercooked poultry is eaten, or if contaminated chicken is not properly stored or handled. A case-control study conducted in four urban centres in New Zealand concluded that greater than 50% of all campylobacteriosis cases could be attributed to consumption of raw or undercooked chicken (Eberhart-Phillips *et al.*, 1997). A more recent study comparing 'source attribution' models for human campylobacteriosis in New Zealand concluded that chicken accounted for between 55 and 71% of human cases (depending on the model used) (French *et al*., 2008).

To date, there have been very few published studies exploring the numbers of *Campylobacter* on different portions of the same poultry carcass. As such, there is a need to quantitatively assess the bacterial distribution by mapping *Campylobacter* numbers from different parts of the carcass itself and correlating this data with dressing procedures used by different poultry processors. Data obtained from this study will contribute to risk assessment approaches of *Campylobacter* in poultry and will help inform poultry processors regarding the impact of specific dressing procedures.

A study by Berrang *et al*, (2001) undertook a series of experiments to compare pathogen counts from poultry skin and uncompromised meat beneath. Portions were collected from different points on the production chain and from retail outlets. One of the sampling positions selected was after the inside/outside rinse and immediately before the birds entered the spin chiller. While not specifically comparing bacterial counts between

portions, data is available from breast (meat and skin), thigh (meat and skin) and drumstick (meat and skin) from the same birds. Results indicated that there were minor differences only in the numbers of *Campylobacter* recovered between the portions with 3.8 $\log_{10}$ CFU/part, 3.9 $\log_{10}$ CFU/part and 3.7 $\log_{10}$ CFU/part for individual (n=10) breast, thigh and drumstick respectively. When determining numbers of all organisms tested including: *Campylobacter*, coliforms, *E. coli* and total aerobes, the counts obtained from the drumstick samples were marginally lower than the counts obtained from the breast and thigh samples. With unmatched samples (not from the same carcass), taken from retail outlets, the numbers of *Campylobacter* recovered from the skin-on drumsticks were lower than those recovered from the skin-on thighs and breasts with 2.1 $\log_{10}$ CFU/part, 2.7 $\log_{10}$ CFU/part and 2.8 $\log_{10}$ CFU/part respectively.

A few papers have considered the prevalence and numbers of pathogens on poultry portions in relation to different sampling methods. A study by Gill *et al*, (2005) compared excision (skin and muscle), rinsing and swabbing to quantify aerobic bacteria, coliforms and *E. coli* recovered from unpaired skin-on breasts and thighs, skinned breasts and tumbled breasts. When rinsed, skin-on samples alone were compared; the bacterial recovery for all pathogens tested was marginally higher from the breast, than the thigh. Scherer, (2006) compared different sampling (leg rinse and leg skin homogenisation) and enumeration techniques (MPN and direct plating) for the quantification of *Campylobacter* on raw, retail chicken legs. The authors concluded that there were no significant differences between the leg rinse and skin homogenisation sampling methods with 77% and 70% respectively being positive for *Campylobacter*. Both of these studies (Gill *et al*., 2005; Scherer, 2006) have demonstrated that quantitative analysis of *Campylobacter* from poultry depends on the sampling technique and enumeration methodology used. Jorgensen *et al*, (2002) evaluated methods for the isolation and enumeration of *Salmonella* and *Campylobacter* from raw, retail chicken. When comparing samples with neck-skin, carcass-rinse or carcass-rinse with homogenised skin of the chicken, the authors concluded that *Salmonella* was more frequently isolated from samples containing chicken skin in comparison with those containing carcass-rinse fluid only. In contrast, the likelihood of isolating *Campylobacter* spp. from neck-skin, carcass-rinse or carcass-rinse plus whole skin samples was similar.

Two studies by the same group have investigated the prevalence of *Campylobacter* distributed over retail chicken breast fillets and retail chicken legs with the aim of generating data distributions to be used in risk assessments. *Campylobacter* were shown to be unevenly

distributed over the legs with the majority of contamination being on the surface and only 11% of the samples contaminated on meat interior as well (Luber *et al*., 2005). Similarly, high levels of *Campylobacter* were found on the breast meat surface (87%) in comparison with low levels of deep tissue contamination (20%) (Luber and Bartelt (2007). The authors concluded that cross-contamination during the preparation of contaminated chicken is a more important pathway for consumers' exposure to *Campylobacter* than the consumption of contaminated meat.

In addition to the data available relating to poultry carcasses, a limited amount of information has been published on the distribution of microbial contamination (aerobic plate counts and *E. coli*) on both lamb and beef carcasses in New Zealand R&D Brief 55-Distribution of microbial contamination on the carcass. Ten different areas of beef carcasses were selected, from three dressing lines, and results were used to create a 'map' showing the levels of microbial contamination on the different areas. While all microbial counts were low, the hock consistently carried the highest level of bacterial contamination due to the use of mechanical hock cutters that cut directly through both the hide and the carcass. High contamination was typically found at those sites associated with opening cuts and/or subject to hide contact during hide removal (Bell, 1997). Aerobic plate counts and *E. coli* enumerations were used to determine sources of bacterial contamination during sheep dressing. The fleece was found to be the main source of contamination however, after pelt removal, subsequent dressing operations did not contribute significantly to microbial contamination. Inverted dressing systems, where the carcass hangs from the back legs after the pelt has been removed, produced carcasses with a lower contamination level than conventional systems. The areas of highest contamination were the forequarter region with inverted dressing and the hindquarter region with conventional dressing. Both of these areas being sites where cuts are made through the skin and direct fleece contact occurs (Bell and Hathaway, 1996).

Given the relatively few published studies aimed at mapping *Campylobacter* to different areas of the poultry carcass, this project was initiated to establish the bacterial distribution on various skin-on parts of the whole chicken, including the cavity. Furthermore, this study was conducted with birds taken from two commercial New Zealand poultry processors.

Results from this study will be used by the New Zealand Food Safety Authority to assist in approaches to control *Campylobacter* through the food chain, and will contribute to

ongoing pathogen risk model developments in this area. Furthermore, data obtained from this study will help to inform poultry processors about the impact of some dressing procedures.

2. METHODOLOGY DEVELOPMENT

2.1 Pilot studies

The pilot studies involved two New Zealand poultry processors (Processors A and B). Two pilot studies were conducted and in each study, two birds per processor were sampled.

2.1.1 Pilot study #1

This study was conducted in order to establish the best practice bird collection and sampling methods. Several points were addressed including:

1) The most suitable position and method, in terms of personnel safety and avoidance of carcass cross contamination, for removing birds from the processing line.
2) The best practice for transporting chickens from the line to the laboratory in order to avoid bird-to-bird contact and therefore cross contamination of samples.
3) The precise methodology for aseptically dissecting the carcass and undertaking portion rinsing.
4) The determination of an appropriate volume of rinsate to be used per portion.
5) The time taken to process a single bird.
6) The most suitable number of birds to be sampled per day.
7) The best practice for packaging and sending the samples to ESR, Christchurch to ensure that the NMD requirements for transportation of samples were adhered to (see 2.2.4).

Both birds, from each poultry processor, were sampled during January 2008. The time of year selected for this trial was chosen to coincide with the high peak of human cases typically observed during the summer months. Third cut birds, weighing approximately 3 kg, were selected from flocks with second cut-positive caecal tests. The sites sampled and the volumes of rinsate used are shown in Table 1:

Table 1: Sampling sites and volume of rinsate used for pilot study #1

Sample number	Sample description	Volume of rinsate: Processor A	Volume of rinsate: Processor B
Selected sampling sites			
1	Cavity rinse #1	50 ml	50 ml
2	Cavity rinse #2	50 ml	50 ml
3	Carcass shell	200 ml	150 ml
4	Neck flap/ glove/hook rinse	100 ml	75 ml
5	Vent flap ('Parsons nose') /glove rinse	100 ml	75 ml
6	Remaining skin	100 ml	75 ml
7	Glove and instrument rinse	75 ml	75 ml
Selected portions			
8	Left wing	100 ml	75 ml
9	Right wing	100 ml	75 ml
10	Left drumstick	100 ml	75 ml
11	Right drumstick	100 ml	75 ml
12	Left thigh	100 ml	75 ml
13	Right thigh	100 ml	75 ml
14	Left breast	100 ml	75 ml
15	Right breast	100 ml	75 ml
		Total 1475 ml	**Total 1150 ml**

The reason for the differences in rinsate volume used between poultry processors was because after the first trial (at Processor A), it was decided that the volume chosen was slightly too high. This was modified for pilot #1 at Processor B. Furthermore, to account for the fact that there may be a large amount of variation in the bacterial counts obtained, it was decided that the larger volume, as used by Processor A, might leave low sample counts at, and possibly below, the limit of detection. Figure 1 highlights the importance of using an appropriate volume of rinsate. This needs to be sufficient to almost cover the portion, thereby allowing good agitation to take place with maximum bacterial removal from skin crevices and feather follicles.

Figure 1: Determination of the appropriate rinsate volume for breast (1a and 1b) and thigh (1c and 1d) portions. Portions depicted in 1a and 1c are in 25 ml of rinsate and portions depicted in 1b and 1d are in 100 ml of rinsate.

Figure 1a

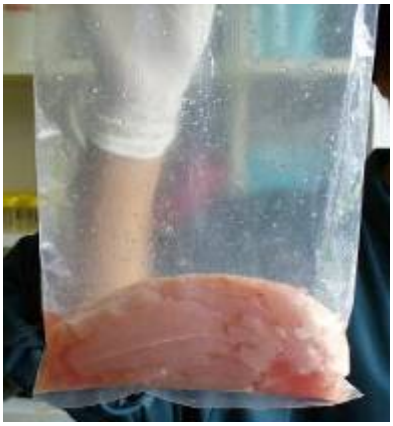


Figure 1b

Figure 1c

Figure 1d

2.1.2 Pilot study #2

A second pilot study was initiated once the results from the first study had been analysed. The second pilot study was necessary to refine the methodology and to ascertain whether some of the samples could be amalgamated or eliminated for the remainder of the trial. These experiments were conducted during February, again using birds from known positive flocks. The number of samples taken were the same as in the first pilot study, and the volume of rinsate, per sample, used by each processor was the same as that used by Processor B in pilot #1 (see Table 1).

2.2 Carcass mapping trial

Following analysis of the results obtained from the two pilot studies, a further 26 birds for Processor A and 27 birds for Processor B were selected for the main experimental trial (making 61 birds in total including the 8 birds from the two pilot studies). These birds were all from known positive flocks (caecal sample positive from the previous cut) and were typically from the third or fourth cut. Birds were selected, portioned and rinsed in groups of three (per week per poultry processor) and the main trial was conducted during the months of April, May and June 2008. A summary of the sampling dates and cut number of birds used by Processor A (Table 2) and Processor B (Table 3) have been tabulated below. In addition, the final sampling sites and volumes of rinsate used for the main trial are given in Table 4.

Table 2: Summary of birds sampled by Processor A

Bird number	Sampling date	Received by ESR	Cut number
1 (pilot #1/1)	8.1.08	9.1.08	3
2 (pilot #1/2)	8.1.08	9.1.08	3
3 (pilot #2/1)	12.2.08	13.2.08	2
4 (pilot #2/1)	12.2.08	13.2.08	2
5	17.4.08	18.4.08	3
6	17.4.08	18.4.08	3
7	17.4.08	18.4.08	4
8	8.5.08	9.5.08	4
9	8.5.08	9.5.08	4
10	8.5.08	9.5.08	4
11	15.5.08	16.5.08	4
12	15.5.08	16.5.08	4
13	15.5.08	16.5.08	3
14	22.5.08	23.5.08	3
15	22.5.08	23.5.08	3
16	22.5.08	23.5.08	3
17	29.5.08	30.5.08	3
18	29.5.08	30.5.08	3
19	5.6.08	6.6.08	3
20	5.6.08	6.6.08	3
21	5.6.08	6.6.08	2
22	19.6.08	20.6.08	5
23	19.6.08	20.6.08	5
24	24.6.08	25.6.08	3
25	24.6.08	25.6.08	3
26	24.6.08	25.6.08	4
27	24.6.08	25.6.08	4
28	26.6.08	27.6.08	3
29	26.6.08	27.6.08	3
30	26.6.08	27.6.08	3

Table 3: Summary of birds sampled by Processor B

Bird number	Sampling date	Received by ESR	Cut number
1 (pilot #1/1)	23.1.08	24.1.08	3
2 (pilot #1/2)	23.1.08	24.1.08	3
3 (pilot #2/1)	2.08	2.08	3
4 (pilot #2/2)	2.08	2.08	3
5	23.4.08	24.4.08	3
6	23.4.08	24.4.08	3
7	23.4.08	24.4.08	3
8	30.4.08	1.5.08	3
9	30.4.08	1.5.08	3
10	30.4.08	1.5.08	3
11	7.5.08	8.5.08	2 (1^{st} +ve)
12	7.5.08	8.5.08	2 (1^{st} +ve)
13	7.5.08	8.5.08	2 (1^{st} +ve)
14	14.5.08	15.5.08	2 (1^{st} +ve)
15	14.5.08	15.5.08	2 (1^{st} +ve)
16	14.5.08	15.5.08	2 (1^{st} +ve)
17	22.5.08	23.5.08	3
18	22.5.08	23.5.08	3
19	22.5.08	23.5.08	3
20	29.5.08	30.5.08	3
21	29.5.08	30.5.08	3
22	29.5.08	30.5.08	3
23	4.6.08	5.6.08	3
24	4.6.08	5.6.08	3
25	4.6.08	5.6.08	3
26	11.6.08	12.6.08	2 (1^{st} +ve)
27	11.6.08	12.6.08	2 (1^{st} +ve)
28	11.6.08	12.6.08	2 (1^{st} +ve)
29	18.6.08	19.6.08	3
30	18.6.08	19.6.08	3
31	18.6.08	19.6.08	3

Table 4: Sampling sites and volumes of rinsate used for the main carcass mapping trial

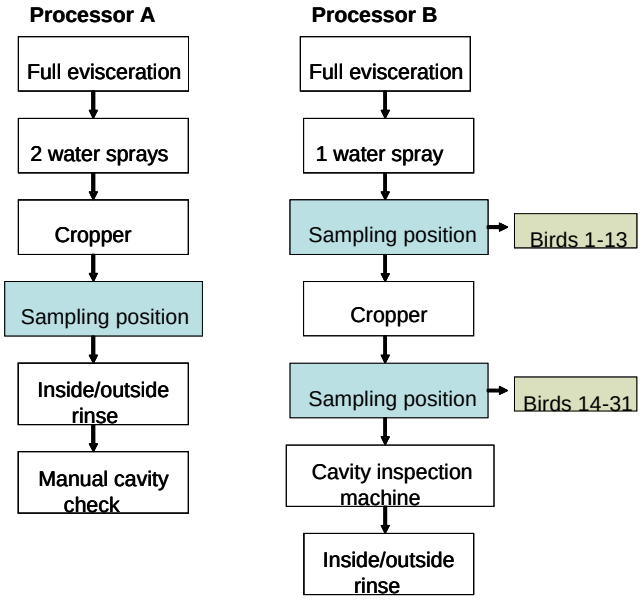
Sample number	Sample description	Volume of rinsate: Processors A and B
Selected sampling sites		
1	Cavity rinse	50 ml
2	Neck flap, glove, hook rinse	75 ml
3	Vent flap ('Parsons nose'), glove rinse	75 ml
4	Remaining skin	75 ml
Selected portions		
5	Left and right wings	150 ml
6	Left and right drums	150 ml
7	Left and right thighs	150 ml
8	Left and right breasts	150 ml
		Total 875 ml

2.2.1 Primary processing sampling point

For both processors, the sampling position of the birds was as similar as was safely and practically possible. For the pilot studies, birds from Processor A were taken after full evisceration, two water sprays and the cropper but before the inside/outside rinses and the manual cavity check. These birds still had some neck skin, vertebrae and part of the oesophagus intact as, despite the sampling position being before the neck breaker and trimmer, this apparatus was de-activated for all of the trial birds (Figure 2). Birds from Processor B were taken after evisceration and one water spray, but before the neck breaker and neck skin trimmer, the cavity inspection machine, the inside/outside rinses and the vent pusher. These birds still had some neck skin present, and the vertebrae were intact. For Processor B, the sampling position of birds 1-13 was before the cropper, but for birds 14-31, this was changed to after the cropper, and after an additional tail wash, in order to better align with the sampling position used by Processor A (Figure 2) (see also Tables 2 and 3 and Appendix 1 and 2 for details of bird numbers). This slight change of sampling position for

Processor B coincided with an intervention strategy alteration with regards to the chemical selection added to the carcass sprays before and after the sampling point.

Figure 2: Sampling position of birds taken from the production line



2.2.2 Removal of birds from production line and transportation to laboratory.

Great care was taken when removing birds from the production line in order to avoid excessive handling and therefore cross contamination of the carcasses. Wearing gloves, the operator firmly grasped the selected birds with both hands around the thigh area and lifted the chicken from the shackles. At both processors a second operator, also wearing gloves, then shackled either the left drum (with the orientation of breast meat facing the operator) (Processor A) or looped 'S'-shaped wire through the left drum (Processor B). Gloves were changed after removal of each bird from the line, and birds were carefully carried back to the laboratory at the processors, ensuring that there was no bird-to-bird contact during transportation. Once in the laboratory, birds were either hung on a wire trolley (Processor A; Figure 3a) or a metal pole (Processor B; Figure 3b) until they were ready to be portioned and rinsed. Care was taken to avoid the carcasses touching each other, and birds were dealt with as soon as possible to avoid unnecessary desiccation with the resulting potential loss of *Campylobacter* from the carcass surface.

Figure 3 Shackling and hanging devices used by Processor A (3a) and Processor B (3b)

Figure 3a

Figure 3b

2.2.3 Carcass portioning and rinsing

ESR staff travelled to both of the processors during October 2007 to discuss the requirements of the project and to practice portioning a carcass in order that a 'preliminary' protocol could be designed. Furthermore, an ESR staff member was present at both of the first pilot trials to demonstrate the precise method by which the birds should be portioned and rinsed.

Birds were handled as little as practically possible during the portioning and rinsing process, and gloves were worn at all times. To reduce cross contamination of samples, gloves were changed, and *Campylobacter* on the gloves was enumerated, after handling potentially heavily contaminated samples (such as the neck and vent), and were rinsed with 70% ethanol after handling all other portions. Where possible forceps were used to hold carcass portions and place them in the bags of rinsate: the smaller surface area of forceps aiding in the reduction of bacterial transfer from the portions themselves. Any instruments used (scalpels and forceps) were dipped in the appropriate rinsate after removal of the portion and were then ethanol-wiped to prevent cross contamination of the next sample.

The samples were collected in the order described below, placed in plastic Whirl-Pak sample bags and rinsed in the appropriate volume of Buffered Peptone Water (BPW), containing 1 ml of a 3 % solution per litre of sodium thiosulphate, (Tables 1 and 4) by manually rocking and massaging the sample for 1 minute. For the pilot studies (birds 1-8), the right and left portions were kept separate, but for the main trial birds (9-61), the two matched portions were rinsed together and treated as one sample. All samples were skin-on unless otherwise stated.

1) The cavity rinse sample was collected by one person gathering up the neck area and inverting the bird while the second person placed the BPW into the internal cavity using a graduated plastic pipette. The vent skin was gathered and the bird rocked gently for 1 minute (Figure 4a). The rinsate was removed using a fresh pipette and, for the pilot studies, this process was repeated. Great care was taken not to allow any rinsate to leak out and contaminate the outside of the carcass during this procedure. The carcass was carefully placed on the boning cone via the vent, and orientated with the breast portions facing the operator. Gloves were dipped in either the neck or the vent rinsate, as appropriate, and discarded.
2) The wing portions were removed by gripping the tips with forceps and carefully cutting through the shoulder joint (Figure 4b).
3) The drumsticks were removed by gripping the drumstick tip with forceps and carefully cutting through the knee joint (Figure 4c).
4) The thighs were removed by gripping the exposed femur tip with forceps and cutting through the hip joint.
5) The breast meat and skin was removed using a scalpel and forceps, cutting as close to the breastbone as possible.
6) The skin from around the neck (neck flap) was carefully removed.
7) The vent flap (defined only as the 'Parsons nose') was removed.
8) Any remaining skin left on the carcass was removed.
9) In the case of the pilot studies only, a final carcass rinse was performed once all of the portions and skin had been removed.

Figure 4: Sampling and portioning of cavity rinse (4a), left wing (4b) and left drumstick (4c) respectively.

Figure 4a

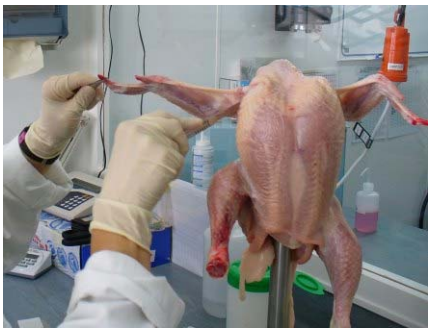


Figure 4b

Figure 4c

2.2.4 Transportation of rinsate samples

The rinsates alone were retained for transportation to the Public Health Laboratory (PHL) at ESR, Christchurch. Volumes (10-20 ml) of rinsate were poured into sterile, leak-proof pots that were completely filled to the top to eliminate headspace. Once collected, the pots were held at 4ºC and dispatched according to the National Microbiological Database (NMD) protocol (http://www.nzfsa.govt.nz/animalproducts/legislation/notices/animal-material-product/nmd/schedule-1-technical-procedures-nmd-final.pdf). Samples were packaged into chilly bins, containing frozen ice packs, and sent by courier to ESR, Christchurch as soon as possible after completion of carcass portioning and rinsing. To confirm that all samples were reaching their destination at temperatures $\leq$ 10°C, either a water blank was included (which was subsequently temperature-tested at ESR), or periodically, Thermochron iButtons® (data loggers) (temperature range -5°C to +26°C) were included in the packaging to record the transportation temperature of the samples, The iButtons® were calibrated against a reference thermometer in ESR Laboratories to ensure that they recorded temperatures within ± 0.5°C of the reference thermometer. Temperature adjustments based on calibration data were made as necessary prior to analysis.

2.2.5 Microbiological analysis of samples

All samples were received at ESR (Christchurch) by 9:00 am on the day following the trial and were processed immediately. This is in line with the NMD requirements of processing samples within 24 hours (30 hours maximum) of birds leaving the production line. The

transportation temperature of the samples was checked upon arrival, either by way of a temperature probe inserted into a water blank that was packaged with the rinsates, or by downloading the information from data loggers. Rinsate samples were plated onto modified charcoal cefoperazone desoxycholate agar (mCCDA) according to NMD protocol (2 ml over 6 plates). The limit of detection therefore depended on the volume of rinsate used. No counts detected represents <25 cfu; <37.5 cfu or <75 cfu for sites rinsed in 50ml, 75ml or 150ml respectively. In addition, 0.1 ml of rinsate was also plated onto each of two plates. For the pilot studies, this was done for every sample, however, for the main trial birds, this was only done for the cavity rinse, neck and vent samples.

Five colonies per bird were selected, re-streaked onto blood agar and their oxidase status confirmed. In addition, these colonies were pooled and used for subsequent *Campylobacter* multiplex PCR confirmation (Wong *et al*., 2004). For each set of samples tested, PCR positive and negative (no DNA, reagents only) controls were included together with controls for Thermotolerant *Campylobacter* species (Therm, 246bp); *C. jejuni* (*lpxA*, 99bp) and *C. coli* (*ceu*, 695 bp). DNA templates were amplified from rinsates using all three primer sets in a multiplex format.

3. RESULTS:

3.1 Pilot study results (birds 1-8)

The results from the 8 birds sampled for the pilot studies are presented in Tables 5 and 6 (Processor A) and 7 and 8 (Processor B). Results obtained from the pilot studies have been reported as 'selected sampling sites' (*Campylobacter* counts from the cavity rinses (3 in total including the final carcass shell rinse)), the skin samples (neck, vent and remaining skin after portion removal) and the glove/instrument rinsates (Tables 5 and 7)) or as 'selected portion samples' (Tables 6 and 8). In addition, the percentage proportion of *Campylobacter* on the carcasses was assessed.

Table 5: Bacterial recovery of *Campylobacter* (log_{10} cfu per total volume of rinsate) from selected sites of pilot birds taken from Processor A. The proportion of *Campylobacter*, expressed as a percentage of the total counts per bird, is shown in parentheses.

	Cavity rinse 1	Cavity rinse 2	Carcass shell	Neck flap	Vent flap	Remaining skin	Gloves etc
	Sample 1	Sample 2	Sample 15	Sample 3	Sample 4	Sample 14	Sample 7
Pilot #1/1	5.24 (56%)	4.44 (9%)	4.71 (16%)	3.68 (2%)	4.20 (5%)	3.47 (1%)	ND (0%)
Pilot #1/2	4.54 (15%)	4.53 (15%)	4.95 (39%)	3.08 (1%)	4.55 (16%)	4.36 (10%)	ND (0%)
Pilot #2/1	3.79 (21%)	3.85 (24%)	3.39 (8%)	2.99 (3%)	3.13 (5%)	NC	ND (0%)
Pilot #2/2	3.68 (9%)	3.60 (8%)	3.95 (17%)	4.38 (47%)	3.13 (3%)	3.33 (4%)	2.18 (0%)
Average of detected counts	4.74	4.26	4.58	3.89	4.13	3.97	2.18

NC – no counts due to a bacterial spreader present on the plates. ND – not detected. For pilot #2/1, the percentage of counts assigned to different rinsates excludes any bacteria present on the skin.

Table 6: Bacterial recovery of *Campylobacter* (log_{10} cfu per total volume of rinsate) from selected portions of pilot birds taken from Processor A. The proportion of *Campylobacter*, expressed as a percentage of the total counts per bird, is shown in parentheses.

	Left wing	Right wing	Left drum	Right drum	Left thigh	Right thigh	Left breast	Right breast	Total bacterial counts per bird (log_{10})
	Sample 5	Sample 6	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	
Pilot #1/1	4.15 (5%)	3.69 (2%)	3.06 (0%)	ND	3.50 (1%)	3.02 (0%)	4.01 (3%)	3.15 (0%)	**5.49**
Pilot #1/2	3.40 (1%)	3.22 (1%)	2.40 (0%)	ND	3.44 (1%)	3.20 (1%)	2.60 (0%)	2.88 (0%)	**5.36**
Pilot #2/1	3.60 (14%)	3.02 (4%)	3.33 (7%)	2.53 (1%)	3.37 (8%)	ND	3.05 (4%)	2.62 (1%)	**4.47**
Pilot #2/2	2.97 (1%)	2.61 (1%)	1.86 (0%)	1.57 (0%)	ND	3.35 (4%)	3.28 (4%)	2.42 (1%)	**4.71**
Average of detected counts	3.73	3.30	2.96	2.27	3.44	3.21	3.54	2.85	

NB: percentages may not always add up to exactly 100 % due to rounding. ND – not detected.

Table 7: Bacterial recovery of *Campylobacter* (log_{10} cfu per total volume of rinsate) from selected sites of pilot birds taken from Processor B. The proportion of *Campylobacter*, expressed as a percentage of the total counts per bird, is shown in parentheses.

	Cavity rinse 1	Cavity rinse 2	Carcass shell	Neck flap	Vent flap	Remaining skin	Gloves etc
	Sample 1	Sample 2	Sample 15	Sample 3	Sample 4	Sample 14	Sample 7
Pilot #1/1	2.86 (66%)	2.35 (20%)	0.70 (0%)	NC	ND	NC	ND
Pilot #1/2	1.70 (10%)	1.40 (5%)	2.48 (60%)	1.18 (3%)	1.57 (7%)	ND	ND
Pilot #2/1	2.24 (11%)	1.88 (5%)	2.83 (41%)	1.88 (5%)	ND	ND	2.18 (9%)
Pilot #2/2	2.44 (3%)	2.44 (3%)	3.54 (36%)	2.05 (1%)	2.35 (2%)	3.68 (51%)	ND
Average of detected counts	2.48	2.18	3.04	1.83	2.12	3.68	2.18

NC – no counts due to a yeast contaminant present on the plates. ND – not detected.
For Pilot #1/1, the percentage of counts assigned to different rinsates excludes any bacteria present on the neck or skin.

Table 8: Bacterial recovery of *Campylobacter* (log_{10} cfu per total volume of rinsate) from selected portions of pilot birds taken from Processor B. The proportion of *Campylobacter*, expressed as a percentage of the total counts per bird, is shown in parentheses.

	Left wing	Right wing	Left drum	Right drum	Left thigh	Right thigh	Left breast	Right breast	Total bacterial counts per bird (log_{10})
	Sample 5	Sample 6	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	
Pilot #1/1	1.88 (7%)	1.88 (7%)	ND	ND	ND	ND	ND	ND	**3.04**
Pilot #1/2	1.88 (15%)	ND	ND	ND	ND	ND	ND	ND	**2.70**
Pilot #2/1	ND	2.05 (7%)	1.57 (2%)	2.53 (21%)	ND	ND	ND	ND	**3.21**
Pilot #2/2	1.57 (0%)	2.05 (1%)	2.05 (1%)	ND	ND	ND	1.88 (1%)	1.57 (0%)	**3.98**
Average of detected counts	1.79	2.0	1.88	2.53	ND	ND	1.88	1.57	

NB: percentages may not always add up to exactly 100 % due to rounding. ND – not detected.

Several preliminary conclusions could be made from these initial experiments, which were subsequently used for defining the exact protocol to be used for the main trial. In summary, the main conclusions were:

1) The highest proportion of bacteria over the carcasses, from both poultry processors, was recovered from the cavity with between 59-65% being recovered from the two cavity rinsates and the whole carcass rinse.

2) When considering the individual portions, with only one exception, the left portions taken from Processor A had a higher proportion of bacteria than their paired right portions. This was not the case for Processor B where *Campylobacter* distribution was more variable and the recovered counts on average lower.

3) The wing samples (combined left and right), from both poultry processors, contained the highest proportion of *Campylobacter* from the portions sampled (6.75-7.25%).

3.2 Pilot study results (birds 1-8)

The data from the pilot and main trials were combined in the results given in this section. To make the pilot data align with the main trial data:

- The left and right portion counts were combined to give a total portion count.
- The first cavity rinse only in the pilot trial was used for the cavity rinse sampling site data.

The site specific proportion of the total *Campylobacter* detected on each carcass was represented as a percentage of the carcass loading;

$$\text{Carcass site loading (\%)} = 100 \quad * \quad \frac{\textit{Campylobacter}\ \text{cfu count from site rinse}}{\text{Total of rinse counts from given carcass}}.$$

Figure 5 shows the distribution of the site loadings for the two poultry processors. The full data set from which Figure 5 was generated is summarised in Appendix 1 and 2. Any birds where count estimates could not be obtained from individual rinsate samples were omitted, as results are presented as a percentage *Campylobacter* distribution over the entire carcass.

Table 9 shows the aggregated data for all the carcass samples where all site rinsate counts could be estimated. The percentage of total load was calculated as follows;

$$\text{Site percentage of total load (\%)} = 100 \; * \; \frac{\sum^{carcasses} \text{Site rinse count}}{\sum^{carcasses} \text{Total of site rinse counts for each carcass}}$$

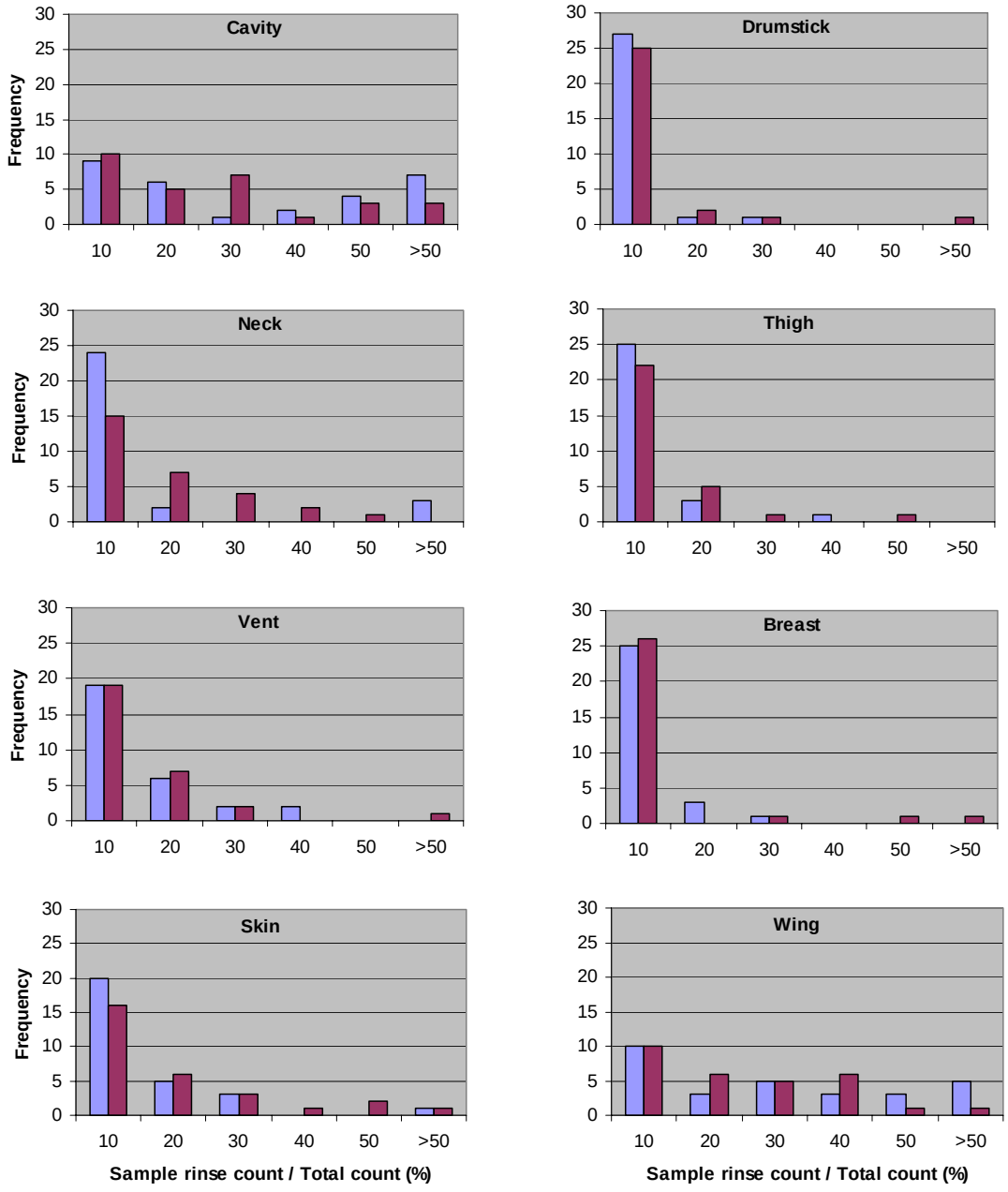
In the case of Processor B, bird 8 yielded exceptionally high *Campylobacter* counts at all sample sites on the carcass. This bird had a large effect on the results in Table 9 with the approach used to display the results. Table 9 therefore represents the data for Processor B with and without bird 8 data included to allow a more representative comparison of the poultry processors for most of the birds sampled.

Table 9: Aggregated proportion of *Campylobacter* from each sample site expressed as a percentage of total counts per processor (all birds) from data given in Appendix 1 and 2.

Sampling site	Percentage of total load		
	Processor A (29 birds)	**Processor B (29 birds) (bird 8 included)**	**Processor B (28 birds) (bird 8 excluded)**
Selected sampling sites			
Cavity rinse	44.7%	39.3%	28%
Neck flap, glove, hook rinse	9.9%	6.6%	17.6%
Vent flap, glove rinse	11.1%	0.5%	3.4%
Remaining skin	8.8%	37.7%	11.2%
Selected portions			
Wings	15.4%	5.8%	15.22%
Drums	1.5%	2.03%	2.4%
Thighs	4.4%	1.6%	11.1%
Breasts	4.3%	6.4%	11%

NB: percentages may not always add up to 100% due to rounding.

Figure 5 Percentage site distribution of *Campylobacter* over the poultry carcass calculated from the sample rinse counts (log_{10} cfu) / total counts (log_{10} cfu) per bird from Processor A (blue bars) and Processor B (red bars).



The Wald-Wolfowitz Runs Test (Cohen & Holliday 1996) was used to determine if there was a difference between the percentage distribution of carcass counts from different sample rinsates.

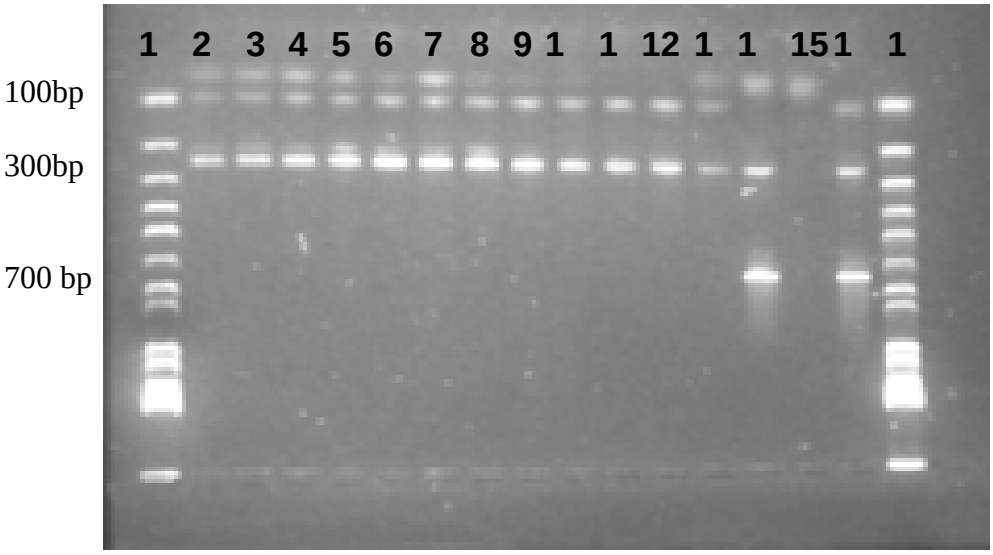
The following conclusions can be made from the above results

1) There were no significant differences (5% level) between the poultry processors for all the sampling sites tested with the exception of the neck (Figure 5). For processor A, the majority of birds had less than or equal to 10% total carcass distribution of *Campylobacter* at this site, while for processor B, the percentage distribution was more variable.
2) When considering the individual sample sites, most of the breasts, thighs and drumsticks harboured ten percent or less of the total carcass *Campylobacter* distribution. Fewer of the skin, neck and vent samples harboured ten percent or less of the total carcass *Campylobacter* distribution while the cavity and wing harboured the least total carcass *Campylobacter* distribution at ten percent or less.
3) The wings and cavity rinse had similar *Campylobacter* distribution patterns with almost one third of birds, from each of the poultry processors, having between greater than 10 percent and greater than 50 percent of their *Campylobacter* counts attributable to those sites. Based on the distributions displayed in Figure 5, the cavity and wings are the sites carrying the highest proportion of the overall counts across all carcasses for both poultry processors. These have been defined as 'hotspots'. This is consistent with the data for the cavity, and wings for Processor A, shown in Table 9. The numerical data in Table 9 for Processor B is consistent for the cavity, but is skewed by bird 8.

3.3 *Campylobacter* identification (complete trial)

All colonies tested (5 per bird) were oxidase positive.
All DNA extractions from pooled colonies were positive for *C. jejuni*. In addition, DNA extractions from colonies recovered from 2 birds from Processor A and 2 birds from Processor B were positive for both *C. jejuni* and *C. coli*. A representative gel, showing bands from *C. jejuni* positive birds only, including all the appropriate controls is presented in Figure 6.

Figure 6: Representative agarose gel showing *C. jejuni* positive samples confirmed by *Campylobacter* multiplex PCR.



Lane key:

1 and 17: 1 kb ladder
2 – 12: test
13 *C. jejuni* positive control
14 *C. coli* positive
15: PCR negative control
16: PCR positive control (*C. jejuni* and *C. coli*).

4. DISCUSSION

Currently, quantitative data on the distribution of *Campylobacter* over different areas of the poultry carcass is limited. In this study, a comparison was made between two New Zealand poultry processors to ascertain the distribution of *Campylobacter* on various parts of poultry carcasses and therefore to better understand how and where contamination of poultry is likely to occur. While the data gained can be viewed as informative, it is prudent to consider that the sampling point used for these experiments was chosen because of the high probability of sampling birds with *Campylobacter* contamination. As such, these results may not directly correlate with any *Campylobacter* carcass mapping results obtained post spin-chill when further intervention strategies have taken place.

4.1 Pilot studies

The initial pilot study, comprising four birds per poultry processor, was conducted in order to optimise the carcass mapping protocol and to determine which, and how many, sampling sites were to be selected for the main trial. Several points of interest arose as a result of these experiments that could warrant further investigation.

The internal cavity was rinsed three times, including the final carcass frame rinse, with very little reduction observed in the resulting *Campylobacter* counts between the first and third rinse. To date, there have been very few published studies exploring the bacterial recovery of different pathogens, including *Campylobacter*, after consecutive whole poultry carcass rinses. The limited information available for various pathogens would suggest that numbers of bacteria in successive rinses of the same carcass have generally been reported to decline at a modest rate (Mead and Thomas, 1973; Notermans and Kampelmacher, 1975; Rigby *et al*., 1982; Lillard., 1987; Lillard., 1989; Izat *et al*., 1991; Jorgensen *et al*., 2002). The study by Jorgensen *et al*., (2002) compared consecutive rinses of *Campylobacter* spp. to obtain an estimate of the proportion of bacteria remaining on the carcass after one rinse. These authors concluded that the $\log_{10}$ geometric mean of *Campylobacter* recovered from thirteen carcasses in the first rinse was 4.8 (SD = 0.4), while the second and third rinses contained 4.5 (SD = 0.4) and 4.2 (SD = 0.5) respectively. A similar pattern of results was observed in the pilot carcass mapping study with, on average, a difference of only 0.2 $\log_{10}$ and 0.37 $\log_{10}$ cfu recovered after three consecutive rinses of the same carcass (cavity and

final rinse) from Processor A and Processor B respectively. This information would be valuable for the poultry industry in order to establish what proportion of bacteria remains on the carcass after a single rinse and therefore, how the counts obtained may relate to the actual number of bacteria present on the birds. Currently, the lack of published literature on this subject, and the preliminary results obtained in this study has led the NZFSA to initiate a small project to investigate the consecutive rinsing of poultry carcasses further.

Interestingly, of the portions sampled, the wings obtained from both poultry processors contained the highest average *Campylobacter* counts. This is discussed in more detail below in relation to the main trial. In addition, the highest counts obtained from the portions were from the left hand side of the bird from Processor A only. From the limited data available, and given that for the remainder of the main trial the left and right portions were combined into a single rinsate, it becomes difficult to make sound statistical conclusions about these results. However, it may be something that could justifiably warrant further investigation as this observation may correlate with dressing differences between poultry processors that impact on *Campylobacter* recovery. Currently it doesn't appear that this issue has been addressed as there is no published literature available on the subject.

4.2 Complete carcass mapping trial

Campylobacteriosis in humans is highly seasonal with a summer peak and a winter trough (ESR Annual Report, 2007) therefore the intention was to conduct the carcass mapping trial during the summer months. While the pilot studies were conducted during January and February, the main trial was completed during the autumn/winter months April, May and June.

The flocks were identified as *Campylobacter* positive in previous cuts by direct plating onto selective media and subsequent confirmation by biochemical analyses. Additionally, the sampling position was before the spin chiller and any subsequent *Campylobacter* control measures meaning that there was a high probability of recovering *Campylobacter* from the rinsates of selected birds.

Both poultry processors that were asked to participate in the trial were using, and continually modifying, various intervention strategies during the course of the carcass mapping experimental work. This was inevitable as New Zealand poultry processors have made major attempts recently to reduce the bacterial load on retail birds, and therefore the

risk to the consumer. Clearly, it has not been possible to document and account for every change that has been made during the trial period moreover, the results were analysed as they became available making it possible to establish a general overview of *Campylobacter* distribution on various parts of the poultry carcass from birds sampled at two different New Zealand poultry processors.

The conclusions from the carcass mapping study suggest that the only site where there were differences in the distribution of *Campylobacter* between processors was the neck skin. Both processors sampled birds that had neck skin intact as this was a requirement of the protocol therefore the reasons for these differences are unclear. It is possible that neck skin might be consumed if a whole bird was purchased, but this is often trimmed by the processor. It is however possible that neck skin may represent a site that could be considered important with respect to cross contamination issues. Reassuringly, the slight change in the sampling position made by Processor B (after bird 13) did not have any statistically significant effect on the recovery of *Campylobacter* from the neck sampling sites.

Of the rinsates collected, the cavity and the wings were identified as carcass 'hot-spots' in terms of *Campylobacter*. Given the trial sampling position of the birds at the plant (after full evisceration, but before inside/outside rinses), it would not seem surprising that the cavity counts had relatively high levels of contamination, with more than 15 percent of birds having greater or equal to 50 percent of their bacterial counts attributed to this area. The sampling position selected for this trial was post evisceration therefore any leakages during this procedure could potentially have contaminated primarily the cavity but also potentially the outer carcass area. Any contamination would most likely be reduced during subsequent washing steps and processor intervention strategies. The large proportion of *Campylobacter* on wing portions may be more important in terms of any potential risks to consumers. Relatively little has been published in relation to prevalence and persistence of *Campylobacter* on poultry wings. A study by Constantin *et al*., (1983) found that *C. jejuni* could be isolated from 82.9% of chicken wings purchased from retail outlets in California USA on the day of arrival at the supermarket. Another study (Reiter *et al*., 2005) looked at the occurrence of *Campylobacter* in various samples taken from water, surfaces and poultry products in a processing plant in Spain. The prevalence of *Campylobacter* on post-chill carcasses was 6/30 (20%), and on chicken parts was: fresh wing 2/30 (6.7%), fresh breast 1/30 (3.3%), and fresh leg 2/30 (6.7%).

In the exposure assessment of *Campylobacter* spp. in broiler chickens published by the FAO/WHO in 2005 (FAO/WHO, 2005) two approaches to assessing undercooking were discussed. One of these, the "protected areas approach", postulated that cells that are in an area of the bird that affords them some level of protection from direct heat may survive cooking. These areas may include "visceral cavities, crevices and areas around joints or in cut and bruised tissues". The anatomy of the wings on carcasses could lead to partial protection of the 'underside' region from rinsing and interventions used during processing, and additionally to heat transfer during cooking. As such, it would be interesting to determine whether a similarly higher proportion of *Campylobacter* counts occurred on wings of birds taken either post chiller or at retail.

The data obtained in the current study has provided valuable information for the poultry industry and has highlighted particular areas that potentially warrant further investigation.

5. CONCLUSIONS

This report provides data on the distribution of *Campylobacter* on different portions and areas of the poultry carcass, using birds sampled from two commercial New Zealand processors at a point after full evisceration but before inside/outside rinses. It is important to note that samples were intentionally collected prior to a number of processing steps aimed at reducing the level of *Campylobacter* on poultry and thus total counts observed on carcasses are not necessarily representative of counts observed on chickens or portions available to consumers. Of the paired portions mapped, the wings were identified as a potential 'hot spot' for *Campylobacter*. Likewise, there was a high proportion of bacterial counts recovered from the internal cavity, with approximately one third of the birds sampled having greater than forty percent of the total *Campylobacter* on the carcass attributable to this site. The fact that *Campylobacter* may be present in varying numbers on different portions of the carcass has implications for the consumer with respect to potential cross contamination issues. Furthermore, the cavity may be more protected from heat during cooking than other areas of the carcass which raises the possibility of exposure through undercooking. If similar results were observed from carcasses taken at the NMD sampling position, or using retail birds, this could correlate with particular areas of the chicken with an increased risk of *Campylobacter* food-borne exposure to the consumer.

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APPENDIX 1: PROCESSOR A LOG_{10} COUNTS PER RINSATE FOR ALL BIRDS SAMPLED.

	Sample								Total (per bird)
Bird	**Cavity**	**Neck**	**Vent**	**Wing**	**Drum**	**Thigh**	**Breast**	**Skin**	
1 (Pilot #1/1)	5.24	3.68	4.20	4.28	3.06	3.62	4.07	3.47	**5.37**
2 (Pilot #1/2)	4.54	3.08	4.55	3.62	2.40	3.64	3.06	4.36	**5.02**
3 (Pilot #2/1)	3.79	2.99	3.13	3.70	3.39	3.37	3.19	NC	
4 (Pilot #2/2)	3.68	4.38	3.13	3.13	2.05	3.35	3.34	3.33	**4.58**
5	5.42	4.30	4.85	4.81	3.57	4.15	4.51	4.48	**5.70**
6	4.43	2.83	3.31	3.77	2.65	3.99	3.58	3.73	**4.74**
7	5.08	3.83	4.03	4.90	3.38	4.43	3.78	3.93	**5.41**
8	5.15	2.94	3.11	3.37	3.02	4.23	3.95	3.41	**5.24**
9	3.54	ND	2.48	2.95	1.88	2.99	2.48	2.97	**3.84**
10	4.12	2.94	3.71	4.11	2.18	3.29	3.81	3.38	**4.63**
11	2.18	ND	ND	ND	ND	ND	ND	ND	**2.18**
12	1.70	ND	1.88	2.18	ND	ND	ND	ND	**2.44**
13	4.60	2.48	4.49	4.03	3.18	3.51	3.32	3.57	**4.96**
14	4.09	5.22	3.15	3.64	2.72	2.48	2.88	3.11	**5.28**
15	3.62	4.01	2.53	3.24	2.18	2.72	1.88	2.83	**4.25**
16	3.42	3.69	3.23	3.91	2.57	3.98	2.88	3.45	**4.49**
17	3.01	3.22	2.92	4.18	2.88	2.88	1.88	3.83	**4.43**
18	3.28	2.27	3.05	3.77	2.18	2.35	2.18	2.57	**4.00**
19	3.14	2.27	1.88	2.88	1.88	2.18	2.18	2.18	**3.46**
20	2.48	ND	1.57	3.37	ND	2.18	2.18	1.88	**3.48**
21	2.85	1.88	1.57	3.31	2.18	2.35	ND	3.01	**3.63**
22	2.48	ND	2.27	2.92	2.72	ND	ND	ND	**3.26**
23	2.65	ND	2.27	3.85	2.18	ND	2.18	2.27	**3.92**
24	5.15	2.18	4.51	3.58	3.26	3.24	2.18	3.96	**5.28**
25	3.73	2.18	4.30	4.59	3.27	3.92	3.49	4.18	**4.97**
26	3.91	2.92	3.37	4.15	3.60	3.43	4.22	3.91	**4.76**
27	4.05	3.55	4.06	4.74	4.17	4.01	4.23	4.21	**5.14**
28	5.28	4.28	4.72	4.39	3.26	3.24	2.78	4.03	**5.48**
29	0.00	2.69	2.18	3.22	2.57	2.72	2.65	2.92	**3.65**
30	3.97	2.05	3.56	4.48	3.20	3.54	2.88	4.92	**5.12**
Sample total	**6.08**	**5.43**	**5.48**	**5.62**	**4.60**	**5.08**	**5.07**	**5.38**	

NC – no counts available (due to yeast contamination or bacterial 'spreader')

ND – no counts detected.

Highlighted row refers to data not included in the final analysis (Figure 5 and Table 9).

Data from pilot study birds includes the first cavity rinse only. Counts from the individual paired portions have been summed to give one figure (see Tables 5-8 for full pilot results).

APPENDIX 2: PROCESSOR B LOG_{10} COUNTS PER RINSATE FOR ALL BIRDS SAMPLED.

	Sample								Total (per bird)
Bird	**Cavity**	**Neck**	**Vent**	**Wing**	**Drum**	**Thigh**	**Breast**	**Skin**	
1 (Pilot #1/1)	2.86	NC	ND	2.18	ND	ND	ND	NC	
2 (Pilot #1/2)	1.70	1.18	1.57	1.88	ND	ND	ND	ND	**2.27**
3 (Pilot #2/1)	2.24	1.88	ND	2.05	2.57	ND	ND	ND	**2.87**
4 (Pilot #2/2)	2.44	2.05	2.35	2.18	2.05	ND	2.05	3.68	**3.95**
5	3.93	4.70	2.83	4.38	4.03	4.50	3.93	4.31	**5.19**
6	4.49	4.40	2.57	4.20	3.20	4.30	3.76	4.01	**5.04**
7	4.07	5.04	3.17	3.94	3.18	4.59	5.13	4.33	**5.52**
8	TNTC*	5.91	4.50	5.86	5.48	4.96	5.96	TNTC*	**7.18**
9	5.01	4.39	4.06	4.46	2.35	3.87	3.24	4.71	**5.36**
10	4.49	3.67	3.85	3.37	3.98	3.44	ND	3.62	**4.79**
11	2.89	4.11	2.57	4.20	1.88	3.71	3.58	3.71	**4.64**
12	4.33	3.79	3.65	4.45	3.24	3.51	3.39	3.73	**4.86**
13	4.03	3.44	3.74	4.16	2.95	3.65	3.26	3.43	**4.63**
14	1.88	1.57	2.65	1.88	2.48	2.35	3.32	2.78	**3.59**
15	1.88	2.42	1.88	2.72	1.88	ND	ND	2.94	**3.27**
16	2.57	3.78	1.88	3.05	1.88	1.88	ND	3.66	**4.09**
17	1.40	ND	ND	ND	ND	ND	ND	ND	**1.40**
18	1.40	ND	ND	1.88	ND	ND	ND	ND	**2.00**
19	0.00	ND	1.57	ND	ND	ND	ND	ND	**1.57**
20	4.06	3.15	3.46	3.66	0.00	2.95	2.35	3.28	**4.37**
21	3.21	2.75	2.48	3.02	1.88	1.88	1.88	2.99	**3.68**
22	3.11	3.01	2.48	2.92	0.00	2.18	2.18	2.75	**3.63**
23	3.19	3.24	3.09	3.49	0.00	2.83	2.78	3.43	**4.06**
24	2.72	2.80	2.48	2.18	2.18	2.18	2.78	2.42	**3.44**
25	2.70	2.18	2.72	2.57	0.00	2.78	2.18	NC	
26	3.33	4.01	3.29	4.50	3.02	4.71	3.94	4.23	**5.09**
27	5.34	4.14	3.73	4.04	2.92	3.70	3.94	4.15	**5.45**
28	3.56	3.05	3.62	3.64	3.52	2.78	2.78	3.04	**4.27**
29	3.60	3.14	3.01	3.96	3.84	2.72	2.92	2.83	**4.39**
30	3.23	4.25	3.61	4.65	2.95	4.07	3.27	4.06	**4.97**
31	3.38	2.94	3.48	3.63	2.35	2.88	2.48	2.99	**4.11**
Sample total	**6.82**	**6.05**	**4.95**	**5.99**	**5.54**	**5.44**	**6.04**	**6.81**	

NC – no counts available (due to yeast contamination or bacterial 'spreader').

ND – no counts detected.

TNTC* Values set to 6.2 log_{10} cfu per rinsate for plots and calculations (based on bacterial plate counts of more than 100 per cm^2) (Compendium of methods for the Microbiological examination of foods, 2001). Highlighted row refers to data not included in the final analysis (Figure 5 and Table 9). Data from pilot study birds includes the first cavity rinse only. Counts

from the individual paired portions have been summed to give one figure (see Tables 5-8 for full pilot results).