



**PRELIMINARY STUDY ON *CAMPYLOBACTER* RECOVERY FROM POULTRY
CARCASSES.**

FINAL REPORT

Prepared for New Zealand Food Safety Authority under project FW09040 “Preliminary study on *Campylobacter* recovery from poultry carcasses” as part of overall contract for scientific services

by

Dr Susan Paulin

August 2010

Client Report
FW09040

**PRELIMINARY STUDY ON *CAMPYLOBACTER* RECOVERY FROM POULTRY
CARCASSES.**

FINAL REPORT

Stephen On
Food Safety Programme Leader

Dr Susan Paulin
Project Leader

Dr Rob Lake
Peer Reviewer

DISCLAIMER

This report or document (“the Report”) is given by the Institute of Environmental Science and Research Limited (“ESR”) solely for the benefit of the New Zealand Food Safety Authority (“NZFSA”), Public Health Services Providers and other Third Party Beneficiaries as defined in the Contract between ESR and the NZFSA, and is strictly subject to the conditions laid out in that Contract.

Neither ESR nor any of its employees makes any warranty, express or implied, or assumes any legal liability or responsibility for use of the Report or its contents by any other person or organisation.

ACKNOWLEDGEMENTS

The author would like to thank:

- The staff at the poultry processors who participated in this trial for their hard work and support.
- The PHL staff at ESR for microbiological analysis of the samples.
- Beverley Horn for assistance with data analysis and graph preparation.

CONTENTS

LIST OF FIGURES	ii
LIST OF TABLES	ii
SUMMARY	iii
1.....INTRODUCTION	1
1.1 General introduction	1
1.2 Bacterial recovery after consecutive poultry carcass rinses.....	2
1.3 Pathogen entrapment within poultry skin crevices and feather follicles	3
1.3.1 Microscopy studies	4
1.3.2 Non-rinsing <i>Campylobacter</i> recovery methods	4
1.4 Summary of literature search information	5
2.....METHOD DEVELOPMENT	7
2.1 Experimental protocol.....	7
2.1.1 Primary processing sampling point:	7
2.2 Trial 1:.....	7
2.3 Trial 2:.....	8
2.4 Transportation of rinsate samples:	9
2.5 Microbiological analysis of samples:.....	10
3.....RESULTS	11
3.1 Trial 1 results (birds 1-8):	11
3.2 Trial 2 results (birds 9-12):	14
4.1 Trial 1:.....	19
4.2 Trial 2:.....	20
5.....CONCLUSIONS	22
6.....REFERENCES	24
APPENDIX 1: bacterial plate counts from 2 ml rinsate. ALL birds sampled FOR TRIAL 1 (2 ml over 6 plates only UNLESS OTHERWISE STATED).	27
APPENDIX 2: bacterial plate counts from 2 ml rinsate. ALL birds sampled FOR TRIAL 2 (2 ml over 6 plates only UNLESS OTHERWISE STATED).	28

LIST OF FIGURES

Figure 1:	Sampling of thoracoabdominal cavity tissue (a) in situ and (b) excised from the bird.	9
Figure 2:	Recovery of <i>Campylobacter</i> (bacterial plate counts from 2 ml rinsate over 6 plates) from consecutive rinses of whole poultry carcasses and macerated skin sampled from two different processors.	12
Figure 3:	Recovery of <i>Campylobacter</i> (bacterial plate counts from 2 ml rinsate over 6 plates) from consecutive rinses of whole poultry carcasses and macerated/homogenised skin.	13
Figure 4:	Recovery of <i>Campylobacter</i> (CFU/rinsate) from rinsates of cavity, cavity tissue segment and homogenised skin (birds 9 – 12).	16
Figure 5:	Recovery of <i>Campylobacter</i> (CFU/rinsate) from rinsates of cavity, cavity tissue segment and homogenised skin from birds 13 - 18.	17

LIST OF TABLES

Table 1:	Recovery of <i>Campylobacter</i> (bacterial plate counts from 2 ml rinsate over 6 plates) illustrating differences in bacterial recovery between rinse 1 and rinse 10.	14
Table 2:	Recovery of <i>Campylobacter</i> (CFU/rinsate) from whole bird rinsate and combined homogenised skin, cavity and cavity tissue rinsates together with the percentage of initial bacteria removed from the birds following a single NMD rinse (birds 9 – 18).	15

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 before or during primary processing. While many of these bacteria will be removed as a result of intervention strategies in place during processing, some appear to strongly adhere to the skin surface and/or become entrapped within feather follicles or skin crevices.

This study was initiated after preliminary results from an NZFSA-funded project indicated that numbers of *Campylobacter* recovered from the thoracoabdominal cavities of poultry did not decrease consistently over three consecutive cavity rinses (Paulin and Wong, 2008). This supports published work reporting that the numbers of various different pathogens recovered do not typically decrease after several consecutive whole bird rinses (Mead and Thomas, 1973; Notermans, 1975; Rigby, 1982; Jorgensen *et al.*, 2002; Chantarapanont *et al.*, 2003; Jang *et al.*, 2007). Bacterial persistence on carcasses may increase the risk to the consumer through undercooking or cross contamination if chicken is not adequately stored or handled.

The aim of this work was to determine how the numbers of *Campylobacter*, quantified by the NMD sampling procedure, related to actual bacterial counts present on the carcass of birds obtained from four New Zealand poultry processors. Birds were collected at positions on the production line that were likely to optimise the chances of obtaining quantifiable numbers of *Campylobacter* from rinsates and macerated and/or homogenised skin samples.

In total eighteen birds were sampled. The first trial, comprising eight birds, was conducted with the aim of determining how many consecutive rinses were necessary before the bacterial numbers recovered from the carcass started to decline, and to determine whether the skin was acting as a reservoir for *Campylobacter*. Of the five birds with sufficient *Campylobacter* to be quantified, the numbers of bacteria recovered remained similar between the first and tenth rinses. However, the quantifiable bacterial numbers recovered from the rinsates for all but one bird were very low and as such this result may not be the same for

more heavily contaminated birds. Where detected, *Campylobacter* numbers recovered by macerating or homogenising the entire skin (removed after all rinsing) were similar to those recovered from single whole bird rinsates (Figures 2 and 3).

The second trial, comprising ten birds, was conducted with the aim of extending the results obtained in the first trial by quantifying *Campylobacter* recovered from a select number of anatomical sites only and from a position on the processing chain where higher counts in the initial rinsate were expected. For all of these birds, when considering the bacterial numbers per total rinsate volume, *Campylobacter* was quantified in the highest numbers from the initial whole bird rinsate followed by the skin and thoracoabdominal cavity rinse. Homogenates of segments of thoracoabdominal cavity tissue yielded the least bacteria (Table 2 and Figures 4 and 5). The percentage of *Campylobacter* recovered from the whole bird rinsate, compared with the combined skin, thoracoabdominal cavity and segment, was between 77 and 92%. This suggests that the majority of *Campylobacter* were recovered from the carcass in the initial rinsate (Table 2).

1. INTRODUCTION

1.1 General 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).

Campylobacter spp. are commonly found in the intestinal flora of many wild and domesticated animals and birds, including chickens. Poultry and poultry products represent an important risk factor for human *Campylobacter* infections in New Zealand. *Campylobacter* can be transferred onto poultry via fluid and faeces from the gastro-intestinal tract of infected birds before or during poultry processing. Many of these bacteria will be removed during later processing steps such as spray washing and spin chilling. The bacteria on carcasses taken before these later steps would also be removed by a sampling rinse, although some bacteria will remain attached to the carcass surface. Bacterial attachment to meat surfaces is believed to be a result of both initial physical forces and a time-dependent process characterised by an increased strength of attachment due to polysaccharide formation (Firstenberg-Eden, 1981). Variations in attachment may occur depending on the organism, the meat surface, the temperature and rinsate solution used. *Campylobacter* adherence to the skin surface and/or entrapment within feather follicles or skin crevices would likely protect the bacteria during the washing and rinsing steps that occur at processing (Mead and Thomas, 1973; Notermans, 1975; Rigby, 1982; Jorgensen *et al.*, 2002; Chantarapanont *et al.*, 2003; Jang *et al.*, 2007). Bacterial persistence on carcasses may increase the risk to the consumer resulting from undercooking or cross contamination if chicken is not adequately 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).

1.2 Bacterial recovery after consecutive poultry carcass rinses

To date, there have been very few published studies exploring the bacterial recovery of different pathogens, including *Campylobacter*, after consecutive whole poultry carcass rinses. This is important information for the poultry industry, and for the development of risk management strategies. National Microbiological Database (NMD) counts of *Campylobacter* on carcasses are derived from a single rinse procedure. It would be useful to know how these results relate to residual numbers of bacteria remaining on the carcass.

Preliminary information, from a small number of samples only, indicated that *Campylobacter* counts recovered from the thoracoabdominal cavities of poultry are amongst the highest found on any part of the birds. Furthermore, these counts did not decrease consistently, if at all, following three consecutive rinses (Paulin and Wong, 2008). These data are supported by a limited number of published reports that have shown the numbers of bacteria in successive rinses of the same poultry carcass decline at a modest rate. This has been reported for aerobic bacteria and *Enterobacteriaceae* (Lillard, 1987; 1989), *Salmonella* (Rigby, 1982; Izat *et al.*, 1991), *Campylobacter* (Jorgensen *et al.*, 2002) and coliforms (Mead and Thomas, 1973; Notermans and Kampelmacher, 1975). Despite an overall pattern of a slow reduction in numbers of pathogens recovered from the carcass, Cason *et al.*, (2006) concluded that none of the above studies reported a significant statistical difference between numbers of bacteria recovered in any two successive rinses. Examination of rinses by light microscopy has shown that the gradual release of bacterial cells on successive rinses does not seem to be associated with excessive shedding of epithelial cells (Lillard, 1987).

A study by Rigby, (1982) showed that *Salmonella* could be enumerated on processed chicken carcasses (stored at either 6 °C or -1 °C) rinsed once at each of 4 successive 24-hour intervals. There were no significant differences in numbers of salmonellae recovered at each time point, suggesting that each rinse removed only a small proportion of the total bacterial population. In another study (Izat *et al.*, 1991), carcasses were successively rinsed four times. A large variation in MPN results among individual carcass rinses was observed and in several cases, salmonellae were not recovered in the initial rinse, but were recovered from subsequent rinsates of the same carcass. Similar results were observed by Lillard, (1989) who found that attached salmonellae were not always recovered in the first whole carcass rinse, but were sometimes recovered in the later rinses. The data led the authors to conclude that only a percentage of the total *Salmonella* present on carcasses were recovered with each consecutive rinse and that a single, whole carcass rinse can result in false negative results for salmonellae. Lillard, (1987) continued to recover between 7.17 and 6.24 log₁₀ CFU/carcass of

aerobic bacteria and 5.96 and 5.12 log₁₀CFU/carcass of *Enterobacteriaceae* for up to 10 whole carcass rinses. After rinsing one chicken 40 consecutive times the difference was only 0.93 log₁₀CFU/carcass (6.08 log₁₀CFU/carcass first rinse) for *Enterobacteriaceae* and 0.85 log₁₀CFU/carcass (5.47 log₁₀CFU/carcass first rinse) for aerobes from the first to the last rinse.

There appear to be only two published papers that have specifically quantified the numbers of *Campylobacter* recovered from carcasses during consecutive rinsing. A study by Jorgensen *et al.*, (2002) compared consecutive rinses of chicken, and isolation of *Campylobacter* spp., to obtain an estimate of the proportion of bacteria remaining on the carcass after one rinse. The geometric mean of *Campylobacter* recovered from 13 carcasses in the first rinse was 4.8 log₁₀ CFU (SD = 0.4), while the second and third rinse contained 4.5 log₁₀ CFU (SD = 0.4) and 4.2 log₁₀ CFU (SD = 0.5) respectively. A study by Cason (2006) compared bacterial counts of *Campylobacter* and incidence of *Salmonella* in rinses of the same carcasses taken at 0 and 24 hours after chilling. The author found no differences in numbers of *Campylobacter* or incidence of *Salmonella* between rinses taken at the two time points.

A series of detailed studies have recently been conducted at ESR to quantify the recovery of *Campylobacter* from artificially contaminated skin-on chicken breast portions after domestic/commercial freezing and thawing (McIntyre, 2008a; 2008b; 2009). The *Campylobacter* numbers recovered in the rinsate of time zero samples (following a 2 minute rinse in 30 ml BPW and centrifugation-concentration of rinsate) were 1.5 log₁₀ CFU lower than the inoculum level employed (approximately 10⁷ CFU) meaning that most of the bacteria were not removed from the samples by the initial rinse (Dr L. McIntyre, *personal communication*). These results however cannot be totally reflective of the multi-factorial issues that contribute to natural carcass contamination.

1.3 Pathogen entrapment within poultry skin crevices and feather follicles

Live broilers typically enter the processing plants contaminated with various microorganisms (Kotula and Pandya, 1995) and during processing, additional contamination can result from bird debris, faecal material, crop and intestinal contents and carcass-to-carcass contact (Capita *et al.*, 2004). While rinsing and intervention strategies are currently in place to reduce superficially-attached pathogens on poultry skin, they may be ineffective against bacterial cells that are either firmly attached to the skin itself or entrapped within skin crevices or feather follicles. Bacteria that have attached to the poultry skin early in the processing (ie at evisceration) may not be easily removed however; continuous spray

cleaning, during poultry processing, could have the result of preventing firm surface bacterial attachment (Firstenberg-Eden, 1981). Strongly attached bacteria are likely to pose less of a danger for the consumer. Loosely attached bacteria are more likely to be transferred onto hands, surfaces and utensils while strongly attached bacteria are not easily transferred to the drip water. A detailed study by Lillard, (1989) concluded that while numbers of *Salmonella*, aerobes and *Enterobacteriaceae* are reduced during processing, a substantial number of bacteria are still attached to carcasses at the end of the process. Buhr *et al.*, (2003; 2005) and Cason *et al.*, (2004) used a strain of genetically featherless broiler chickens to demonstrate that the presence of empty feather follicles in the skin of processed broiler carcasses was not a significant factor in overall carcass bacterial contamination levels.

1.3.1 Microscopy studies

Several microscopic studies have visually demonstrated the ability of pathogens to become entrapped in skin crevices and feather follicles. It has been shown that collagen fibres, associated with chicken muscle connective tissue, expand when immersed in water. Laboratory cultures of *E. coli*, *Salmonella* and *Campylobacter* become firmly entrapped within this expanded collagen network when suspended in distilled water (Thomas and McMeekin, 1981; Campbell *et al.*, 1987). These studies suggest that changes in the microtopography of tissue surfaces affects contamination of carcasses. A study by Chantarapanont *et al.*, (2003) used green fluorescent protein-labelled *Campylobacter* and confocal microscopy to demonstrate how viable bacteria became located in crevices and feather follicles and subsequently entrapped in a watery biofilm. These authors demonstrated a greater number of *Campylobacter* cells on chicken skin at a depth of 0 to 10µm in feather follicles, which can remain in place after rinsing, than on the skin surface. These follicles and crevices provide a suitable microenvironment for bacteria to survive and a potential niche for *C. jejuni* present on chicken skin. Kim *et al.*, (1996) similarly used confocal microscopy to demonstrate the presence of *Salmonella* located in the skin crevices and follicles of poultry carcasses. Bacteria were observed floating freely in a watery biofilm even after the skin had been thoroughly rinsed.

1.3.2 Non-rinsing *Campylobacter* recovery methods

Several studies have directly compared bacterial recovery following carcass rinsing and maceration of the removed skin. Lillard, (1987) showed that whole carcass rinse and stomaching for 1 minute (300 ml peptone solution; wash in tap water; wash for 1 minute in 0.85% saline and repeat 5 times) or blending (0.1% peptone – 1:3 ratio on high speed for 1 minute) of excised skin resulted in the isolation of comparable numbers of aerobes and

Enterobacteriaceae from broilers taken after leaving the final washer and before entering the chiller. Jorgensen *et al*, (2002) sampled chilled raw frozen chickens from retail outlets in the UK and found that *Salmonella* was more frequently isolated from samples containing stomached chicken skin (buffered peptone water for 2 minutes) than carcass-rinse fluids (skin-on birds) only, but they found that the probability of isolating *Campylobacter* spp. from the neck-skin, carcass-rinse or carcass-rinse plus whole skin samples was similar. Another study by Scherer, (2006) compared different sampling techniques and enumeration methods for the quantification of *Campylobacter* on raw retail chicken legs. The authors concluded that there were no significant differences in *Campylobacter* prevalence between rinse and skin samples, with 77% and 70% positive respectively. Both rinsing and skin homogenisation/maceration were suitable for the isolation of *Campylobacter* but to detect superficially-attached bacteria as well as those lodged in skin crevices, Scherer *et al*, (2006) and Firstenberg-Eden, (1981) concluded that homogenisation (sample placed in a stomacher bag and homogenised with 225 ml Preston broth for 2 minutes) or maceration (using a blender) were the preferred methods. Firstenberg-Eden, (1981) concluded that loosely-attached bacteria were easily removed by shaking alone however the firmly-attached bacteria could only be removed by blending the meat.

1.4 Summary of literature search information

Taken together, it appears clear from the studies cited above that pathogens, including *Campylobacter*, are protected from removal from poultry skin prior to processing or during the processing procedure itself. These pathogens can resist complete removal from the carcass during processing, and therefore may present a risk to the consumer. It could be speculated however that loosely-attached bacteria may present a higher risk to the consumer, than strongly attached bacteria, as these pathogens could be transferred to drip water and therefore will be more likely to cross contaminate hands, surfaces and utensils. Strongly adherent organisms in biofilm-covered skin crevices may be protected from heat exposure but may also be less available for cross contamination. While a limited number of studies have compared bacterial counts obtained from whole carcass rinses, followed by skin removal and homogenisation, there do not appear to be any published studies that have directly quantified numbers of *Campylobacter* recovered from both consecutively rinsed carcasses and the homogenised skin removed from these birds.

This study was conducted in order to enumerate *Campylobacter* present in consecutive rinsates of poultry, obtained from New Zealand processors, and to determine the numbers of bacteria that remain strongly associated with the external poultry carcass using

more aggressive removal methods such as maceration or homogenisation. This will clarify the relationship between the single rinse NMD poultry sampling procedure and the study results.

Results from this study will be used by the New Zealand Food Safety Authority (NZFSA) to assist in control of *Campylobacter* through the food chain, and will contribute to ongoing pathogen risk model developments in this area.

2. METHOD DEVELOPMENT

2.1 Experimental protocol

The experimental part of this project was split into two distinct sections and involved four New Zealand poultry processors. During Trial 1 eight whole bird carcasses were consecutively rinsed 10 times in total prior to skin removal, skin maceration/homogenisation and enumeration of *Campylobacter*. During Trial 2 ten birds were sampled and rinsed once, followed by a cavity rinse, and then removal of both a skinless cavity tissue segment and the entire carcass skin for homogenisation and enumeration of *Campylobacter*.

All birds were either second or third cut and were sampled from flocks that were assumed to be *Campylobacter* positive based on caecal testing results from the previous cut where available. During the course of the trial mandatory caecal testing of every cut was stopped meaning that carcasses were only sampled from flocks where the previous cut NMD rinse counts had been positive for *Campylobacter*. Birds for Trial 1 were sampled between June and October 2008 while those selected for Trial 2 were sampled between April and August 2009.

2.1.1 Primary processing sampling point:

For Trial 1, initially four birds were sampled: three from post spin chiller, pre Sanova and one after full evisceration but before the inside/outside washes. As *Campylobacter* in the rinsates from these birds was either not detected or the level of bacteria was extremely low, two birds were subsequently sampled from each of two different processors at the same post spin chiller, pre Sanova position (Birds 1-8, Appendix 1). For Trial 2, ten birds were sampled at a position after full evisceration and inside/outside washes but before the spin chiller or Sanova treatment (Birds 9-18, Appendix 2).

Birds were removed from the production line, at the appropriate position, directly into a sterile bag according to NMD protocol (<http://www.nzfsa.govt.nz/animalproducts/legislation/notices/animal-material-product/nmd/schedule-1-technical-procedures-nmd-final.pdf>) (January 2008).

2.2 Trial 1:

This study was conducted in order to establish the best practice sampling methodology for enumerating both numbers of *Campylobacter* present in consecutive whole

bird rinses and numbers that remained on the skin after rinsing. A secondary aim was to determine whether the number of consecutive rinses performed needed to be modified for Trial 2.

Carcasses were consecutively rinsed 10 times according to the NMD procedure. The first rinse was taken within 30 minutes of the bird being removed from the line, and each bird was rinsed in 400 ml Buffered Peptone Water (BPW) for 2 minutes. After each rinse, the corner of the sampling bag containing the bird was cleaned with sterile “Mediwipe” swabs, the corner was cut using sterile scissors and the rinsate for sampling drained into a sterile pot. Excess liquid was discarded, the bag was split open and the bird transferred into a fresh sampling bag without touching the carcass. This procedure was used for conducting all ten whole carcass rinses. Birds were then carefully lifted from the sampling bag, by a gloved operator, and positioned onto a boning cone by grasping the neck tissue only. The skin was removed from the entire carcass, using sterile forceps and scalpel, transferred into a fresh bag with 400 ml BPW and macerated in a stomacher for 2 minutes making eleven samples in total. For birds 7 and 8, after stomaching the skin was transferred into fresh BPW, rinsed again in 400 ml BPW and finally blended in a sterile metal homogeniser in 400 ml BPW, making thirteen samples in total.

2.3 Trial 2:

This work was conducted as an extension to Trial 1 above, using ten birds sampled from two different processors. The aim of this work was to quantify the numbers of *Campylobacter* recovered from a single whole bird rinsate, a thoracoabdominal cavity rinse, the homogenised skin and a sample of tissue taken from the cavity. The sampling position was changed to pre spin chiller to increase the probability of obtaining birds with high *Campylobacter* carcass counts. Whole bird rinsates were sampled using NMD procedures as described above. The birds were then carefully removed from the bag by gathering up the neck, with sterilised gloved hands, and inverting the chicken while a second person poured 50 ml of BPW into the thoracoabdominal cavity. The vent skin was gathered and the bird rocked gently for 2 minutes. The rinsate was removed using a sterile 60 ml 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 a boning cone and the skin removed as described above. It should be noted that while every attempt was made to remove as much skin from the carcass as possible, skin from the extremities (i.e. wing, drum and

“Parsons nose” tips) was very difficult to remove completely. An estimate of the skin remaining on the carcass after this procedure was conducted would be approximately 5 to 8%. After removal, the skin was homogenised in 400 ml BPW as above. The remaining carcass was then removed, positioned breast-down on a clean bench and sterilised poultry shears were used to split the bird longitudinally up the backbone. The carcass was then opened to expose the thoracoabdominal cavity. Two portions of tissue were removed from either side of the distal sternum cartilage and homogenised in 50 ml BPW. The tissue portions represented approximately one sixth of the thoracoabdominal cavity area, weighed between 7 and 9 grams each and measuring approximately 15 cm in width x 40 cm in length (Figures 1a and 1b).

Figure 1: Sampling of thoracoabdominal cavity tissue (a) in situ and (b) excised from the bird.

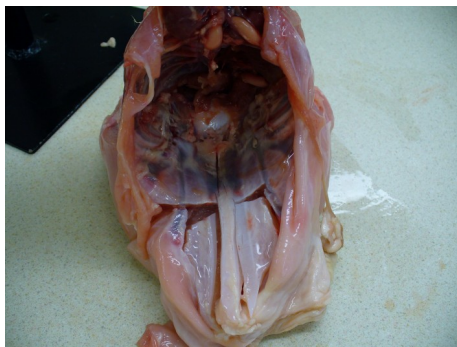


Figure 1a



Figure 1b

2.4 Transportation of rinsate samples:

The rinsates and the samples containing skin and cavity tissue for homogenisation 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 while skin and tissue samples, together with the BPW, were poured into leak-proof Whirl-Pak sterile bags. Once collected, the samples were held at 4°C and dispatched according to the NMD protocol. Samples were packaged into chilly bins, containing frozen ice packs, and either sent by courier or taken directly to ESR, Christchurch as soon as possible after completion of carcass portioning and rinsing. To confirm that samples sent by courier were reaching their destination at temperatures $\leq 10^{\circ}\text{C}$, a water blank was included which was subsequently temperature-tested at ESR.

2.5 Microbiological analysis of samples:

All samples were received at ESR, Christchurch either by 9:00 am on the day following the trial (if sent by courier) or on the same day as the trial. Samples were processed as soon as they arrived at the PHL lab 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 desoxycholate agar (mCCDA) (2 ml over 6 plates). The limit of detection therefore depended on the volume of rinsate used. Not detected (ND) results represented <200 Colony Forming Units (CFU) for samples rinsed in 400 ml BPW and <25 CFU for samples rinsed and homogenised in 50 ml. In addition, 0.1 ml of rinsate was plated onto each of two plates to obtain a 1:10 dilution.

Five colonies per bird were selected, from positive plates, 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).

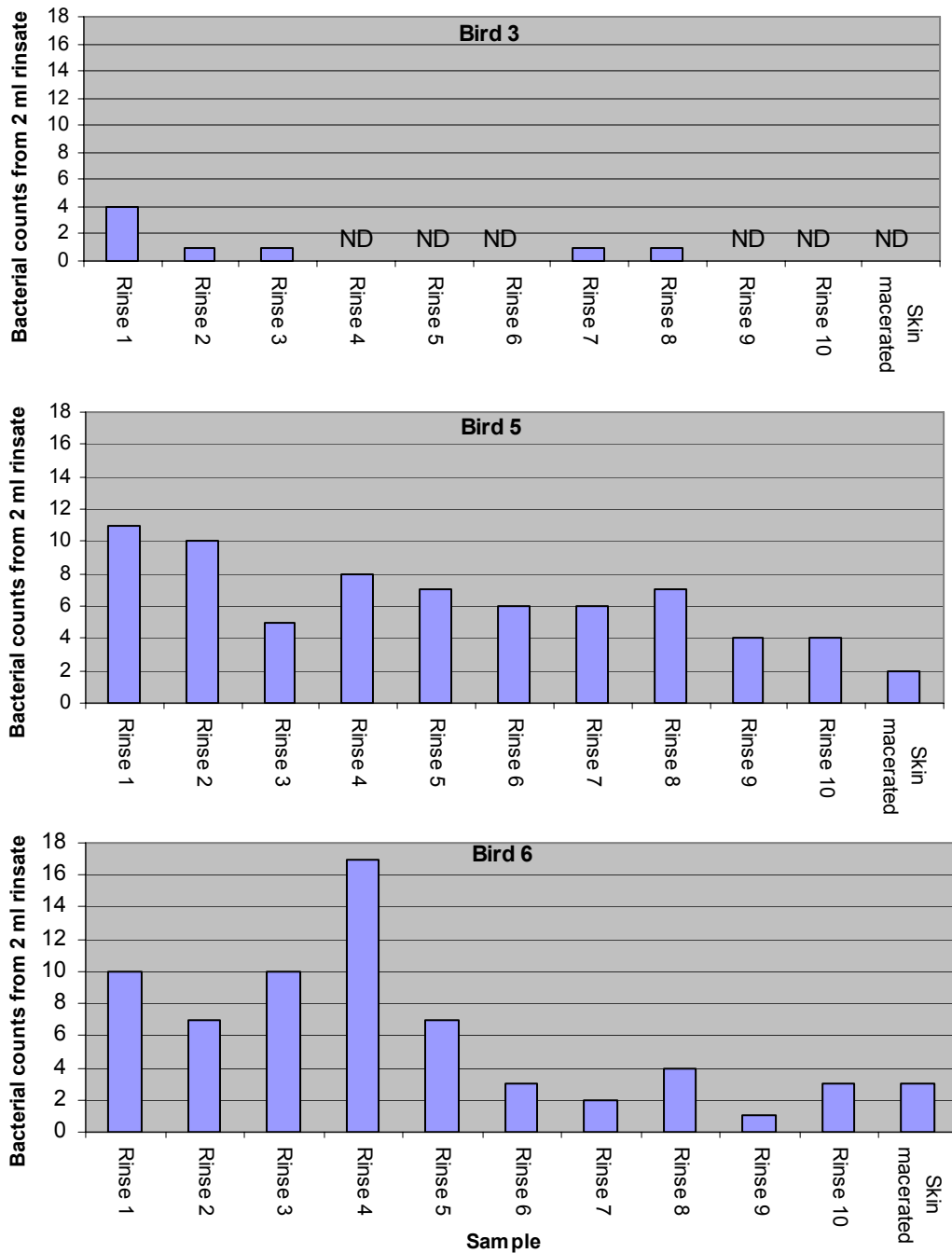
3. RESULTS

The complete set of *Campylobacter* counts for all birds sampled are presented in Appendix 1 (Trial 1) and Appendix 2 (Trial 2). Out of the eighteen birds sampled, from the four poultry processors, *Campylobacter* could be recovered from the rinsates and macerated/homogenised samples of only fifteen chickens (five in Trial 1 and ten in Trial 2). These results have been presented in Figures 2-5 and Tables 1 and 2.

3.1 Trial 1 results (birds 1-8):

The numbers of *Campylobacter* present in the consecutive rinsates and macerated skin from birds 1-4 were either very low or below the level of detection therefore, results for only one bird (Bird 3) are presented in Figure 2. The numbers of *Campylobacter* present in the rinsates from birds 5 and 6 are also presented in Figure 2 (presented as bacterial plate counts from 2 ml rinsate spread over 6 plates). In addition to the rinsates, two extra samples (skin rinsed and skin homogenised) were taken from birds 7 and 8 and the numbers of *Campylobacter* present in the samples from these birds are presented in Figure 3. To illustrate differences in bacterial recovery from consecutive rinses, *Campylobacter* numbers in rinsates 1 and 10 are presented in Table 1.

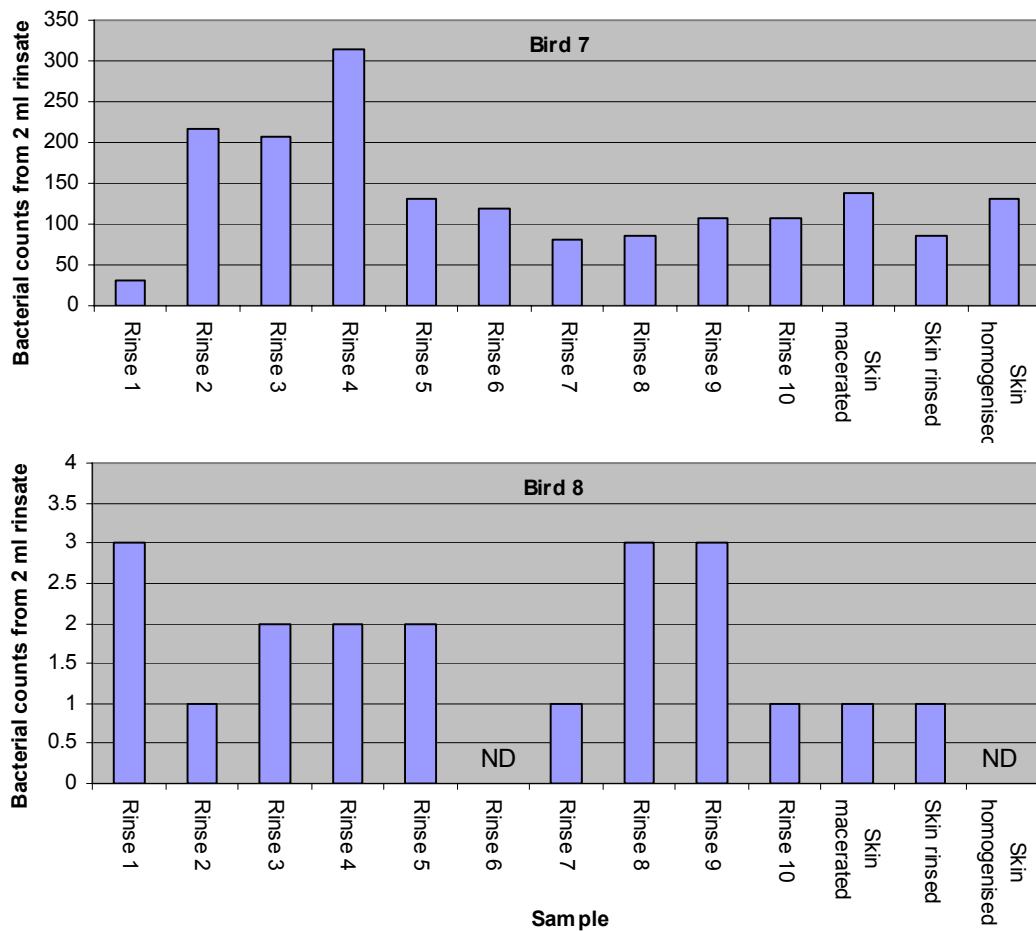
Figure 2: Recovery of *Campylobacter* (bacterial plate counts from 2 ml rinsate over 6 plates) from consecutive rinses of whole poultry carcasses and macerated skin sampled from two different processors.



ND – not detected

All of the samples were rinsed in 400 ml BPW

Figure 3: Recovery of *Campylobacter* (bacterial plate counts from 2 ml rinsate over 6 plates) from consecutive rinses of whole poultry carcasses and macerated/homogenised skin.



ND – not detected

All of the samples were rinsed in 400 ml BPW

The homogenised skin samples were diluted 1:10 before plating therefore the figures used for the graph represent the actual plate counts multiplied by ten.

Table 1: Recovery of *Campylobacter* (bacterial plate counts from 2 ml rinsate over 6 plates) illustrating differences in bacterial recovery between rinse 1 and rinse 10.

Bird number	3	5	6	7	8
Sample					
Whole bird rinse 1	4	11	10	31	3
Whole bird rinse 10	ND	4	3	107	1

ND – not detected

There are insufficient positive results in Trial 1 to conduct statistical analyses however, the following conclusions can be drawn from the above results (Figures 2 and 3 and Table 1):

- *Campylobacter* could be consistently recovered from almost all of the consecutive whole bird rinsates indicating, as expected, that the NMD single rinse does not remove all of the *Campylobacter* from the poultry carcass.
- Although *Campylobacter* continued to be recovered in consecutive rinses, the overall numbers were modest. The exception was Bird 7, where higher numbers of bacteria were recovered, and in contrast to the other four birds, the count from rinsate 1 was lower than the subsequent rinsates.
- *Campylobacter* was recovered from most of the macerated and/or homogenised skin rinsates in similar, or slightly lower, numbers to those found in the whole carcass rinsates despite the more aggressive approach to bacterial removal from these samples. For birds 7 and 8, three of the four skin treatment samples taken after the initial “skin macerated” sample (i.e. stomached), recovered further bacteria.

3.2 Trial 2 results (birds 9-12):

For Trial 2, the sampling position was changed to increase the possibility of obtaining birds with high *Campylobacter* carcass counts. Furthermore, the number of samples taken per bird was reduced as results from Trial 1 clearly indicated that even after several whole bird rinses bacteria could still be recovered from the chicken carcass. Results derived from the carcasses of birds 9-18 are presented in Table 2 and Figures 4 and 5. Birds were sampled from two different poultry processors and are presented as separate Figures.

Table 2: Recovery of *Campylobacter* (CFU/rinsate) from whole bird rinsate and combined homogenised skin, cavity and cavity tissue rinsates together with the percentage of initial bacteria removed from the birds following a single NMD rinse (birds 9 – 18).

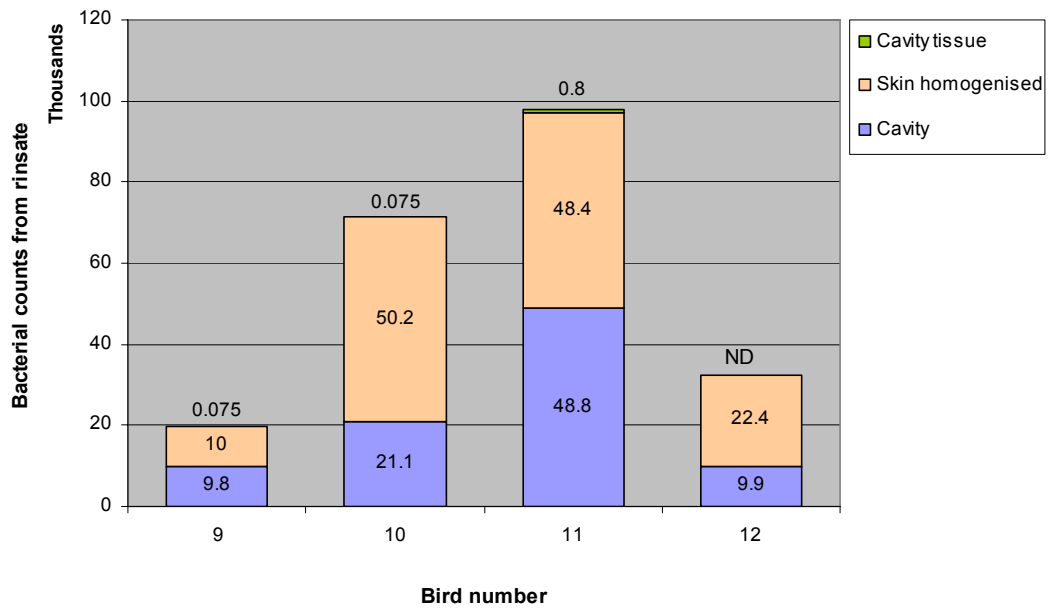
Bird number	9	10	11	12	13	14	15	16	17	18
Sample										
Whole bird rinse	124800	*426000	*336000	242400	*141400 0	13000	153200	1600	8600	*762000
Combined cavity rinse; skin and cavity tissue homogenate	19900	71375	*97925	32275	*124975	3525	28975	375	1325	*170575
Percentage of initial <i>Campylobacter</i> removed following a single whole bird rinsate (%)	86.2	85.6	77.4	88.2	91.9	78.7	84.1	81.0	86.6	81.7

*1:10 dilution counts (cavity rinse and/or whole bird rinse only) used if neat was too numerous to count

Percentage of initial *Campylobacter* removed derived from whole bird rinse / whole bird rinse + combined samples *100.

Most of the *Campylobacter* were removed following the initial whole bird rinse therefore Figures 4 and 5 represent the bacterial counts per rinsate recovered from the remaining samples (cavity, cavity tissue segment and skin).

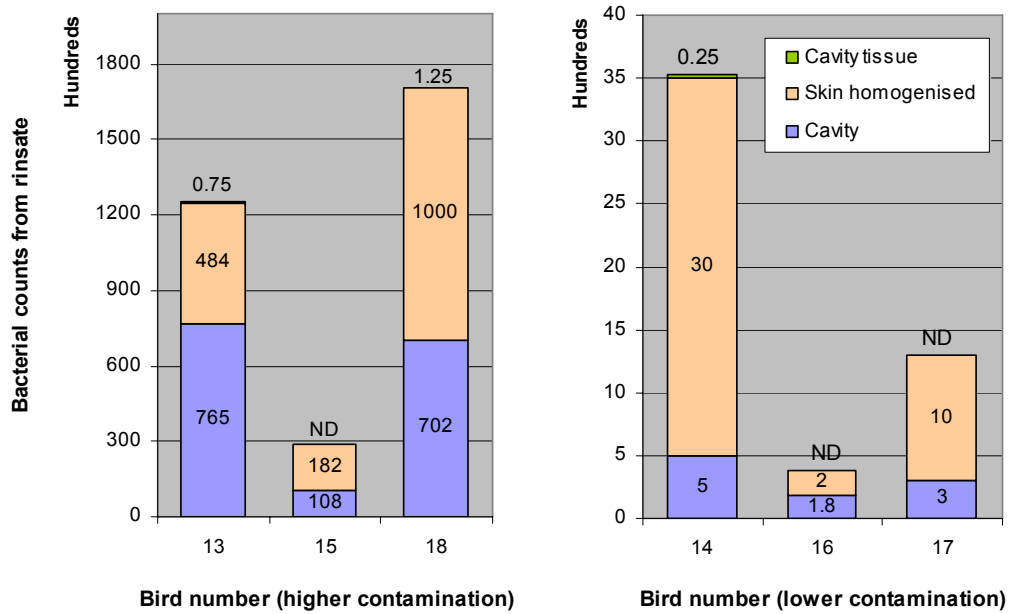
Figure 4: Recovery of *Campylobacter* (CFU/rinsate) from rinsates of cavity, cavity tissue segment and homogenised skin (birds 9 – 12).



ND – no counts detected from cavity rinse sample

The cavity rinsate from bird 11 represents bacterial counts taken from the minus one dilution (as the colonies from the initial rinse were too numerous to count).

Figure 5: Recovery of *Campylobacter* (CFU/rinsate) from rinsates of cavity, cavity tissue segment and homogenised skin from birds 13 - 18.



ND – no counts detected from cavity rinse samples

Cavity rinsates from birds 13 and 18 represent bacterial counts taken from the minus one dilution (as the colonies from the initial rinse were too numerous to count).

The following conclusions can be drawn from the above results (Table 2 and Figures 4 and 5):

- The numbers of *Campylobacter* recovered from the initial whole bird rinsate, for all of the birds sampled, were higher than for the combined subsequent skin homogenate, cavity and cavity tissue rinses (Table 2). The combined subsequent bacterial counts do exclude small amounts of skin present on the wing and drum extremities, the remainder of the thoracoabdominal cavity tissue and the Parsons nose tip (estimated as approximately 5 - 8% of the total carcass skin).
- The percentage of *Campylobacter* recovered following the initial single whole bird rinsate ranged from 77.4 to 91.9% suggesting that for more heavily-contaminated birds the initial rinse removes the majority of *Campylobacter* present on the carcass.
- The cavity tissue segment samples yielded only low or not detectable numbers of *Campylobacter*.

4. DISCUSSION

Currently, quantitative data on the bacterial recovery of *Campylobacter* from consecutive whole poultry carcass rinses and poultry skin are limited. However, a few studies have demonstrated that bacteria can adhere to and/or become entrapped within feather follicles or skin crevices meaning that these pathogens resist removal from the carcass during the washing and rinsing steps that occur at processing (Mead and Thomas, 1973; Notermans, 1975; Rigby, Firstenberg-Eden, 1981; 1982; Jorgensen *et al.*, 2002; Chantarapanont *et al.*, 2003; Jang *et al.*, 2007).

It is important to remember that this study was conducted with samples taken before processing steps that would reduce *Campylobacter* numbers (Trial 1 carcasses were taken pre-Sanovia, and Trial 2 carcasses were taken pre-spin chill and Sanovia). This was done to increase the probability of obtaining positive results, but means that the results may not directly correlate with those obtained from retail birds.

4.1 Trial 1:

The original aim of Trial 1 was to determine how many whole bird rinses would be required before a “not detected” result was obtained, as a guide for further studies. However, despite selection of birds from presumed *Campylobacter*-positive flocks, it proved difficult to obtain birds that had detectable bacterial counts in the whole carcass rinsates. The low numbers and high proportion of not detected results may have been due to the post spin chill sampling position.

From the five birds that did have quantifiable bacterial counts in the whole carcass rinsates, there were only minor differences in *Campylobacter* numbers recovered from rinse one compared to rinse ten. It should however be noted that the bacterial recovery from most of the birds was only slightly higher than the limit of detection. This is consistent with results obtained by Jorgensen *et al.*, (2002) who found a reduction of 0.6 log₁₀ CFU of *Campylobacter* on whole bird carcasses between rinse 1 and rinse 3. Similarly, only a modest decrease in the recovery of various pathogens, after multiple poultry carcass rinses, has been reported by Lillard (1987; 1989), Rigby (1982), Izat *et al.* (1991), Mead and Thomas (1973), and Notermans and Kampelmacher (1975). However as the *Campylobacter* carcass loading on all but one of the positive birds was only just above the level of bacterial detection, these

results may not be representative of more heavily contaminated birds. The *Campylobacter* counts from Bird 7 were lowest in the first rinsate, which is different from the other four birds in trial 1, suggesting that this first carcass rinse may not have been conducted in the same way as the others. Operator variation in such a manual procedure would be expected.

For all five birds in this trial (and indeed in trial 2), *Campylobacter* were always recovered from the first rinse, where bacteria were also recovered from later rinses or samples. This was not the case in studies by Izat *et al.*, (1991) and Lillard, (1989) who observed that recovery of *Salmonella* was sometimes not detected from the first rinse but may be present in subsequent consecutive carcass rinses. This offers reassurance that any birds positive for *Campylobacter* will be detected by the current NMD sampling procedure.

The *Campylobacter* numbers in the macerated and homogenised skin samples were, where detected, similar or slightly lower than the numbers associated with the whole carcass rinsates. This is consistent with the study by Lillard (1987) who demonstrated that whole carcass rinse and stomaching or blending of excised skin resulted in the isolation of comparable numbers of *Enterobacteriaceae* from broilers prior to entering the spin chiller.

The similar recovery using more aggressive treatments suggests that bacteria adhering to the poultry skin and/or entrapped within feather follicles and skin crevices, will be difficult to remove by the rinsing and washing procedures used by processors to reduce carcass bacterial loads.

A recent study evaluated the potential benefit of adding sand to whole carcass rinsates and concluded that bacterial levels with sand added to the broth were significantly higher than peptone-only rinsates (Hannah *et al.*, 2008). The addition of an abrasive substance to the rinsate may have the effect of loosening, and therefore removing, the strongly-attached bacteria or disrupting bacterial migration into and out of the watery biofilm overlaying skin crevices and feather follicles.

4.2 Trial 2:

As the first trial demonstrated that rinsing whole carcasses ten times continued to result in the presence of detectable numbers of bacteria in most of the samples, the protocol for Trial 2 was modified to gather additional information on cavity rinses and cavity tissue. The change in sampling position was aimed at obtaining birds with heavier contamination levels than those used in Trial 1.

The numbers of *Campylobacter* recovered from the whole carcass rinse in trial 2 were higher than the aggregate of the three subsequent samples. This suggests that for more heavily contaminated carcasses, the first rinse removes most of the bacteria, with declining recovery from subsequent samples. This conclusion needs to be treated with caution, as the methodology for Trials 1 and 2 was not the same. As Trial 1 and a previous study have indicated, repeated treatments of skin and repeated rinsing of the thoracoabdominal cavity will recover more bacteria following each separate rinse (Paulin and Wong, 2008). It could however be hypothesised that more aggressive treatments, such as maceration or homogenisation, may recover more *Campylobacter* than repeated whole bird rinses would have done, particularly in the case of bacteria strongly attached to the skin surface.

It was not possible to completely remove all of the skin from the carcass following the first rinse and as such it becomes difficult to suggest how bacterial loading on the extremities might influence the overall *Campylobacter* counts from the poultry skin. However, a previous study has recognised the “Parsons nose” as an area of potentially high carcass contamination (Paulin and Wong, 2008), and thus the omission of this site and extremities from the excised skin may have contributed to the lower combined counts.

5. CONCLUSIONS

The aim of this work was to determine how the numbers of *Campylobacter* quantified by the NMD sampling procedure related to actual bacterial counts present on the carcass. Birds were sampled from four New Zealand poultry processors and were, particularly in the case of Trial 2, intentionally selected from sampling positions prior to a number of processing steps aimed at reducing the level of *Campylobacter* on poultry in order to increase the probability of obtaining quantifiable counts. As such, total counts on carcasses found in these experiments are not necessarily representative of counts observed on chickens available to consumers.

It is encouraging that in this study, first rinsates were always positive when subsequent samples were positive (the only exception being bird 1 from Trial 1 where a single colony was detected in rinse 9), thus supporting the validity of the NMD sampling procedure. The results of Trial 2 suggest that for more heavily contaminated birds, the first rinsate recovers a higher proportion of the total bacteria compared to birds carrying lower numbers, and this improves confidence in the use of single rinse data to estimate risk, which largely derives from more heavily contaminated carcasses. However, the numerical relationship between the numbers of bacteria obtained in the first rinsate to those recoverable by further sampling, or remaining on the carcass, is not completely defined by these results. It is apparent that there is a population of bacteria which remains on the carcass following the first rinse, and the persistence of recovered bacteria in consecutive rinsates and skin samples suggests that completely eliminating these bacteria during full primary processing would be very difficult. The numbers of these strongly attached or trapped bacteria may also be underestimated by the aggressive treatments of the skin in this study, since these treatments themselves may destroy the bacterial cells. It could also be argued that if these bacteria are strongly attached to the skin then the likelihood of cross contamination may also be reduced.

In exposure assessments of *Campylobacter* spp. from 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 bacteria 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 and crevices”. If it is assumed that the bacteria recovered from the macerated and homogenised skin samples in this study are those entrapped within skin crevices and feather follicles, then these data may be useful for modelling purposes.

The number of bacteria on the carcass is clearly higher than those recovered from a single rinse. The significance of this in terms of the risk of human exposure is uncertain. Models estimating exposure from cross contamination that use single rinse data for the numbers available for transfer may be underestimating exposure. A conversion factor may therefore be required. The risk from undercooking may also be underestimated by these data, since the numbers of bacteria still on the carcass, and perhaps protected from heat in follicles and crevices, are uncertain.

Further research to investigate these issue could include:

- Examining the effect of adding an abrasive material such as sand, to enhance recovery from rinsates;
- Performing experiments that mimic undercooking, to determine survival of any bacterial cells.
- Sample a small number of extra birds, as per Trial 1, selecting chickens from a sampling position more likely to yield *Campylobacter*-positive rinsates.

6. REFERENCES

- Buhr, R.J., Berrang, M.E., Cason, J.A. (2003). Bacterial recovery from breast skin of genetically feathered and featherless broiler carcasses immediately following scalding and picking. *Poultry Science*. **82**: 16410-1647.
- Buhr, R.J., Bourarra, D.V., Northcutt, J.K., Hinton, A., Ingram, K.D., Cason, J.A. (2005). Bacterial recovery from genetically feathered and featherless broiler carcasses after immersion chilling. *Poultry Science*. **84**: 1499-1504.
- Campbell, S., Duckworth, S., Thomas, C.J., McMeekin, T.A. (1987). A note on adhesion of bacteria to chicken muscle connective tissue. *Journal of Applied Bacteriology*. **63**: 67-71.
- Capita, R., Prieto, M., Alonsi-Calleja, C. (2004). Sampling methods for microbiological analysis of red meat and poultry carcasses. *Journal of Food Protection*. **67**: 1303-1308.
- Cason, J.A., Hinton, A., Buhr, R.J. (2004). Impact of feathers and feather follicles on broiler carcass bacteria. *Poultry Science*. **83**: 1452-1455.
- Cason, J.A., Berrang, M.E., Smith, D.P. (2006). Recovery of bacteria from broiler carcasses rinsed zero and twenty-four hours after immersion chilling. *Poultry Science Association Inc.* 333-336.
- Chantarapanont, W., Berrang, M., Frank, J.F. (2003). Direct microscopic observation and viability determination of *Campylobacter jejuni* on chicken skin. *Journal of Food Protection*. **66**: 2222-2230.
- Eberhart-Phillips J, Walker N., Garrett N., Bell D., Sinclair D., Rainger W., Bates M. (1997). *Campylobacteriosis* in New Zealand: results of a case control study. *Journal of Epidemiology and Community Health*, 51:686-691.
- ESR (2007) Notifiable and other diseases in New Zealand. Annual Report 2007. ESR Client Report FW08034. Porirua: ESR.
- FAO/WHO (2005). Hazard identification, hazard characterisation, and exposure assessment of *Campylobacter* spp. in broiler chickens. Joints FAO/WHO Activities on Risk Assessment of Microbiological Hazards in Foods. Preliminary Report. MRA 01/05.
- Firstenberg-Eden R. (1981) Attachment of bacteria to meat surfaces: a review. *Journal of Food Protection*, 44:602-607.

- French N., Carter P., Collins-Emerson J., Midwinter A., Mullner P., Wilson D. (2008). Comparing source attribution models for human campylobacteriosis. Poster presentation at the Society for Veterinary Epidemiology and Preventive Medicine conference. Liverpool, UK.
- Hannah, JF., Fletcher, DL., Cox, NA., Smith, DP., Cason, JA., Northcutt, JK., Richardson, LJ., Buhrt, RJ. (2008). Effect of sand and shaking duration on the recovery of aerobic bacteria, coliforms and *Escherichia coli* from prechill broiler whole carcass rinsates. *Journal of Applied Poultry Research*. **17**: 272-277.
- Izat, AL., Yamaguchi, W., Kaniawati, S., McGinnis, JP., Raymond, SG., Hierholzer, RE., Kopek, JM., Mauromoustakos, A. (1991). Use of consecutive carcass rinses and a most probable number procedure to estimate salmonellae contamination of inoculated broilers. *Poultry Science*. **70**: 1448-51.
- Jang, KI., Kim, MG., Ha, SD., Kim, KS., Lee, KH., Chung, DH., Kim, CH, Kim, KY. (2007). Morphology and adhesion of *Campylobacter jejuni* to chicken skin under varying conditions. *Journal of Microbiol and Biotechnology*. **17**: 202-206.
- Jorgensen, F., Bailey, R., Williams, S., Henderson, P., Wareing, DRA., Bolton, FJ., Frost, JA., Ward, L., Humphry, TJ. (2002). Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. *International Journal of Food Microbiology*. **76**: 151-164.
- Kotula, KL., Pandya, Y. (1995). Bacterial contamination of broiler chickens before scalding. *Journal of food Protection*. **58**:1326-1329.
- Kim, KY., Frank, JF., Craven, SE. (1996). Three-dimensional visualisation of *Salmonella* attachment to poultry skin using confocal scanning laser microscopy. *Letters in Applied Microbiology*. **22**: 280-282.
- Lillard, HS. (1987). Comparison of sampling methods and implications for bacterial decontamination of poultry carcasses by rinsing. *Journal of Food Protection*. **51**: 405-408.
- Lillard, HS. (1989). Incidence and recovery of salmonellae and other bacteria from commercially processed poultry carcasses at selected pre-and post-evisceration sites. *Journal of Food Protection*. **52**: 88-91.

- McIntyre, L. (2008a). Quantifying the reduction of *Campylobacter jejuni* on skin-on chicken breasts frozen and stored for up to ten weeks in a domestic freezer. Client report FW0776. Prepared by ESR for the NZFSA
- McIntyre, L. (2008b). Quantifying the reduction of *Campylobacter jejuni* on skin-on chicken breasts commercially frozen and stored for up to ten weeks in a domestic freezer. Client report FW0799. Prepared by ESR for the NZFSA
- McIntyre, L. (2009). Quantifying the reduction of *Campylobacter jejuni* on skin-on chicken breasts frozen and stored for up to ten weeks at -12°C. Client report FW0915. Prepared by ESR for the NZFSA
- Mead, GC., Thomas, NL. (1973). The bacteriological condition of eviscerated chickens processed under controlled conditions in a spin-chilling system and sampled by two different methods. *British Poultry Science*. **14**: 413-419.
- Notermans, S., Kampelmacher, EH. (1975). Further studies on the attachment of bacteria to skin. *British Poultry Science*. **16**: 487-96.
- Paulin, S.M., Wong, T. (2008). Quantification of *Campylobacter* from internal and external carcass rinses. Client report FW0879. Prepared by ESR for the NZFSA
- Rigby, CE. (1982). Most probable number cultures for assessing *Salmonella* contamination of eviscerated broiler carcasses. *Canadian Journal of Comprehensive Medicine*. **46**: 279-282.
- Scherer, K., Bartelt, E., Sommerfeld, C., Hildebrandt, G. (2006). Comparison of different sampling techniques and enumeration methods for the isolation and quantification of *Campylobacter* spp. in raw, retail chicken legs. *International Journal of Food Microbiology*. **108**: 115-119.
- Thomas, CJ., McMeekin, TA. (1981). Attachment of *Salmonella* spp. to chicken muscle surfaces. *Applied and Environmental Microbiology*. **42**: 130-134.
- Wong TL., Devane ML., Hudson JA., Scholes P., Savill MG., Klena JD. (2004). Validation of a PCR method for *Campylobacter* detection in poultry packs. *British Food Journal*, **106**: 642-650.

APPENDIX 1: BACTERIAL PLATE COUNTS FROM 2 ML RINSATE. ALL BIRDS SAMPLED FOR TRIAL 1 (2 ML OVER 6 PLATES ONLY UNLESS OTHERWISE STATED).

Sample	Bird number	Processor A				Processor B		Processor C	
		1	2	3	4	5	6	7	8
Volume									
rinse 1	400 ml	ND	ND	4	ND	11	10	31	3
rinse 2	400 ml	ND	ND	1	ND	10	7	216	1
rinse 3	400 ml	ND	ND	1	ND	5	10	207	2
rinse 4	400 ml	ND	ND	ND	ND	8	17	315	2
rinse 5	400 ml	ND	ND	ND	ND	7	7	132	2
rinse 6	400 ml	ND	ND	ND	ND	6	3	120	0
rinse 7	400 ml	ND	ND	1	ND	6	2	80	1
rinse 8	400 ml	ND	ND	1	ND	7	4	86	3
rinse 9	400 ml	1	ND	ND	ND	4	1	107	3
rinse 10	400 ml	ND	ND	ND	ND	4	3	107	1
skin (macerated)	400 ml	ND	ND	ND	ND	2	3	139	1
skin (rinsed)	400 ml	NS	NS	NS	NS	NS	NS	85	1
skin (homogenised)	400 ml	NS	NS	NS	NS	NS	NS	*130	*ND

* 1:10 dilution used samples as neat samples were TNTC

NS – not sampled

ND – no counts detected

APPENDIX 2: BACTERIAL PLATE COUNTS FROM 2 ML RINSATE. ALL BIRDS SAMPLED FOR TRIAL 2 (2 ML OVER 6 PLATES ONLY UNLESS OTHERWISE STATED).

	Processor C					Processor D				
	9	10	11	12	13	14	15	16	17	18
Sample										
rinse 1	624	*2130	*1680	1212	*7070	65	766	8	43	*3810
cavity	393	844	*1950	395	*3060	20	431	7	13	*2810
skin (homogenised)	50	251	242	112	242	15	91	1	5	501
cavity tissue	3	3	31	ND	3	1	ND	ND	ND	5

* 1:10 dilution used for samples as neat samples were TNTC

NS – not sampled

ND – no counts detected