Longitudinal Mapping of *Campylobacter* on Poultry Carcasses

Final Report

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SUMMARY

Campylobacteriosis is a leading cause of foodborne disease worldwide, and is the most frequently reported bacterial illness in New Zealand (http://www.surv.esr.cri.nz/PDF_surveillance/AnnualRpt/AnnualSurv/2009/2009AnnualSurvRpt.pdf). 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 before or during poultry processing. Bacterial persistence on carcasses can 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 handled and stored. A key element in controlling this disease is to reduce the numbers of *Campylobacter* on poultry carcasses during slaughter. As such, it is important to identify possible intervention sites in processing plants in order to understand which dressing procedures may contribute to increased carcass loading and which operations may reduce bacterial contamination.

This study was conducted in order to quantitatively assess the changes in bacterial carcass loading, as defined by rinsate counts, during different stages of poultry processing and to correlate these data with dressing procedures used by two New Zealand poultry processors (three including the pilot study). Rinsate samples were taken from three separate stages of processing (Stage 1 = post de-feathering; Stage 2 = post full evisceration and Stage 3 = post spin-chilling) and included selected sites (cavity; neck; vent; wings; legs and skin).

A pilot study was conducted initially (Processor A), which consisted of six birds (2 from each of 3 stages of processing), aimed at developing methodology and best practice sampling procedure. The main longitudinal mapping study consisted of ninety birds (15 per processor from each of three stages of processing) (Processors B and C). At each stage birds were assessed visually for carcass contamination and rinsates, taken from various carcass sites, were enumerated for *Campylobacter*. Only one bird had visible faecal carcass contamination on the vent tissue. Intervention strategies in place between Stage 2 and Stage 3 appeared to be highly effective in reducing *Campylobacter* levels on all birds. However, there was a difference in the bacterial carcass loading between the processors at sampling Stages 1 and 2. *Campylobacter* was recovered in higher numbers at Stage 1, compared with Stage 2, from the birds at Processor B while the *Campylobacter* counts were similar at these stages

with birds sampled from Processor C. Birds sampled from Processor B had a higher proportion of *Campylobacter* counts associated with the internal cavity post evisceration and post spin-chilling compared with birds sampled from Processor C. Furthermore, the birds sampled from Processor C, at Stage 1, had more bacteria associated with the neck tissue while those sampled from Processor B had more bacteria associated with the vent and skin.

Results from this study will be used by the New Zealand Food Safety Authority (NZFSA) to evaluate the impact of processing steps and will contribute to risk management of *Campylobacter* in the food chain by providing improved knowledge of the effects of commercial poultry dressing.

1. INTRODUCTION

Campylobacteriosis is a leading cause of foodborne disease worldwide, and is the most frequently reported notifiable disease in New Zealand with 7,176 cases reported during 2009 (a rate of 166.3 per 100,000 population). This is a significant increase on the 2008 rate of 156.8 per 100,000 population (6694 cases) (http://www.surv.esr.cri.nz/PDF_surveillance/AnnualRpt/AnnualSurv/2009/2009AnnualSurvRpt.pdf) yet a large decrease from the 2006 rate of 383.5 per 100,000 population (15,873 cases) http://www.surv.esr.cri.nz/PDF_surveillance/AnnualRpt/AnnualSurv/2007/2007AnnualSurvRpt.pdf.

Campylobacter spp. are commonly found in the intestinal flora of many wild and domesticated animals and birds, including chickens. Poultry and poultry products therefore represent an important risk factor for human *Campylobacter* infections as the bacteria can be transferred onto poultry via fluid and faeces from the gastro-intestinal tract of infected birds before or during poultry processing. Bacterial persistence on carcasses can provide a major source of *Campylobacter* and can potentially create a risk for consumers if undercooked poultry is eaten, or if contaminated meat is not properly handled and stored. A case-control study, conducted in four urban centres in New Zealand, concluded that at the time 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). Furthermore, a survey carried out to determine the prevalence and concentration of *Campylobacter* on retail chicken products in New Zealand found that *Campylobacter* was isolated from 44.8% of carcass rinse samples collected (Chrystal *et al*., 2008). The authors concluded that these figures are similar to levels reported in other developed countries.

A recent study has provided detailed information on the distribution of *Campylobacter* on various parts of the poultry carcass prior to spin chilling (Paulin and Wong, 2008). The results highlighted the wings and the internal cavity as areas of high *Campylobacter* contamination. To date there have also been several published studies quantifying *Campylobacter* on whole carcasses, and portions thereof, at different stages of the processing chain (Stern *et al*., 2001; Hinton *et al.*, 2004; Rosenquist *et al*., 2006; Berrang

et al., 2007; Reich *et al*., 2008). The findings of these studies will be discussed in detail below.

During growing, and transport of birds to the processing plant the skin and feathers of broilers and their environment are likely to become contaminated by faeces from the gastrointestinal tract of the birds. The concentration of *Campylobacter* present in the gastrointestinal tract of poultry at slaughter can exceed 8.0 $\log_{10}$ CFU/g (Rosenquist *et al*., 2006). Some of these pathogens may become firmly attached to the skin prior to arrival at the slaughterhouse. Within the processing plant birds pass through a series of dressing stages that can both reduce the external carcass bacterial load, through interventions, or increase the bacterial load through contamination from faecal leakage, cross contamination from other birds, the environment, personnel or equipment.

There are several key stages during processing that contribute to an increase in carcass bacterial load including stunning, scalding, de-feathering and evisceration while washing and chilling operations, together with the use of chlorination and biocides, contribute to a reduction in carcass contamination (Bryan and Doyle, 1995; Berrang *et al*., 2007). Carcass excrement, from defecation, may be problematic during the stunning process. Inadequate water temperature and irregular water replacement can lead to a build up of pathogens in the scald tanks. Carcass cross contamination of soil and faecal organisms and equipment contamination are potentially problematic following de-feathering while transfer of pathogens from the hands of workers, equipment and surfaces together with spillage of intestinal contents represent potential contamination areas following evisceration.

A study by Reich *et al*, (2008) demonstrated a positive correlation between the numbers of *Campylobacter* present in the caeca and the numbers present on carcasses at various stages of processing. These results agree with the findings of Rosenquist *et al*, (2006) and Allen *et al*, (2007) and led the authors to reach the conclusion that a reduction in *Campylobacter* during meat processing is possible by reducing the bacterial numbers in caecal contents. The reduction in *Campylobacter* prevalence within broiler flocks may be achieved by improved on-farm biosecurity, such as the strict exclusion of flies from broiler housing; improved hygiene measures, aimed at reducing cross contamination both on the farm and in the processing plant and more efficient processing equipment. Reich *et al*., 2008 confirmed that the scalding water may represent an important area of cross contamination as even during the slaughter of negative flocks the majority (77.8%) of scalding water samples

were positive for *Campylobacter*. This finding was confirmed by Stern *et al*, (2001) who demonstrated that up to 25% of post-scalding water and up to 15% of post-chilling water in their study was contaminated with *Campylobacter*. These authors highlighted the importance of processing negative flocks before positive flocks in an attempt to reduce carcass contamination within the processing plant, although there appears to be no general consensus on the value of this point.

At all stages of poultry dressing, processor-specific technology could account for differences observed at various stages of the slaughter procedure. For example, Reich *et al*, (2008) and Hinton *et al*, (2004) observed a decrease in *Campylobacter* numbers after evisceration while Rosenquist *et al*, (2006) found an increase in concentration at this stage of 0.5 $\log_{10}$ CFU/g, in one out of two processing plants sampled. Carcass washing, followed by chilling caused a reduction in *Campylobacter* concentration by up to 0.97 $\log_{10}$ CFU/g. Hinton *et al*, (2006) conducted a detailed study to examine the spread of *Campylobacter* during poultry processing in different seasons. The authors concluded that poultry flocks may introduce different strains of *Campylobacter* into the processing facilities. Furthermore, different populations of the pathogen may be carried into the processing plant by successive flocks and some strains of *Campylobacter* may reappear in the same processing facility during different times of the year. Hunter *et al*, (2009) found that the genetic diversity of *Campylobacter* decreased as carcasses proceeded through processing suggesting that some subtypes were unable to survive processing whereas others may persist on the carcass or even within the equipment despite stressors encountered in the processing environment.

Campylobacteriosis continues to be a major human health issue in New Zealand and a key element in controlling this disease is to reduce the concentration of *Campylobacter* on chicken carcasses during slaughter. To identify possible intervention sites in New Zealand plants, it is crucial to understand which dressing procedures may contribute to increased carcass loading and which operations may reduce bacterial contamination. As such, this project was initiated to quantitatively assess the changes in bacterial carcass loading during different stages of poultry processing and to correlate these data with dressing procedures used by two commercial New Zealand poultry processors (and a third for the pilot study). Data from this study will be used by the New Zealand Food Safety Authority to evaluate the impact of processing steps and will contribute to risk management of *Campylobacter* in the food chain by providing improved knowledge of the effects of commercial poultry dressing.

2. METHODOLOGY DEVELOPMENT

The experimental methodology for this project was split into two distinct parts. 1) The pilot study, which consisted of six birds (2 from each of 3 stages of processing) conducted at a New Zealand poultry processor (Processor A), aimed to develop methodology and best practice sampling procedure. 2) The main longitudinal carcass mapping study consisted of ninety birds (15 per processor from each of 3 stages of processing) conducted at two New Zealand poultry processors (Processors B and C). Rinsates taken from different processing sampling positions and various carcass sites were enumerated for *Campylobacter*. Birds were mostly second or third cut (three sets of birds were fourth cut from Processor C) and were sampled from flocks that were assumed to be *Campylobacter* positive based on caecal testing results from the previous cut where available. Birds for the pilot study were sampled during December 2008, while those selected for the main longitudinal mapping trial were sampled between May and September 2009.

2.1 Pilot study

The pilot study was conducted in order to develop a suitable and practical study protocol to provide robust data on visible contamination and *Campylobacter* loading on defined parts of the poultry carcass using six birds (2 from each of 3 sampling positions) selected from Processor A. The specific objectives of the pilot longitudinal carcass mapping study were:

1) To develop a suitable scoring scheme for visually assessing the level of contamination present on poultry carcasses at specific stages of processing.
2) To evaluate the practicalities of sampling birds at various positions along the processing line.
3) To develop suitable sampling methodology to enable aseptic portioning and rinsing of various carcass sites.
4) To provide data on the numbers of *Campylobacter* recovered from sample rinsates derived from six pilot birds.

The methodology detailed in section 2.2 (longitudinal carcass mapping study) was the same for both the pilot study and the full longitudinal carcass mapping study.

2.2 Longitudinal carcass mapping study

Following analysis of the results obtained from the pilot study, a further 45 birds, (15 birds each from three different sampling positions) were selected from Processors B and C for the main longitudinal carcass mapping study (making 96 birds in total including the 6 birds from the pilot study). Birds were selected, portioned and rinsed in groups of three (1 bird per sampling position) and all sets of three birds were selected from the same flock.

2.2.1 Primary processing sampling points

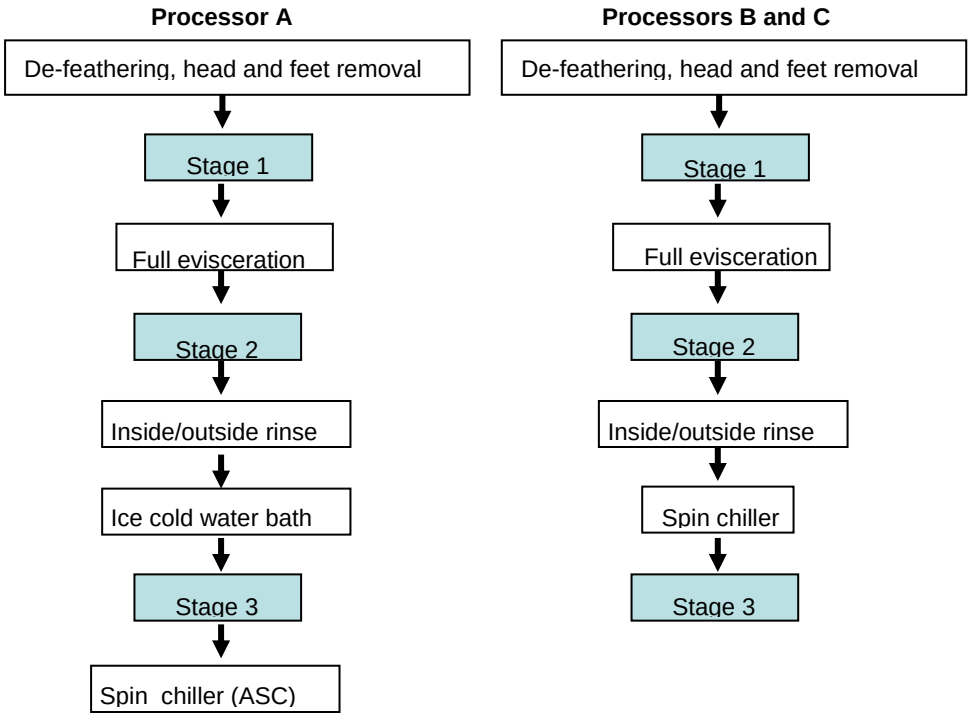
Three different sampling positions were selected from the processing line as follows:

Stage 1: After de-feathering, head and feet removal

Stage 2: After full evisceration, but before the inside/outside rinses

Stage 3: After spin chiller but before Acidified Sodium Chlorite (ASC) treatment. This final sampling position varied slightly depending on the individual processor layout (Figure 1).

Figure 1: Sampling positions of birds taken from the production line



NB: For Processor B, the Acidified Sodium Chlorite tank was pre spin chilling. Stage 3 birds were therefore selected before the Acidified Sodium Chlorite treatment and manually placed in the spin chiller.
NB: Processor B has no washing facilities prior to scalding whereas Processor C does wash birds at this stage of processing. In addition, Processor B does not have an inside/outside washer, post evisceration, the birds are just spray rinsed at this stage.

2.2.2 Dressing procedures used by different processors

Processor-specific details, including brand, type of equipment, processing speed, changes and improvements in intervention strategies will not be disclosed due to the commercially-sensitive nature of this information. It should however be noted that at the time of undertaking this trial, the evisceration equipment used by Processor B was old and has since been replaced.

2.2.3 Removal of birds from the production line and transportation to the 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 either lifted the chicken from the shackles (Stages 1 and 2) or out of the tumbler/spin chiller (Stage 3). Gloved operators either carefully carried the birds back to the on-site laboratory, ensuring that there was no bird-to-bird contact during transportation, or undertook the experimental procedures in the primary-processing department (due to logistical difficulties in removing the birds from the plant). Once in the laboratory, or the primary-processing area, a sterile hook was placed through the drum of the right leg. Birds were then hung on a metal trolley or pole until they were ready to be portioned and rinsed (Figure 2). Care was taken to avoid the carcasses touching each other, and birds were dealt with as soon as possible after collection to avoid unnecessary desiccation with the potential loss of *Campylobacter* from the carcass surface.

Figure 2: Shackling and hanging devices used within the laboratory or primary-processing area.

2.2.4 Scoring system for visually assessing the level of carcass contamination

A modification of the scoring system already in place at Processor A was designed in order to semi-quantitatively assess the visible contamination present on the carcasses at each of the sampling stages. Carcasses were assessed for faecal and ingesta contamination at Stage 1 and for both faecal and ingesta contamination and full evisceration (ie no intestines, liver, heart or gizzard) at Stages 2 and 3. In addition to the whole carcass score, each of the selected sampling sites was scored for contamination as described above. Results were recorded as either 'yes' (visible faecal/ingesta material / residual intestines present) or 'no' (visible faecal/ingesta material / residual intestines absent) in order that the scoring scheme be transferable, easy and consistent to use for different staff members at different processing plants.

2.2.5 Carcass portioning and rinsing

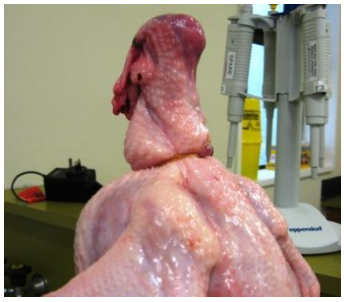
Birds were handled as little as possible during the portioning and rinsing process and gloves were worn at all times. To reduce cross contamination of samples gloves were either rinsed with 70% ethanol or were cleaned with an alcohol wipe after handling all the portions. Where possible, forceps were used to hold the 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. *Campylobacter* on gloves was enumerated after handling potentially heavily-contaminated samples, such as the neck and vent.

In order to reduce cross contamination, the 'cleanest' Stage 3 birds were sampled first, followed by the Stage 2 and Stage 1 birds. The samples were aseptically collected in the order described below, placed in Whirl-Pak sample bags and rinsed in the appropriate volume of Buffered Peptone Water (BPW) (Table 1) by manually rocking and massaging the samples for one minute. All samples were skin-on unless otherwise stated. For Stage 1 birds, all samples were collected while the birds were hanging from the frame. As these birds still had the gastro-intestinal tract intact, it was not possible to perform a cavity rinse or to place the birds on the boning cone for portioning. The samples were collected in the same order as for the Stage 2 and Stage 3 birds (described below) with the exception that both leg samples could not be collected together as one remained shackled to keep the bird hanging. The unshackled leg was removed and placed in BPW first. The skin was then removed and finally, the second leg was removed, unshackled and dropped carefully into the Whirl-Pak bag containing the other leg. Care was taken to avoid the carcass dropping onto the floor immediately after all the samples had been removed.

For Stage 2 and Stage 3 birds, the sampling procedure was as follows:

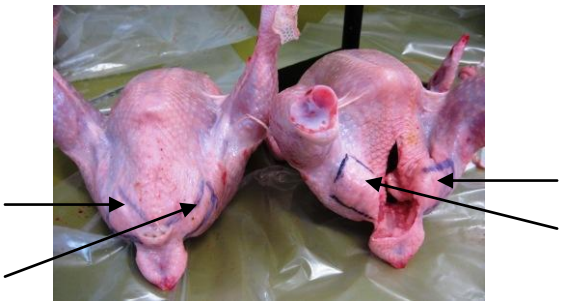
1) **Cavity rinsate:** this sample was collected with the aid of two people - one person gathered up the neck area and inverted the bird while the second person placed the BPW into the internal cavity via a syringe or pipette. The vent skin was gathered and the bird rocked gently for 1 minute. The rinsate was carefully removed using a fresh pipette or syringe. 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 sterile de-boning cone via the vent (Processor C), for removal of the remaining portions, or the remaining portions were removed while the birds were still shackled and only the torso placed on the de-boning cone for final skin removal (Processor B). Gloves were dipped in either the vent or neck rinsate and subsequently discarded.
2) **Neck:** an elastic band was carefully placed around the base of the neck (Figure 3) and the length of neck tissue (including skin and vertebrae) was measured for each bird. Any excess tissue present on the Stage 1 birds, compared to the Stage 2 and Stage 3 birds, was removed and discarded (as this was considered not to be available for human consumption).

Figure 3: Sampling of neck tissue



3) **Vent:** the removal of the vent tissue was standardised between all birds. Vent tissue was defined as the 'Parsons nose' and the two easily defined areas of skin and underlying tissue either side of the vent itself (Figure 4). For the Stage 1 birds, great care was taken to avoid disrupting the gastro-intestinal tract and subsequently releasing faecal material over the outside of the carcass (only vent tissue likely to be used for human consumption was selected).

Figure 4: Sampling of vent tissue (tissue sampled included the "Parsons nose" and the two defined areas (arrows) either side of the vent itself)).



4) **Wing portions** (x 2) were removed by gripping the wing tips with forceps and carefully cutting through the shoulder joint using either a scalpel blade (Processor C) or a pair of stainless steel poultry shears (Processor B).
5) **Legs** (thighs and drumsticks x 2) were removed by gripping the drumstick tip with forceps and carefully cutting through the hip joint using either a scalpel blade (Processor C) or a pair of stainless steel poultry shears (Processor B).
6) **Remaining skin** left on the carcass was carefully removed using a scalpel blade.

Table 1: Sampling sites and volumes of diluent added for the Stage 1, 2 and 3 birds (NB: no cavity rinse was sampled for the Stage 1 birds).

Selected sample sites	Volume of diluent added
Cavity rinse	50 ml
Neck (skin and vertebrae as appropriate)	75 ml
Vent ('Parsons nose' and defined areas as detailed above	75 ml
Wings x 2	150 ml
Legs x 2 (thighs and drumsticks)	300 ml
Remaining skin	75 ml
	Total rinsate volume used 725 ml

2.2.4 Transportation of rinsate samples

The rinsates alone, with the exception of the skin samples which included both skin and rinsate, were retained for transportation to the Public Health Laboratory (PHL) at ESR, Christchurch. Volumes (10-20 ml) of the rinsate sample were poured into sterile, leak-proof pots that were completely filled to the top to eliminate headspace. Skin samples, together with the appropriate volume of BPW, were transported in Whirl-Pak bags for subsequent homogenisation in the laboratory. Once collected, the pots and bags 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 from Processors B and C (the main longitudinal carcass mapping study) were packaged into chilly bins, containing frozen ice packs, and sent by overnight 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 $\leq$ 10ºC, a water blank was included with each consignment which was subsequently temperature-tested at ESR immediately upon receipt.

2.2.5 Microbiological analysis of samples

All samples were received at ESR, Christchurch by 9:00 am on the day following the sampling (if sent by courier) or on the same day as the trial (pilot study). Samples 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. Rinsate samples were plated onto modified charcoal cefoperazone deoxycholate agar (mCCDA) (2 ml over 6 plates). The limit of detection therefore depended on the volume of rinsate used. Not detected results represented <25 Colony Forming Units (CFU); <37.5 CFU; <75 CFU or <150 CFU for sites rinsed in 50 ml, 75 ml, 150 ml or 300 ml respectively. In addition, 0.1 ml of rinsate was plated onto each of two plates to obtain a 1:10 dilution to accommodate heavily contaminated samples (including vent, cavity, neck and skin). For select samples, a 1:100 dilution was also plated onto each of two plates (Processor B only).

Five colonies per bird were selected from positive plates and re-streaked onto blood agar to confirm their oxidase status. In addition, these colonies were pooled and used for subsequent *Campylobacter* multiplex PCR confirmation (Wong *et al*., 2004).

3. RESULTS

3.1 Assessment of visible contamination

An assessment of the visible contamination present on each of the whole carcasses, and portions thereof, was conducted using a modification of the system employed by Processor A. Birds were examined thoroughly, while shackled, for the presence of any external faecal/ingesta contamination and any remaining internal intestinal contamination.

Processor A: None of the birds selected for the pilot study had any visible contamination present on the outside of the carcass, and all of the birds from Stages 2 and 3 were fully eviscerated.

Processor B: Only one of the birds (Bird 37; Stage 1) had visible faecal contamination present on the vent. All of the birds from Stages 2 and 3 were fully eviscerated.

Processor C: None of the birds had any visible faecal/ingesta contamination present on the outside of the carcass and all of the birds from Stages 2 and 3 were fully eviscerated. However, a few other observations were made, relating to the carcass condition. These included: Bird 2 (Stage 2) still had the neck attached at sampling; Bird 16 (Stage 1) had a few residual carcass feathers; Birds 19 and 31 (Stage 1) had feathers present on the drumsticks; Bird 38 (Stage 2) had feathers around vent and one leg; Birds 40 (Stage 1) and 41 (Stage 2) had a few residual feathers on the body and legs and Bird 28 (Stage 1) had some bruising on the wings.

3.2 Standardisation of bird neck length

The total length of neck skin (including vertebrae) was measured for all of the birds. The necks of the Stage 1 and Stage 2 birds were trimmed to the same length as the Stage 3 birds for each set.

Processor A: The trimmed length of all the chicken necks (Stages 1, 2 and 3) used for the pilot study was 11 cm.

Processor B: The range of trimmed lengths of chicken necks (Stages 1, 2 and 3) used for the main Longitudinal carcass mapping trial was 6 cm – 10 cm.

Processor C: The range of trimmed lengths of chicken necks (Stages 1, 2 and 3) used for the main Longitudinal carcass mapping trial was 8 cm – 11 cm.

3.3 Pilot study results

The complete results from the rinsates of 6 birds sampled for the pilot study are presented in Appendix 1. As only 2 birds per stage were selected, from Processor A, these results have not been included in any data analysis relating to site distribution of *Campylobacter*.

3.4 Main experimental trial results

The complete set of results obtained from the main longitudinal carcass mapping trial are presented in Appendix 2 (Processor B) and Appendix 3 (Processor C). Section 3.4 summarises the data and compares the results between different steps in the processing line and between the two independent lines used by Processors B and C. It should be noted that one set of three birds, from Processor C, arrived at ESR frozen. These results have been excluded from the data analysis.

Figure 5 shows the cumulative distribution of counts for each carcass excluding the cavity. The x-axis of the cumulative frequency graph gives the sum of the rinsate counts from the neck, vent, wings, legs and skin. The cavity was not included to enable the *Campylobacter* counts at Stage 1 (post de-feathering, viscera intact) to be compared against the counts derived from Stages 2 and 3 (viscera absent).

The y-axis represents a proportion of the birds sampled (15 sets from Processor B and 14 sets from Processor C). Hence a value of 1 represents all the carcasses that were sampled and a proportion of 0.5 represents half or 50% of the carcasses that were sampled. The graph provides an indication of the proportion of carcasses that had summed counts less than or equal to the counts given in the x-axis. For example, in

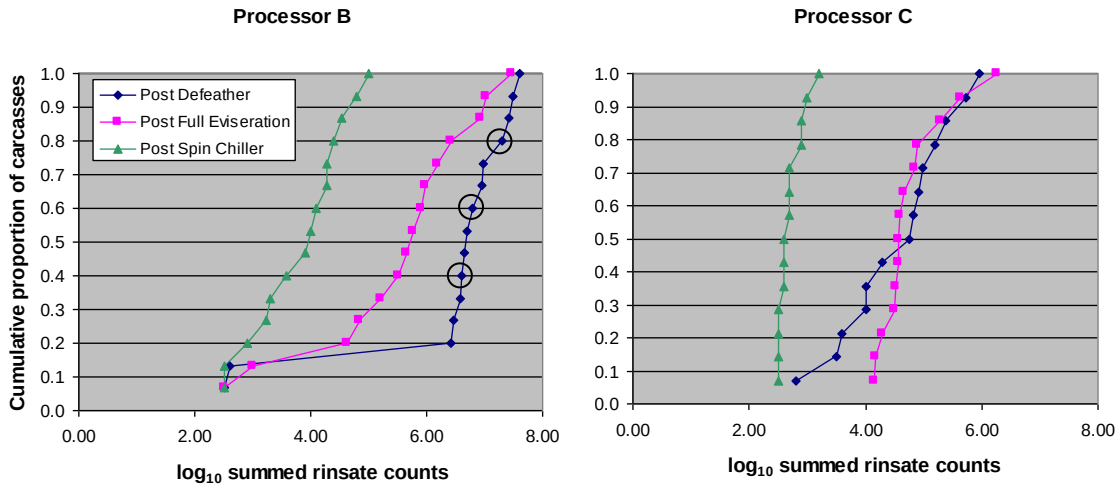
Figure 5, Processor B, 60% of carcasses had summed counts of less than 4 $\log_{10}$ CFU after the spin chiller compared to summed *Campylobacter* counts of less than 6 $\log_{10}$ CFU after evisceration. The steeper the plot, the less variation in the counts across the carcasses sampled.

The results are presented as a cumulative distribution to allow the data to be compared incorporating “less than” and “greater than” results. For this plot the “not detected” results were set at the limit of detection for the rinsate (section 2.2.5) and the “greater than” results were set to the minimum count for the rinse given greater than 100 CFU in a square

centimetre of the plate, as indicated by circles on the figure. A value of 337.5 CFU (2.5 $\log_{10}$ CFU) is equal to the sum of the limit of detection for all the rinses excluding the cavity.

Birds with detectable counts, sampled from both processors, had an approximate 2 $\log_{10}$ reduction in the *Campylobacter* numbers recorded in the total rinse between Stages 2 (post evisceration) and 3 (post spin–chill). However, there was a difference between the processors when comparing Stages 1 (post de-feathering) and 2 (post evisceration). The distribution of counts recorded from Processor C were similar for Stages 1 and 2. In contrast, the data from Processor B showed that the Stage 1 bacterial count distribution had higher *Campylobacter* counts than those quantified at Stage 2.

Figure 5: Cumulative distribution of *Campylobacter* for the summed rinse counts of the neck, vent, wings, legs and skin, at different points in the processing line for Processors B and C.



Circles represent greater than values.

Figure 6 shows the cumulative distribution of counts found in the cavity of carcasses, for the two processors, expressed as a percentage of the summed counts from all of the rinsates for a given carcass. For example, post evisceration, 40% of Processor B carcasses had 10% or less of the total carcass rinsate count located in the cavity rinse. Some of the "not detected" rinsates may have been contaminated with *Campylobacter*, but the concentration and absence/presence is not known. It was therefore decided to set the "not detected" results to zero and express percentages in terms of countable CFU in the rinsates. Hence, the "not detected" results have been represented as zero on the percentage axis.

Carcass cavity loading (%) = *Campylobacter* CFU count from cavity rinse
Total of the rinse counts from given carcass

Post evisceration, most of the carcasses from both processors had some proportion of the *Campylobacter* counts found in the cavity, with processor C having less of the carcass loading in the cavity compared to Processor B. There was more variation in the percentage of the summed carcass counts found in the cavity for Processor B than Processor C. These trends continued post spin chiller when 85% of the cavity rinses for Processor C resulted in "not detected" values.

Figure 6: Cumulative distribution of the proportion of *Campylobacter* counts for the cavity of carcasses sampled at Stages 2 (post evisceration) and 3 (post spin-chiller) for Processors.

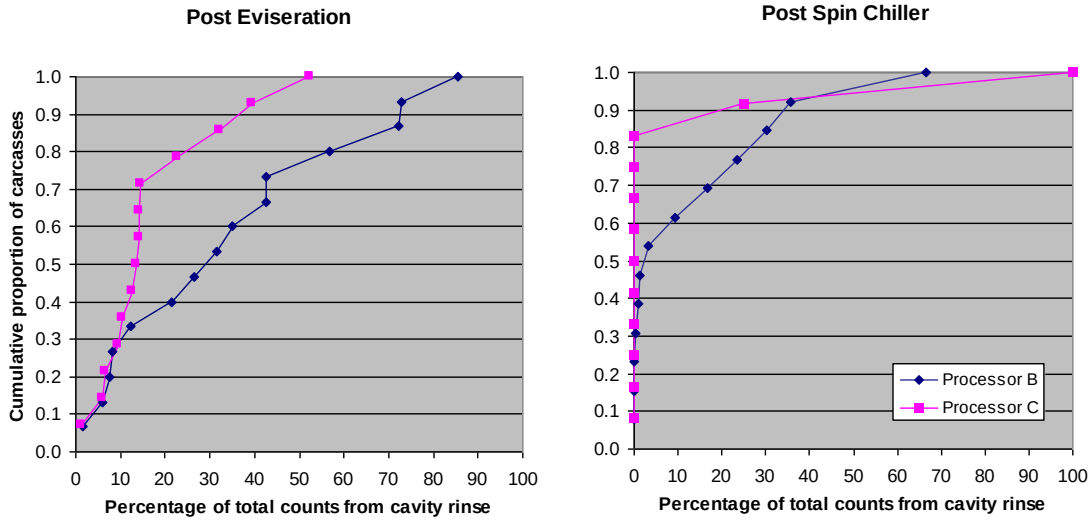


Figure 7 shows the spread of the rinsate counts over the carcass. For each carcass sampling portion, and processing step, a similar cumulative plot as used in Figure 6 is given for the portion rinsate counts as a percentage of the summed counts from the neck, vent, wings, legs and skin rinsates. The cavity rinse was not sampled for Stage 1 due to the presence of intact viscera. Not detected results were also set to zero percent for this figure.

Carcass site loading (%) = *Campylobacter* CFU count from site rinse
Total of the rinse counts from given carcass (excluding cavity)

When interpreting these graphs it should be remembered that the plots show the percentage spread of the rinsate counts across a carcass and do not reflect the actual *Campylobacter* count numbers. A carcass with only one countable rinse will have one portion rinse as 100%

in one plot and 0% in the remaining plots. However, the detectable counts may be just over the limit of detection, and the actual counts may be evenly spread over the bird. Refer to Appendix 2 and 3 for rinsate count data.

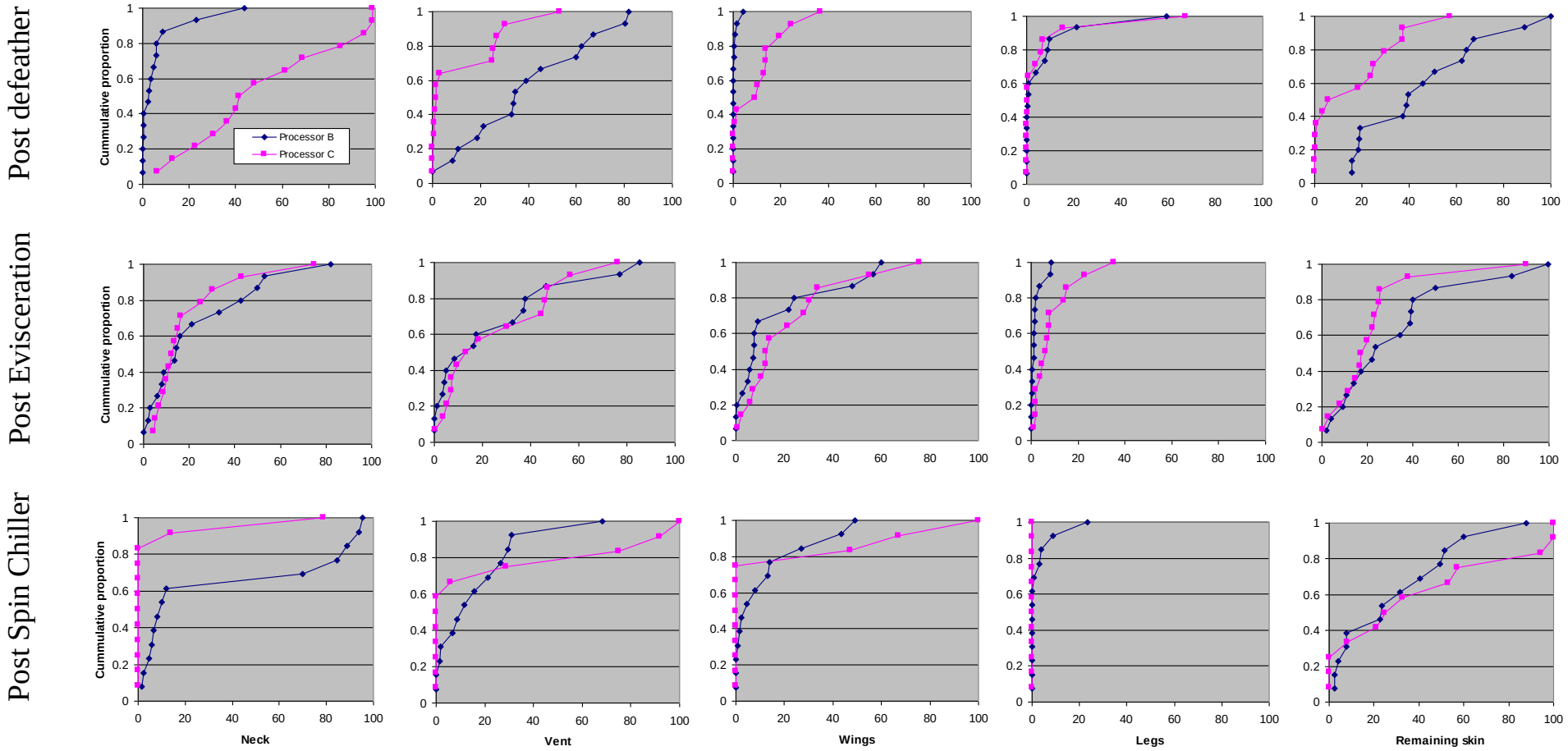
At Stage 1 (post de-feathering) the neck, vent and skin showed the greatest variation in the percentage of the rinsate counts found for these sites compared to the rest of the carcass. Many of the rinsates from the wings and legs resulted in not detected results.

Processor B differed from Processor C in the percentage of the counts from a carcass rinsed from the neck, vent and skin. Processor B had very little of the total detectable *Campylobacter* in the neck and wings compared to Processor C, but had greater percentages of the counts in the carcass rinsates from the vent and skin rinsates.

At Stage 2 (post evisceration) there were no major differences between the two processors with respect to the percentage of counts from rinsates of the different sample sites.

At Stage 3 (post spin chiller) the most variation in the loading of the different portions was from the skin rinse. While most of the carcasses had small percentages of the summed carcass rinsate count detected from the neck, vent and wing rinsates, each of these portions did provide the highest percentages for a few of the carcasses tested.

Figure 7: Cumulative distribution of the proportion of *Campylobacter* counts from selected sample site rinsates, excluding the cavity, of carcasses taken from different stages in the processing line for Processors B and C.



4. DISCUSSION

Campylobacter spp. represent common contaminants of live broiler chickens. Commercial processor dressing operations, including scalding, de-feathering, evisceration and chilling may influence the level of *Campylobacter* carcass contamination, one of the aims of processing being to reduce the final numbers of bacteria present on retail birds. The potential for processing operations to spread carcass contamination to other birds, equipment, personnel, the environment and water has been reported (Stern *et al*., 2001; Hinton *et al.*, 2004; Rosenquist *et al*., 2006; Berrang *et al*., 2007; Reich *et al*., 2008). Processed poultry products contaminated with *Campylobacter* may then serve as vehicles for campylobacteriosis outbreaks in humans (Bryan and Doyle, 1995).

This study has generated information on the concentration of *Campylobacter* present on selected sampling sites of broiler carcasses during specific slaughter operations at two commercial New Zealand poultry processing plants. The data have revealed that there are differences between the processors that can be attributed to specific stages of the dressing procedure. It should however be noted that certain parts of the processing equipment in Plant B were old and have recently been replaced, leading to an improvement in performance since this trial was conducted.

Sample site-specific *Campylobacter* loading differences appear to occur, particularly at the de-feathering stage, which may have an influence on the overall bacterial numbers remaining on the carcass after spin-chilling. For example, while there was a clear reduction in *Campylobacter* counts, from birds at both processors, between the post evisceration and post spin-chilling stages, the bacterial counts also reduced by approximately 1 $\log_{10}$ between de-feathering, and evisceration at Processor B. This was in contrast to the *Campylobacter* counts, on birds at Processor C that remained consistent and lower than those from birds at Processor B, following both de-feathering and post evisceration. This result would suggest that as the numbers of *Campylobacter* present at de-feathering were greater on the birds selected from Processor B, intervention strategies around this stage were efficient at reducing carcass bacterial numbers. Whether the higher number of bacteria found initially on the birds from Processor B was a result of contamination from the early stages of processing or from different processing conditions is unclear. It is possible that *Campylobacter* contamination of poultry could be introduced indirectly via catchers, during transportation or via equipment

used at the start of the processing chain (Tecklok Wong, personal communication from data generated during 2005/2006 prior to the introduction of *Campylobacter* performance targets in April 2008).

These findings are in line with other published results and may simply be a result of variations in plant machinery and processor operations. Rosenquist *et al*, (2006) made a comparison of published data on the influence of selected processing operations on *Campylobacter* contamination of broiler carcasses and concluded that post de-feathering and post evisceration the carcass counts appeared to be the most variable. Some of the studies reported a decrease in bacterial numbers at this point and some reported very little change. Interestingly Izat *et al*, (1988) and Stern and Robach, (2003) have reported an increase in the *Campylobacter* numbers on carcasses during the evisceration operation which would likely be a result of viscera rupture during processing. All of the papers surveyed by Rosenquist *et al*, (2006) reported a significant decrease in *Campylobacter* numbers on carcasses after washing and cooling which concurs with the findings of the longitudinal mapping study. An interesting observation from the review by Rosenquist *et al*, (2006) was the large decrease in bacterial numbers following bleeding and the uniform increase after scalding. As carcass counts at these points were not quantified on the birds sampled in the present study, it was not possible to determine how this may correlate with published results.

At the post evisceration and post spin-chiller stages of sampling, there was a difference in the percentage *Campylobacter* counts from the cavity rinse between the processors with carcasses selected from Processor B having a higher percentage of total counts from this site. It is possible that the greater level of cavity contamination associated with Processor B after Stage 2 also contributed to the higher level associated with birds from this Processor at Stage 3. Post evisceration clearly represents a crucial stage in processing and one that lends itself to the possibility of carcass contamination if viscera are not removed intact. A previous report has shown the cavity of birds post evisceration to be a carcass 'hot spot' for *Campylobacter* (Paulin and Wong, 2008). High intestinal content *Campylobacter* concentrations have been correlated with high bacterial carcass counts after the defeathering stage (Rosenquist *et* al., 2006), suggesting that a reduction in the concentration of *Campylobacter* on slaughtered chickens can be obtained by reducing the numbers of *Campylobacter* in the intestines of living birds. When considering any correlation between visible contamination of carcasses and increased *Campylobacter* numbers, it is interesting to

note that, with the exception of one bird, none of the carcasses sampled in the trial had any visible faecal or ingesta contamination.

The current study has also highlighted some interesting observations relating to processor-specific differences in the cumulative distribution of the proportion of *Campylobacter* on different sites of the birds particularly after the de-feathering stage. Processor C had greater percentages of the bacterial counts on the neck while Processor B had greater percentages of the bacterial counts on the vent and skin. Individual dressing procedures may account for these differences for example; it is noteworthy that the range of trimmed neck lengths measured was longer for birds sampled at Processor C. This could suggest that the larger surface area sampled represented the potential for recovery of a greater percentage of the bacterial counts on this tissue. Both processors used three banks of rubber finger pluckers for de-feathering. It could be speculated that, as the birds from Processor B were more heavily contaminated at all stages sampled, the plucking machinery may be identified as a source for the potential contamination of other carcasses if birds arriving at the slaughterhouse had high levels of *Campylobacter* present externally. This could also have resulted in a higher percentage of the bacterial counts being associated with the vent and skin following de-feathering. Results from both processors showed a low percentage of *Campylobacter* associated with the legs, from all stages sampled, which is in agreement with the findings of a previous study (Paulin and Wong, 2008).

Risk management decisions, aimed at reducing campylobacteriosis cases in humans, have been centred on the hypothesis that a reduction in the number of *Campylobacter* on chicken meat would cause a significant decrease in the number of human campylobacteriosis cases. The development of a quantitative risk model to investigate *Campylobacter* contamination in the processing and consumption stages of the New Zealand poultry food chain during 2003-2006 concluded that reductions in flock prevalence, freezing of the poultry supply or primary processing interventions are the most effective methods of reducing bacterial numbers on carcasses (Lake *et* al., 2007). The results of the current study indicate the effectiveness of decontamination at reducing numbers of *Campylobacter* on birds between the post de-feathering and the post spin-chilling stages. However, there do appear to be differences in *Campylobacter* carcass loading between the processors that can be attributed to specific stages of the dressing procedure and to specific sites on the carcass. Whether these differences correlate with a high bacterial load upon plant entry or with procedures within the slaughterhouse remains unclear.

We believe that 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. It would seem prudent to conclude that a high level of slaughterhouse hygiene and appropriate processing intervention strategies are crucial for the reduction of *Campylobacter* in the food chain.

5. CONCLUSIONS

This report provides data on the changes in *Campylobacter* carcass loading during the different stages of poultry processing at two New Zealand poultry slaughterhouses. The dressing steps, equipment brand, model and age used by different processors will vary but all aim to have acceptable procedures in place that will reduce the numbers of bacterial contamination from birds entering the plant to those on birds packaged or portioned for retail sale.

The birds sampled from Processor B had a greater loading of *Campylobacter*, as defined by rinsate counts, at all stages of processing sampled compared with the birds from Processor C. The numbers of *Campylobacter* on the carcasses of birds from Processor B, with detectable results, were reduced by approximately 1 $\log_{10}$ following full evisceration and by approximately 2 $\log_{10}$ following spin-chilling. In contrast, despite the initial lower carcass bacterial numbers associated with birds from Processor C, there appeared to be no obvious reduction in bacterial counts between the de-feathering and full evisceration stages yet, of the birds with detectable results, there was approximately 2 $\log_{10}$ reduction following spin chilling. Furthermore, the birds sampled from Processor B had a higher percentage of total bacterial counts associated with the internal cavity post evisceration and post spin-chilling. There were clear differences in the spread of the *Campylobacter* counts over the carcass sites, at different stages of processing, which may correlate with differences in dressing procedures. Of greatest significance was the percentage of rinsate counts post de-feathering where birds from Processor C had more bacteria associated with the neck and birds from Processor B had more bacteria associated with the skin and vent.

These results indicate the effectiveness of primary processing in reducing numbers of *Campylobacter* on birds between the post de-feathering and the post spin-chilling stages. However, there do appear to be differences in *Campylobacter* carcass loading between the processors that can be attributed to specific stages of the dressing procedure and to specific sites on the carcass. Whether these differences correlate with a high bacterial load upon plant entry or with procedures within the slaughterhouse remains unclear.

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

		Sample					
Stage of sampling		**Cavity**	**Neck**	**Vent**	**Wings**	**Legs**	**Skin**
Stage 1	Bird 1	N/A	4.37	5.99*	4.51	4.91	6.37*
(after de-feathering)	Bird 4	N/A	4.56	5.13	4.49	4.31	4.95
Stage 2	Bird 2	5.03	4.17	4.18	4.67	4.06	4.28
(after full evisceration)	Bird 5	5.13	4.02*	4.74	3.82*	4.26*	4.36
Stage 3	Bird 3	3.66	3.15*	2.83*	3.05*	3.18*	3.26*
(Pre spin chiller)	Bird 6	4.39	3.39	2.88*	2.92*	3.13*	2.85*

Key to all appendix tables:

N/A – no rinse taken.

* - Counts which are estimates because of one of the following reasons: less than 25 colonies being present in 1ml of rinsate; more than 350 colonies countable on a single plate or 4 x $1cm^2$ areas counted and used to estimate the total plate count. All viable count data have been reported according to the recommendations in the Compendium of methods for the Microbiological examination of foods, (2001).

< – Counts which were not detectable under the conditions used. Therefore, for data analysis purposes, the counts were assumed to be less than the limit of detection for that rinsate (section 2.2.5).

> – Counts which were too numerous to count.. Therefore, for data analysis purposes, the counts were assumed to be greater than the maximum number detectable for that rinsate assuming a maximum countable number of 100 colonies per cm^2.

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

		Sample					
Stage of sampling		**Cavity**	**Neck**	**Vent**	**Wings**	**Legs**	**Skin**
Stage 1 (after de-feathering)	Bird 1	N/A	6.33*	6.56*	4.24	4.05	6.54*
	Bird 4	N/A	5.77	7.32*	4.43	4.56	6.60*
	Bird 7	N/A	5.01	6.37*	4.09	3.61	5.66
	Bird 10	N/A	<1.57	1.57*	<1.87	<2.18	1.88*
	Bird 13	N/A	5.33*	6.48*	2.99	5.64	5.92
	Bird 16	N/A	6.25*	>6.63	4.39	6.20*	>7.1
	Bird 19	N/A	<1.57	<1.57	<1.87	<2.18	1.57*
	Bird 22	N/A	3.99	6.60*	3.53	5.53	6.65*
	Bird 25	N/A	3.79	6.20*	3.13*	4.18	6.02*
	Bird 28	N/A	5.35	>6.1	3.97	5.9	6.16*
	Bird 31	N/A	5.46*	6.49*	5.32	5.65	5.98*
	Bird 34	N/A	5.25	>6.1	4.74	6.60*	>6.1
	Bird 37	N/A	5.20	7.15*	4.42	5.59	7.42*
	Bird 40	N/A	5.08	6.51	4.61	4.01	7.43*
	Bird 43	N/A	6.23	5.5	4.79	4.41	6.25*
Stage 2 (after full evisceration)	Bird 2	6.03*	5.05	5.58	4.79	4.85	5.29
	Bird 5	6.96*	5.91	4.27	5.52	4.45	5.53
	Bird 8	6.38*	7.10*	6.71*	5.12	5.13	7.06*
	Bird 11	2.63*	1.88*	1.57*	2.65*	<2.18	2.57*
	Bird 14	4.5	3.83	4.2	3.59	3.18*	4.17
	Bird 17	4.31	4.66	4.03	5.28	4.42	4.65
	Bird 20	2.3*	1.57*	<1.57	<1.87	<2.18	1.57*
	Bird 23	4.39	3.62	4.72	3.52*	2.88*	3.87
	Bird 26	5.16	2.97*	4.14	3.93	2.65*	6.94*
	Bird 29	5.84*	4.3	5.90*	4.41	3.68*	4.93
	Bird 32	4.92	5.69*	4.69	4.65	3.48*	4.04
	Bird 35	>6.45	5.96*	6.23*	6.77*	5.03	6.25*
	Bird 38	6.15*	5.94*	6.00*	5.80*	4.53	5.01
	Bird 41	4.16	3.68	3.91	4.06	3.38*	5.13
	Bird 44	6.07	4.98	5.17	4.4	3.84	5.25
Stage 3 (post spin chiller)	Bird 3	3.40*	2.57*	2.97*	3.55	<2.18	3.52
	Bird 6	3.57	2.42*	3.74	3.41*	3.64*	3.77
	Bird 9	<1.4	3.54	4.03	3.20*	3.02*	4.25
	Bird 12	<1.4	<1.57	<1.57	<1.87	<2.18	<1.57
	Bird 15	3.05	1.57*	1.57*	2.18*	<2.18	2.53*
	Bird 18	3.22	2.48*	2.78*	3.27*	2.18*	2.95*
	Bird 21	<1.4	<1.57	<1.57	<1.87	<2.18	<1.57
	Bird 24	<1.4	4.96	3.21	2.18*	<2.18	3.61
	Bird 27	3.80	3.90	3.38	2.18*	<2.18	2.94*
	Bird 30	2.78*	3.04*	4.11	2.65*	2.18*	3.63
	Bird 33	2.10*	3.96	2.94*	<1.87	<2.18	2.42*
	Bird 36	2.30*	3.22*	<1.57	2.18*	<2.18	2.18*
	Bird 39	2.0*	3.48*	<1.57	<1.87	<2.18	4.34
	Bird 42	2.72*	4.77	3.02*	2.65*	<2.18	3.17*
	Bird 45	<1.4	1.57*	2.65*	2.35*	2.18*	2.92*

APPENDIX 3: PROCESSOR C LOG_{10} COUNTS PER RINSATE FOR ALL BIRDS SAMPLED

		Sample					
Stage of sampling		**Cavity**	**Neck**	**Vent**	**Wings**	**Legs**	**Skin**
Stage 1 (after de-feathering)	Bird 1	N/A	4.48	4.4	4.00	4.18	4.26
	Bird 4	Sample arrived frozen so not included in analysis					
	Bird 7	N/A	4.65	2.99*	4.10	3.65*	3.37
	Bird 10	N/A	5.56*	5.68*	3.13*	3.62*	4.72
	Bird 13	N/A	3.31	<1.57	2.48*	<2.18	2.99*
	Bird 16	N/A	3.41	3.78	3.41*	3.08*	3.87
	Bird 19	N/A	5.36	3.84	3.22*	3.22*	2.83*
	Bird 22	N/A	5.40*	3.51	4.86	3.32*	5.29
	Bird 25	N/A	4.28	4.32	4.31	3.52	4.30
	Bird 28	N/A	5.22	3.19*	2.35*	<2.18	2.57*
	Bird 31	N/A	4.76	1.57*	1.88*	2.18*	2.57*
	Bird 34	N/A	3.64	2.18*	<1.87	<2.18	3.78
	Bird 37	N/A	2.80*	<1.57	2.18*	3.82*	3.39
	Bird 40	N/A	2.18*	2.05*	2.18*	<2.18	<1.94
	Bird 43	N/A	3.51	1.57*	2.72*	<2.18	<1.57
Stage 2 (after full evisceration)	Bird 2	4.61	3.57	4.24	3.71	3.35	3.93
	Bird 5	Sample arrived frozen so not included in analysis					
	Bird 8	4.04	4.21	3.42	4.02	3.72	3.47
	Bird 11	5.29	4.91	6.15	4.06	4.54	5.50
	Bird 14	3.35	3.76	3.26	3.76	2.88	3.69
	Bird 17	2.72	3.77	4.22	3.92	3.26	3.76
	Bird 20	3.39	4.05	3.30	3.02	2.48	2.65
	Bird 23	4.43	4.36	3.01	4.01	3.91	5.59
	Bird 26	4.5	4.68	4.76	4.37	4.13	4.68
	Bird 29	3.52	3.75	3.22	4.54	3.56	2.24
	Bird 32	3.82	3.28	3.90	2.92	2.18	3.50
	Bird 35	3.65	3.70	3.34	4.02	3.85	3.79
	Bird 38	3.71	3.36	3.79	4.26	3.41	3.59
	Bird 41	4.07	3.79	3.57	3.93	4.39	4.54
	Bird 44	4.7	3.94	4.55	3.91	4.07	4.11
Stage 3 (post spin chiller)	Bird 3	<1.4	<1.57	<1.57	2.48*	<2.18	2.53*
		Sample arrived frozen so not included in analysis					
	Bird 9	<1.4	<1.57	<1.57	<1.87	<2.18	2.05*
	Bird 12	<1.4	<1.57	<1.57	<1.87	<2.18	1.57*
	Bird 15	<1.4	<1.57	<1.57	1.88*	<2.18	1.57*
	Bird 18	<1.4	2.75*	<1.57	<1.87	<2.18	2.18*
	Bird 21	1.70*	<1.57	2.05*	<1.87	<2.18	1.57*
	Bird 24	<1.4	1.57*	1.88*	<1.87	<2.18	2.18*
	Bird 27	<1.4	<1.57	<1.57	<1.87	<2.18	<1.57
	Bird 30	1.40*	<1.57	<1.57	<1.87	<2.18	<1.57
	Bird 33	<1.4	<1.57	2.35*	<1.87	<2.18	<1.57
	Bird 36	<1.4	<1.57	1.88*	<1.87	<2.18	3.09*
	Bird 39	<1.4	<1.57	2.62*	<1.87	<2.18	1.57*
	Bird 42	<1.4	<1.57	<1.57	2.35*	<2.18	<1.57
	Bird 45	<1.4	<1.57	<1.57	<1.87	<2.18	<1.57