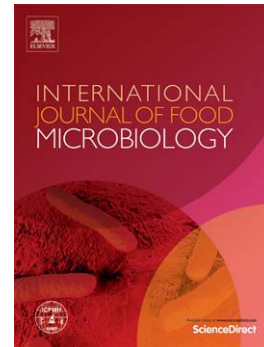# Accepted Manuscript

Quantitative effects of in-line operations on *Campylobacter* and *E. coli* through two Australian broiler processing plants

Lesley L. Duffy, Patrick J. Blackall, Rowland N. Cobbold, Narelle Fegan

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Quantitative effects of in-line operations on *Campylobacter* and *E. coli* through two Australian broiler processing plants

Running Title: *Campylobacter* through poultry processing

Short Communication

Lesley L. Duffy[a,c]*, Patrick J. Blackall[b], Rowland N. Cobbold[c], Narelle Fegan[d]

[a]*CSIRO Animal, Food and Health Sciences, 39 Kessels Road, Coopers Plains, Brisbane, Australia 4108. Email: Lesley.Duffy@csiro.au; Ph: +61 7 3214 2055; Fax: +61 7 3214 2150

[b]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, 41 Boggo Road, Dutton Park, Brisbane, Australia 4102. Email: pblackall@uq.edu.au

[c]The University of Queensland, School of Veterinary Science, Gatton, Australia 4343. (rcobbold@uq.edu.au)

[d]CSIRO Animal, Food & Health Sciences, Werribee, Australia. (Narelle.Fegan@csiro.au)

Abstract

Campylobacter is an important food borne pathogen, mainly associated with poultry. A lack of through-chain quantitative *Campylobacter* data has been highlighted within quantitative risk assessments. The aim of this study was to quantitatively and qualitatively measure *Campylobacter* and *Escherichia coli* concentration on chicken carcasses through poultry slaughter. Chickens (n=240) were sampled from each of four flocks along the processing chain, before scald, after scald, before chill, after chill, after packaging and from individual caeca. The overall prevalence of *Campylobacter* after packaging was 83% with a median concentration of 0.8 $\log_{10}$ CFU/mL. The processing points of scalding and chilling had significant mean reductions of both *Campylobacter* (1.8 and 2.9 $\log_{10}$ CFU/carcase) and *E. coli* (1.3 and 2.5 $\log_{10}$ CFU/carcase). The concentration of *E. coli* and *Campylobacter* was significantly correlated throughout processing indicating that *E. coli* may be a useful indicator organism for reductions in *Campylobacter* concentration. The carriage of species varied between flocks, with two flocks dominated by *C. coli* and two flocks dominated by *C. jejuni*. Current processing practices can lead to significant reductions in the concentration of *Campylobacter* on carcasses. Further understanding of the variable effect of processing on *Campylobacter* and the survival of specific genotypes may enable more targeted interventions to reduce the concentration of this poultry associated pathogen.

1. Introduction

Campylobacter is the leading cause of bacterial gastroenteritis in many industrialised countries including Australia (Allos, 2001; EFSA, 2012; Stafford et al., 2008). Notification rates vary across the globe. The number of cases per 100,000 is reported as 14.3 in the United States (CDC, 2013) , 101.6, Australia (NNDSS, 2013), 162.5, New Zealand (NZPHSR, 2013) in 2012 and 113.4, United Kingdom (EFSA, 2012) in 2010.. Although campylobacteriosis is typically moderate in severity and self-limiting, it is a cause of significant morbidity and sequelae including irritable bowel syndrome, inflammatory bowel disease, reactive arthritis and Guillain-Barré syndrome (GBS) (Jacobs et al., 2008). Although only a small proportion of *Campylobacter* infections go on to develop these severe symptoms, the large number of campylobacteriosis cases means that a notable number of people need substantial on-going care following infection (Jacobs et al., 2008).

Poultry meat is considered the leading source of *Campylobacter* exposure in Europe, the US and Australia (EFSA, 2010c; Friedman et al., 2004; Stafford et al., 2008). The prevalence of *Campylobacter* carriage in poultry at slaughter can vary from 5 to 100%, with a mean across European Member states in 2008 of 75.8% (EFSA, 2010a). The prevalence of *Campylobacter* on poultry at retail or at the end of processing in Australia has been reported from 84.3 to 95.8% (FSANZ, 2010; King and Adams, 2008). It has been recognized in a risk assessment of broilers conducted by the World Health Organization (FAO/WHO, 2009), that there are a lack of quantitative data both on-farm and through primary processing of poultry meat. This lack of data was also highlighted in an Australian risk assessment of broilers conducted by Food Standards Australia and New Zealand (FSANZ) (FSANZ, 2005), such that primary processing stages were only assessed qualitatively. The lack of such data makes it difficult to conclusively identify which stages within poultry processing which may have a

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significant impact on the prevalence or level of *Campylobacter* within Australian poultry processing.

While FSANZ have released a primary production and processing standard for the poultry industry in Australia, there are currently no regulatory measures regarding an acceptable prevalence or concentration of *Campylobacter* or *Salmonella* in poultry. However, poultry growers work towards minimising the introduction and spread of these food borne pathogens by compliance with an industry biosecurity manual (DAFF, 2009). While much work continues on pre-processing controls of *Campylobacter* such as farm biosecurity, the use of Hazard Analysis and Critical Control Point programs within the processing plant should also be highlighted. A reduction of $\log_{10} 2$ on carcass has been suggested to be able to reduce the campylobacteriosis rate in humans by 30 times (Rosenquist et al., 2003). There is a higher difficulty and cost associated with monitoring *Campylobacter* in poultry processing in comparison to *E. coli* (Altekruse et al., 2009; Berrang and Bailey, 2008) which creates a challenge with respect to implementing regulations based on *Campylobacter*.

The Australian chicken meat industry slaughtered 512 mill chickens in 2010 to produce 934 k tonnes of chicken meat (ACMF, 2011). The average dressed weight of chickens at slaughter in Australia in 2010 was 1.82 kg (ACMF, 2011). As a general guide Australian poultry processing plants operate in the following stages; Stunning either electrical or gas, bleeding, scalding between 50 and 58 °C for 2 to 3 min with counter flow multistage tanks, evisceration, pre-wash followed by immersion chilling and/or air chilling. Immersion chill tanks are commonly multistage counter flow with the use of chlorine at a level up to 5 ppm of free available chlorine. This study was designed as a pilot to begin to fill the gap in data availability on *Campylobacter* prevalence through the poultry processing chain and provide quantitative data on processes that effectively reduce *Campylobacter* concentration, to enable future risk assessments. The study also aims to assess the potential use of *E. coli* as

a surrogate measure of the control of *Campylobacter* concentration through broiler processing.

2. Materials and Methods

2.1 Sampling

Whole chickens were collected from poultry processing at Plant A (flocks 1 and 3) and Plant B (flocks 2 and 4). The plants, located in different Australian states, were sampled in order of flock number; November 2009, January 2010, April 2010 and July 2010. Both plants operated with a line speed of approximately 160 chickens per minute. Flocks were not pre-tested for the presence of *Campylobacter* before being sent for slaughter, but were selected on the day of sampling after arrival at the abattoir, as the next flock scheduled for slaughter with a live weight >2.5 kg and were ≥40 days of age. No flocks that were slaughtered at the beginning of a processing shift were selected.

Carcasses (n=10) were collected in-line (approximately every fifth chicken) from five sites sequentially such that the same flock was tested throughout the process at the following sites: immediately before scald but after bleed-out (BS); immediately after scald but before defeathering (AS); after evisceration immediately before immersion chilling (BC); after immersion chilling (AC); and after packaging (AP). After packaging samples were collected immediately before whole chickens were bagged so as to capture the final product before leaving the processing plant. Individual caeca (n=10) were also collected from each flock at the point of evisceration and placed into small stomacher bags (17 x 30 cm; Amyl Media, Victoria, Australia), for a total of 240 samples across all flocks. Caecal samples were held on

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ice, to ensure they were chilled but not frozen before processingg at the Brisbane laboratory within 24 h of collection. Whole birds were placed into individual large stomacher bags (38 x 50 cm; Sarstedt, South Australia, Australia) held at ambient temperature and sampled within 2 h of collection. At sampling sites where chickens with intact feathers, feet, heads, intestinal contents or combinations of these materials were sampled, the chickens were rinsed as is without removal of these parts.

Whole chickens were sampled using the whole bird rinse technique following Australian Standard AS5013.30 (AS5013.30, 2004). Briefly, 500 mL of Buffered Peptone Water (BPW; Oxoid, Basingtoke, UK) was poured into the bag and each chicken was vigorously shaken and hand massaged for 2 mins. A volume of 250 ml (to ensure minimal headspace) of the rinsate was stored in sterile plastic bottles before shipment, on ice to ensure samples were chilled but not frozen, to the Brisbane laboratory. Rinsates were tested within 24 h. Processing conditions including scald and immersion chiller temperatures, immersion chiller pH and the level of free available chlorine, as recorded by Quality Assurance staff, are presented in Table 1. The method of measuring FAC in each plant was not recorded,

2.2 Qualitative Campylobacter *analysis*

Rinsates and caecal contents were tested for *Campylobacter* following a modified Australian Standard (AS5013.6, 2004). A modification was made in the selection of agar plates by replacing Preston Agar with modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA; Oxoid). A 50 ml portion of each rinsate was added to 50 ml of double strength Preston Broth without antibiotics and incubated at 37°C for 2 h. Preston antibiotic supplement (Oxoid) was added and the sample incubated at 42 °C for 46 h under 5% CO_2 (Duffy and Dykes, 2009) atmosphere generated within a CB150 incubator (Binder, Tuttlingen, Germany). Generation

of 5 % CO_2 for the growth of *Campylobacter* has been extensively utilised in our laboratory. All caeca were sampled by aseptically cutting the end of the caecal loop and squeezing the contents into a small stomacher bag. Caecal material and Preston Broths were streaked onto mCCDAwith antibiotic supplement (SR0155E, Oxoid) and Skirrow agar (bioMérieux, France). All plates were incubated with 5% CO_2 at 42°C for 48 h . Presumptive positive colonies were sub-cultured on CCDA (without antibiotics) before storing at -80°C in Protect Bacterial Preserver Beads (Technical Service Consultants, Heywood, UK).

2.3 Quantitative Campylobacter *analysis*

Preston Broth was added to the caecal contents to create a 9:1 wt:wt ratio before being stomached for 2 min. Rinsates and caecal samples were decimal diluted in BPW and 100 µL spread plated on both mCCDA and Skirrow agar. Samples from after chilling and after packaging were additionally analysed by spreading each of six plates of mCCDA and Skirrow agar with 500 µL of rinsate each. The agar, either mCCDA or Skirrow, with the highest confirmed count was used to calculate the CFU/mL before multiplying by 500 to obtain CFU/carcase. The detection limit was 2.22 $\log_{10}$ CFU/carcase. All plates were incubated at 42°C for 48 h under 5% CO_2. Up to 12 presumptive positive colonies from each sample were selected and sub-cultured on CCDA (without antibiotics) before storing at -80°C, in Protect Bacterial Preserver Beads (Technical Service Consultants), for confirmation and speciation. Confirmed colonies were used to correct the count/mL of rinsate before transforming to $\log_{10}$ CFU per carcase. Samples that were positive by enrichment but below the level of quantifiable detection were assigned a value equal to the limit of detection. Negative samples were assigned a value of 1.93 $\log_{10}$ CFU/carcase for calculation of

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medians. As chickens at different sample sites vary with regard to surface area due to presence of feathers, head and legs all counts are expressed as per carcase.

2.4 Quantitative E. coli *analysis*

Rinsates and caecal material diluted in Preston broth as described in 2.3 were 10 fold serially diluted in BPW and 1 mL plated onto *E. coli*/Coliform Petrifilm™ (3M, Australia) following manufacturer's instructions. Petrifilm were incubated at 37°C for 48 h and all blue colonies with or without gas were counted. Concentrations were calculated per mL of rinsate, multiplied by 500, then transformed to $\log_{10}$ CFU per carcase.

2.5 Campylobacter *speciation*

All isolates (up to 12 from each sample) were recovered from -80°C storage by incubation on CCDA (without antibiotics) under 5% CO_2 at 42°C for 48 h. A loopful of culture was then added to 10 ml of Nutrient Broth No. 2 (Oxoid) in a 10 ml tube with limited headspace. The tubes were incubated under 5% CO_2 at 42°C for 48 h. A 1 ml portion was centrifuged at 13,000 g for 3 min, supernatant removed, and the pellet resuspended in 200 µl of sterile distilled water. The sample was boiled for 10 min before centrifuging at 13, 000 g for 5 min and the supernatant used as the DNA template. All isolates were speciated by PCR using the method of Klena et al. (2004) and/or the method of Khan and Edge (2007) for isolates that did not produce a result with the Klena PCR. All products were separated on a 2% (wt:vol) agarose gel, stained with ethidium bromide before visualising under U.V. light (Gene Genius, Syngene, Cambridge, UK).

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2.6 Statistics

Log transformed *Campylobacter* and *E. coli* concentrations across all sampling sites for each flock were assessed for normality (Anderson Darling) and for equality of variance (Levene). Mann-Whitney was used to compare the median at each sampling point within and between each flock. Correlation between *Campylobacter* and *E. coli* concentration was assessed using Spearmans rank order coefficient. Changes in the percentage carriage of each species were tested for significance using Chi-squared. A retrospective power analysis was conducted. All analyses were conducted using Minitab 16 software (Minitab Inc., Minneapolis, USA).

4. Results and Discussion

The prevalence of *Campylobacter* on whole chickens at the end of processing, in this study (82.5%) is comparable to that of other Australian studies (84.3 to 95.8%; (FSANZ, 2010; King and Adams, 2008)). This could be considered high in comparison to some baseline surveys conducted in Canada, UK, USA and Sweden, 75.0, 65.2, 46.6 and 15% respectively (Bohaychuk et al., 2009; Lindblad et al., 2006; UKFSA, 2009; USDA, 2009). Differences in methodology both in isolation such as limit of detection and in process methodology such as the use of chlorine make direct comparisons difficult.

The primary aim of this study was to provide data on the effect of current processing practices (previously suggested to reduce *Campylobacter* levels) on the prevalence and concentration of *Campylobacter* on whole chickens, therefore flocks were selected as described to increase the probability of high levels of *Campylobacter* in the caeca. The increasing age of a flock has previously been linked to an increase in the prevalence of

Campylobacter. The two companies that took part in this study do not require pre-slaughter analysis of *Campylobacter* prevalence or quantification in flocks scheduled for slaughter. Australian poultry flocks are commonly subjected to thinning and this also may have increased the chance of selecting a positive flock. Prevalence in caeca has previously been found to influence the prevalence on carcasses (Allen et al., 2007; Hue et al., 2010; Reich et al., 2008). This is supported by the current study for three of the four flocks, with 100% prevalence in the caeca and on the whole chickens after packaging (Figure 1).The mean concentration of *Campylobacter* in the caeca from Australian chickens has been reported as 6.87 $\log_{10}$ CFU/g (FSANZ, 2010) compared to the current study with median concentration of 7.8, 8.5, 7.0 and 6.3 $\log_{10}$ CFU/g for flocks 1 to 4 respectively (Table 2). Each flock had a significantly (P<0.05) different concentration in the caeca compared to the other flocks (Table 2). While the prevalence of *Campylobacter* in caeca is related to the age of the chickens, the concentration is not (Hue et al., 2010), which suggests that chickens sampled in the previous FSANZ study had not been colonized for the same length of time as those in the current study.

The application and control of specific processing technology along the poultry processing chain within each plant may account for some level of variation although there was no significant (*P*<0.05) effect of plant in this study with each plant having consistent measured processing parameters (Table 1). When comparing a number of studies (Guerin et al., 2010; Rosenquist et al., 2006), defeathering and evisceration have been shown to maintain or increase the concentration of *Campylobacter* in a number of studies. The use of sprays that may contain biocides such as chlorine or best practice use of equipment to minimise rupture of viscera may offer some control through these stages but these may not always be universally well implemented. In the current study there was no significant (*P*<0.05) change in concentration for samples taken after scalding compared to samples taken

before chilling which encompasses the stages of defeathering and evisceration (Table 2). Reductions were noted in most studies through scalding and again through washing and cooling (Guerin et al., 2010; Rosenquist et al., 2006). This is in agreement with the current study where all flocks had a significant ($P<0.05$) decline in concentration following scalding and again following chilling with overall mean decreases of 1.8 and 2.9 $\log_{10}$ CFU/carcase (Figure 2). Control of processing parameters such as scald temperature and chlorine concentration/temperature/pH of immersion chiller water may play a more important role in reducing the concentration of *Campylobacter* on whole chickens within these processing plants than the initial concentration of *Campylobacter* in the caeca. Larger more targeted studies will need to be undertaken to examine these specific production conditions. Within the immersion chiller, variability may be affected by the volume of water used in respect to the number of chickens in the tank, the effectiveness of the auger screw and any congestion points that may prevent the effective washing of all chickens. Cross contamination has also been suggested as a source of contamination in immersion chillers (Reich et al., 2008) although it was not assessed in this study. Attention to detail with hygienic practices throughout production and primary processing and alterations to the immersion-chiller conditions were suggested as key areas that lead to reductions in the prevalence and concentration of *Campylobacter* of whole chicken carcasses in New Zealand (Sears et al., 2011). The mandatory testing of poultry carcass rinsates and setting of performance targets were also key to this improvement (Sears et al., 2011).

Variation in *Campylobacter* concentration occurred within flocks and across sampling sites suggests that the contamination of carcasses does not occur homogenously. The median *Campylobacter* concentration of all samples after packaging was 3.5 (Q1-Q3; 2.89-5.11) $\log_{10}$ CFU/carcase with individual counts ranging from 2.22 to 7.3 $\log_{10}$ CFU/carcase. Variability in *Campylobacter* concentration has been suggested to be related to the variable

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concentration of caecal carriage in individual chickens, variable contamination due to visceral breakage or leakage and to potentially different sensitivities of *Campylobacter* strains to processing stresses (Allen et al., 2007; Stern and Robach, 2003). This variability is of most concern when only one or a few samples are collected (Hansson et al., 2010). A retrospective power analysis indicated that a sample size of 10 had a >60% chance of detecting a difference of at least 1 $\log_{10}$ CFU/carcase. A smaller change in the median values would require larger sample sizes to have adequate power, however changes of < 1 $\log_{10}$ CFU/carcase may not be biologically significant in the poultry processing system. The use of different methods for sampling and calculation of the concentration of *Campylobacter* makes comparison between studies difficult, although a general guide to the effect of individual processing steps can be gained. The systematic review of prevalence of *Campylobacter* through poultry processing by Guerin et al. (2010) highlights the need for standardised testing methods and reporting.

The significant decrease in the concentration of *Campylobacter* at scald and again at immersion chilling for all flocks is in agreement with other published reports (Guerin et al., 2010; Rosenquist et al., 2006). While some studies report increases during to defeathering and / or evisceration (Guerin et al., 2010) the overall effect of these stages on the concentration of *Campylobacter* was not significant (*P*>0.05) in this study which supports the initial study design. A decrease in medians of greater than 3.5 $\log_{10}$ CFU/carcase was recorded after chilling for three of four flocks in this study (Table 2). Cross contamination within immersion chillers has previously been noted (Reich et al., 2008) which may play a role in the different results noted for flock 2. No explanation for this difference in flocks 2 and 4 slaughtered at abattoir B can be suggested as processing controls such as chlorine concentration, pH and temperature of the immersion chillers, as measured by Quality Assurance staff, were identical at both visits (Table 1). Variations in such data are common, with a recent study of UK flocks demonstrating a decline of approximately 1 $\log_{10}$ CFU/g in

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neck and breast skin after chilling in only two of five flocks and an increase of approximately 0.2 to 0.4 $\log_{10}$ CFU/g in the remaining three flocks after chilling (Elvers et al., 2011).

The concentration of *E. coli* on post-chill chicken carcasses has been found to fit a logistic distribution and therefore may have value in process control plans (Altekruse et al., 2009). *E. coli* concentration has been found to decrease concurrently with *Campylobacter* concentration from re-hang to post-chill (Altekruse et al., 2009; Berrang et al., 2007) which is supported by the results of this study. All flocks had a significant ($P < 0.05$) decrease in concentration after scald and again after immersion chilling (Table 3) with a mean decline of 1.3 and 2.5 $\log_{10}$ CFU/carcase respectively. At all sampling sites for all four flocks good correlation ($r^2=0.8$) was noted between *E. coli* and *Campylobacter* concentration (excluding caeca) with similar declines of the two organisms at each processing stage (Figure 3) for samples over the detection limit. This suggests that when *Campylobacter* is present, processes currently operating in these abattoirs that decrease *E. coli* concentration will also affect a similar decrease in *Campylobacter* concentration. A correlation between the concentration of *Campylobacter* and *E. coli* has been recorded (Ghafir et al., 2008; Habib et al., 2012) and this may suggest the use of *E. coli* as an indicator of high concentrations of *Campylobacter* (Habib et al., 2012). Conversely the weak but positive correlation between *E. coli* and *Campylobacter* was suggested to not be a reliable indicator of concentrations of *Campylobacter* (Wiliams and Ebel, 2014).

Differences in risk factors for human exposure have been identified for different *Campylobacter* species and as such, data should be collected for both *C. jejuni* and *C. coli* to improve risk calculations (EFSA, 2010b; Gillespie et al., 2002). The relative prevalence of different species of *Campylobacter* has been reported in some studies (Allen et al., 2007; Huat et al., 2010; Klein et al., 2007; Reich et al., 2008; Wedderkopp et al., 2000) either in caecal contents or after packaging. All isolates in this study (n=2201) were either

C. jejuni or *C. coli* with 39 and 12 % of after packaging samples were contaminated with a single species respectively. A total of 48 % were contaminated with both species. Differences were noted in the proportional carriage of *C. jejuni* and *C. coli* across sampling sites in a flock dependant manner (Figure 1). For the examination of the change in carriage of each species, chickens contaminated with both species were counted for both *C. jejuni* and *C. coli*. The proportional carriage of *C. coli* increased through processing in flock 1 from before scald (56 %) to after chill (81 %) or after packaging (75 %), suggesting that these strains may be better able to survive processing compared to the *C. jejuni* strains contaminating this flock. The increased survival of *C. coli* compared to *C. jejuni* is also suggested in flock 3 with a significant (P<0.05) increase in proportional carriage from 40 % before chilling to 67 % after chilling. The change in carriage of species may be related to the high level of *C. coli* in the caeca of both flock 1 and 3 (98 and 83 % respectively).

This study provides the first published report of through-chain qualitative and quantitative data on *Campylobacter* and *E. coli* in Australian poultry processing plants. As a pilot study, it is not possible to thoroughly examine all aspects of the poultry slaughter process and their effects on campylobacter concentrations. The through-chain quantitative data can be incorporated into future risk assessments enabling a quantitative evaluation of poultry processing stages. The processing steps, scalding and immersion chilling, are highlighted as key control points in the production process. However the decrease was not uniform and further more targeted studies may enhance our understanding of the effect of specific processing factors on the resulting prevalence and concentration of *Campylobacter*. Use of *E. coli* concentration, as an indicator of the effect of processing practices on *Campylobacter* concentration, but not presence/absence, may represent a more applicable and practical solution to monitoring and validating process effects, based on the relative ease and standardisation of *E. coli* enumeration. Further characterization of isolates obtained from this

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study will enable increased understanding of the survival of specific genotypes of *Campylobacter* through the poultry processing chain. This in turn may aid future improved control strategies.

Acknowledgement

References

ACMF. 2011. The Australian chicken meat industry: An industry in profile [Online]. Available at www.chicken.org.au/industryprofile (Accessed: January, 2014).

Allen, V. M., Bull, S. A., Corry, J. E., Domingue, G., Jorgensen, F., Frost, J. A., Whyte, R., Gonzalez, A., Elviss, N., Humphrey, T. J. 2007. *Campylobacter* spp. contamination of chicken carcasses during processing in relation to flock colonisation. *International Journal of Food Microbiology* 113, 54-61.

Allos, B. M. 2001. *Campylobacter jejuni* Infections: update on emerging issues and trends. *Clinical Infectious Diseases* 32, 1201-1206.

Altekruse, S. F., Berrang, M. E., Marks, H., Patel, B., Shaw, W. K., Saini, P., Bennett, P. A., Bailey, J. S. 2009. Enumeration of *Escherichia coli* Cells on chicken carcasses as a potential measure of microbial process control in a random selection of slaughter establishments in the United States. *Applied and Environmental Microbiology* 75, 3522-3527.

AS5013.6. 2004. Australian Standard. Food Microbiology Method 6: Examination for specific organisms - *Campylobacter*.

AS5013.30. 2004. Australian Standard. Food Microbiology. Method 20: Preparation of test samples for microbiological examination - Poultry and poultry products.

Berrang, M. E. and Bailey, J. S. 2008. *Campylobacter*, *Salmonella* and *E. coli* on broiler carcasses from commercial plants under HIMP inspection. *Poultry Science* 87, 134-134.

Berrang, M. E., Bailey, J. S., Altekruse, S. F., Patel, B., Shaw, W. K., Meinersmann, R. J., Fedorka-Cray, P. J. 2007. Prevalence and numbers of *Campylobacter* on broiler carcasses collected at rehang and postchill in 20 US processing plants. *Journal of Food Protection* 70, 1556-1560.

Bohaychuk, V. M., Checkley, S. L., Gensler, G. E., Barrios, P. R. 2009. Microbiological baseline study of poultry slaughtered in provincially inspected abattoirs in Alberta, Canada. *Canadian Veterinary Journal-Revue Veterinaire Canadienne* 50, 173-178.

CDC. 2013. Incidence and trends of infection with pathogens transmitted commonly through food - Foodborne diseases active surveillance network, 10 U.S. sites, 1996-2012. *Morbity and Mortality Weekly Report* 62, 283-287.

DAFF. 2009. National farm biosecurity manual poultry production [Online]. Available at http://www.daff.gov.au/__data/assets/pdf_file/0009/1147554/poultry-biosecurity-manual.pdf (Accessed: 1 August, 2012).

Duffy, L. L. and Dykes, G. A. 2009. The ability of *Campylobacter jejuni* cells to attach to stainless steel does not change as they become nonculturable. *Foodborne Pathogens and Disease* 6, 631-634.

EFSA. 2010a. Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008, Part A; *Campylobacter* and *Salmonella* prevalence estimates. *EFSA Journal* 8, 1503.

EFSA. 2010b. Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008. Part B Analysis of factors associated with *Campylobacter* colonization of broiler batches and with *Campylobacter* contamination of broiler carcases; and investigation of the culture method diagnostic characteristics used to analyse broiler carcass samples. *EFSA Journal* 8, 1522.

EFSA. 2010c. EFSA panel on biological hazards (BIOHAZ); Scientific opinion on quantification of the risk posed by broiler meat to human campylobacteriosis in the EU. *EFSA Journal* 8, 1437.

EFSA. 2012. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. *EFSA Journal* 10, 2597.

Elvers, K. T., Morris, V. K., Newell, D. G., Allen, V. M. 2011. The molecular tracking, through processing, of *Campylobacter* strains colonising broiler flocks. *Applied and Environmental Microbiology* 78, 5722-5729.

FAO/WHO. 2009. Risk assessment of *Campylobacter* spp. in broiler chickens: Technical report. Microbiological risk assessment series, No. 12, Technical report. Geneva. 132pp. [Online]. Available at http://www.who.int/foodsafety/publications/micro/MRA12_En.pdf (Accessed: 1 August, 2012).

Friedman, C. R., Hoekstra, R. M., Samuel, M., Marcus, R., Bender, J., Shiferaw, B., Reddy, S., Ahuja, S. D., Helfrick, D. L., Hardnett, F., Carter, M., Anderson, B., Tauxe, R. V., FoodNet, E. I. P. 2004. Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. *Clinical Infectious Diseases* 38, S285-S296.

FSANZ. 2005. Scientific assessment of the public health and safety of poultry meat in Australia [Online]. Available at http://www.foodstandards.gov.au/_srcfiles/P282_Poultry%20_%20DAR%20Attach3.pdf (Accessed: 1 August, 2012).

FSANZ. 2010. Baseline survey on the prevalence and concentration of *Salmonella* and *Campylobacter* in chicken meat on-farm and at primary processing [Online]. Available at

http://www.foodstandards.gov.au/_srcfiles/Poultry%20survey%20rept%20March%202010.pdf (Accessed: 1 August, 2012).

Ghafir, Y., China, B., Dierick, K., De Zutter, L., Daube, G. 2008. Hygiene indicator microorganisms for selected pathogens on beef, pork, and poultry meats in Belgium. *Journal of Food Protection* 71, 35-45.

Gillespie, I. A., O'Brien, S. J., Frost, J. A., Adak, G. K., Horby, P., Swan, A. V., Painter, M. J., Neal, K. R. 2002. A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: A tool for generating hypotheses. *Emerging Infectious Diseases* 8, 937-942.

Guerin, M. T., Sir, C., Sargeant, J. M., Waddell, L., O'Connor, A. M., Wills, R. W., Bailey, R. H., Byrd, J. A. 2010. The change in prevalence of *Campylobacter* on chicken carcasses during processing: A systematic review. *Poultry Science* 89, 1070-1084.

Habib, I., De Zutter, L., Van Huffel, X., Geeraerd, A. H., Uyttendaele, M. 2012. Potential of *Escherichia coli* as a surrogate indicator for postchill broiler carcasses with high *Campylobacter* counts. *Food Control* 25, 96-100.

Hansson, I., Pudas, N., Harbom, B., Engvall, E. O. 2010. Within-flock variations of *Campylobacter* loads in caeca and on carcasses from broilers. *International Journal of Food Microbiology* 141, 51-55.

Huat, J. T. Y., Aziz, S. A. A., Abu, J., Ghazali, F. M., Chilek, T. Z. T., Ahmad, N., Sandra, A., Nishibuchi, M., Radu, S. 2010. Thermophilic *Campylobacter* spp. occurrence on chickens at farm, slaughter house and retail. *International Journal of Poultry Science* 9, 134-138.

Hue, O., Le Bouquin, S., Laisney, M. J., Allain, V., Lalande, F., Petetin, I., Rouxel, S., Quesne, S., Gloaguen, P. Y., Picherot, M., Santolini, J., Salvat, G., Bougeard, S., Chemaly, M. 2010. Prevalence of and risk factors for *Campylobacter* spp. contamination of broiler chicken carcasses at the slaughterhouse. *Food Microbiology* 27, 992-999.

Jacobs, B. C., von Belkum, A., Endtz, H. P. 2008. Guillain-Barre and *Campylobacter* infection. In: Nachamkin, I., Szymanski, C.M., Blaser, M.J., (Eds.), *Campylobacter*, Third ed. ASM Press, Washington DC.

Khan, I. U. H. and Edge, T. A. 2007. Development of a novel triplex PCR assay for the detection and differentiation of thermophilic species of *Campylobacter* using 16S-23S rDNA internal transcribed spacer (ITS) region. *Journal of Applied Microbiology* 103, 2561-2569.

King, S. and Adams, M. C. 2008. Incidence of *Campylobacter* in processed poultry: Is it a concern for human health? *Journal of Food Safety* 28, 376-388.

Klein, G., Reich, F., Beckmann, L., Atanassova, V. 2007. Quantification of thermophilic *Campylobacter* spp. in broilers during meat processing. *Antonie Van Leeuwenhoek* 92, 267-273.

Klena, J. D., Parker, C. T., Knibb, K., Ibbitt, J. C., Devane, P. M. L., Horn, S. T., Miller, W. G., Konkel, M. E. 2004. Differentiation of *Campylobacter coli, Campylobacter jejuni, Campylobacter lari*, and *Campylobacter upsaliensis* by a Multiplex PCR Developed from the Nucleotide Sequence of the Lipid A Gene *lpx*A. *Journal of Clinical Microbiology* 42, 5549-5557.

Lindblad, M., Lindmark, H., Lambertz, S. T., Lindqvist, R. 2006. Microbiological baseline study of broiler chickens at Swedish slaughterhouses. *Journal of Food Protection* 69, 2875-2882.

NNDSS. 2013. National Notifiable Diseases Surveillance System [Online]. Available at http://www9.health.gov.au/cda/source/rpt_2_sel.cfm (Accessed: January, 2014).

NZPHSR. 2013. New Zealand Public Health Surveillance Report [Online]. Available at https://surv.esr.cri.nz/surveillance/NZPHSR.php?we_objectID=3880 (Accessed: January, 2014).

Reich, F., Atanassova, V., Haunhorst, E., Klein, G. 2008. The effects of *Campylobacter* numbers in caeca on the contamination of broiler carcasses with *Campylobacter*. *International Journal of Food Microbiology* 127, 116-120.

Rosenquist, H., Nielsen, N. L., Sommer, H. M., Norrung, B., Christensen, B. B. 2003. Quantitative risk assessment of human campylobacteriosis associated with thermophilic *Campylobacter* species in chickens. *International Journal of Food Microbiology* 83, 87-103.

Rosenquist, H., Sommer, H. M., Nielsen, N. L., Christensen, B. B. 2006. The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter*. *International Journal of Food Microbiology* 108, 226-232.

Sears, A., Baker, M. G., Wilson, N., Marshall, J., Muellner, P., Campbell, D. M., Lake, R. J., French, N. P. 2011. Marked campylobacteriosis decline after interventions aimed at poultry, New Zealand. *Emerging Infectious Diseases* 17, 1007-1015.

Stafford, R. J., Schluter, P. J., Wilson, A. J., Kirk, M. D., Hall, G., Unicomb, L. 2008. Population-attributable risk estimates for risk factors associated with *Campylobacter* infection, Australia. *Emerging Infectious Diseases* 14, 895-901.

Stern, N. J. and Robach, M. C. 2003. Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses. *Journal of Food Protection* 66, 1557-1563.

UKFSA. 2009. A UK survey of *Campylobacter* and *Salmonella* contamination of fresh chicken at retail sale, 6 Oct 2009, Food Survey information sheet 04/09 [Online]. Available at

http://www.food.gov.uk/science/surveillance/fsisbranch2009/fsis0409
(Accessed: 1 August, 2012).

USDA. 2009. The Nationwide microbiological baseline data collection program: Young
chicken survey [Online]. Available at
http://www.fsis.usda.gov/PDF/Baseline_Data_Young_Chicken_2007-2008.pdf
(Accessed: 1 August, 2012).

Wedderkopp, A., Rattenborg, E., Madsen, M. 2000. National surveillance of
Campylobacter in broilers at slaughter in Denmark in 1998. *Avian Diseases* 44,
993-999.

Wiliams, M. S. and Ebel, E. D. 2014. Estimating the correlation between concentrations
of two species of bacteria with censored microbial testing data. *International
Journal of Food Microbiology* 175, 1-5.

Figure 1: Prevalence of *Campylobacter* spp. at each sampling site and percentage of isolates that are *C. jejuni* and *C. coli*. Line (▲) indicates overall prevalence of *Campylobacter* spp. Dark columns indicate prevalence of *C. coli* and light columns indicate prevalence of *C. jejuni* at each sampling site. A. Flock 1; B. Flock 2; C. Flock 3; D. Flock 4.

Figure 2: The effect of each processing stage on the median concentration of *Campylobacter* and *E. coli* and the cumulative effect of the slaughter process for each flock. Solid bars are the concentration of *Campylobacter* and grey bars are the concentration of *E. coli* A. Flock 1; B. Flock 2; C. Flock 3; D. Flock 4. Error bars represent the 95% confidence interval for the point estimate of the difference in the two population medians at the beginning and end of each process, as calculated in the Mann-Whitney test.

Figure 3: Correlation of *Campylobacter* and *E. coli* levels at all sampling sites within each flock. Only samples with a recorded count of *Campylobacter* are included. Number of samples (n =), pearsons rho (ρ), and r^2 value are displayed for each graph. Sampling sites are indicated by ◇ Before scald, □ After scald, △ Before chill, x After chill and ◯ After packaging.
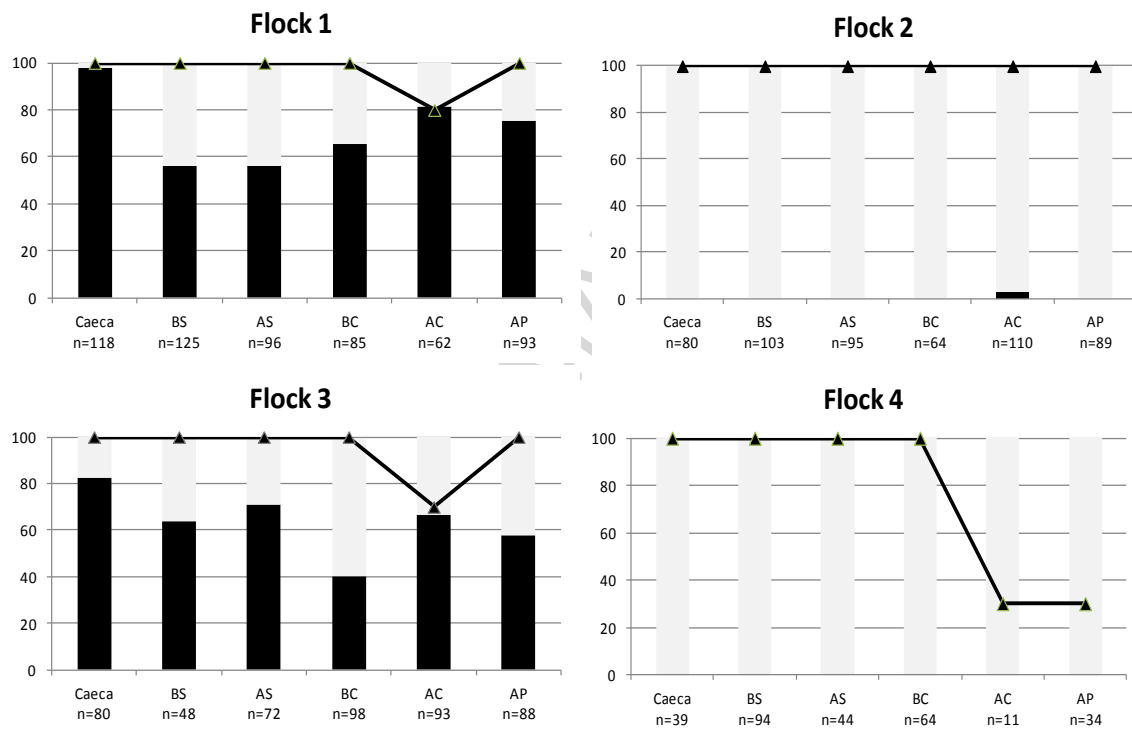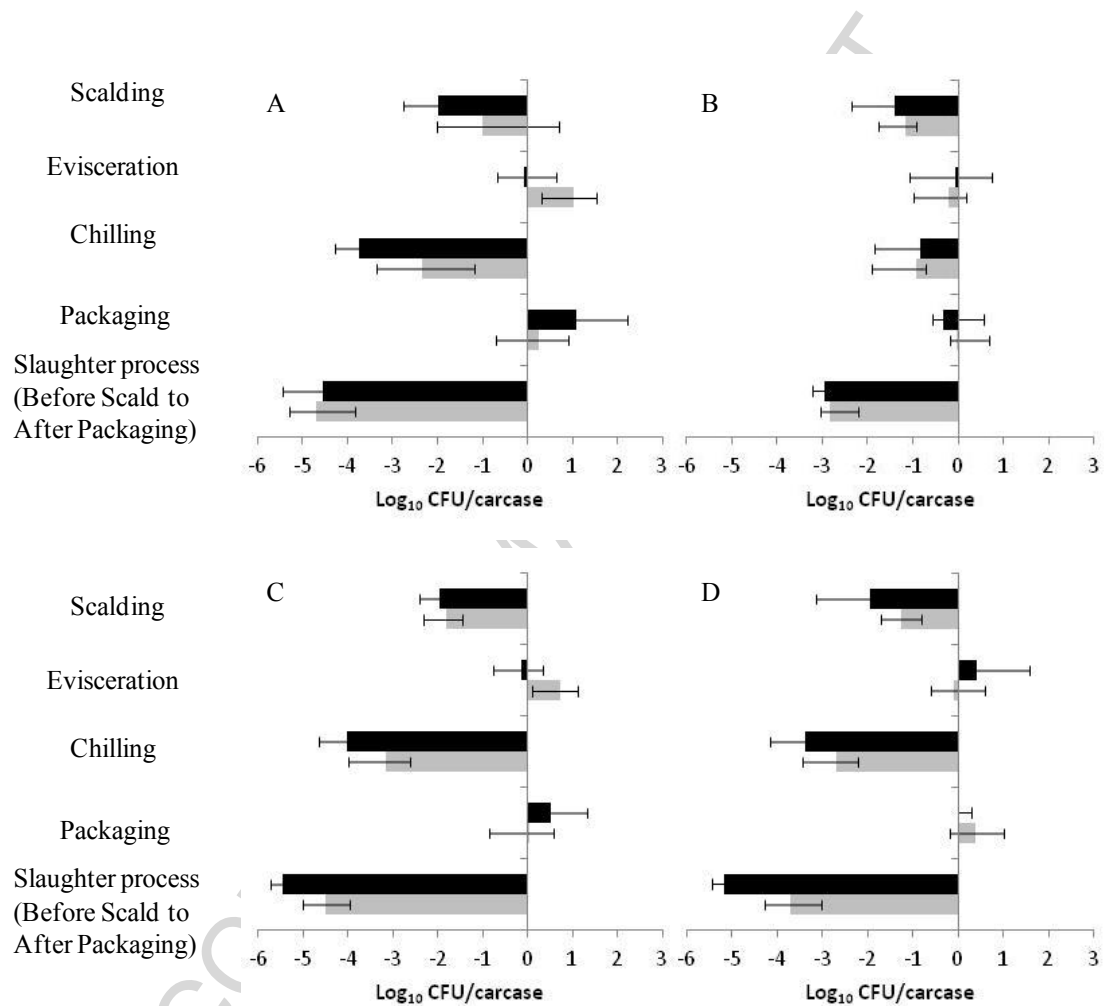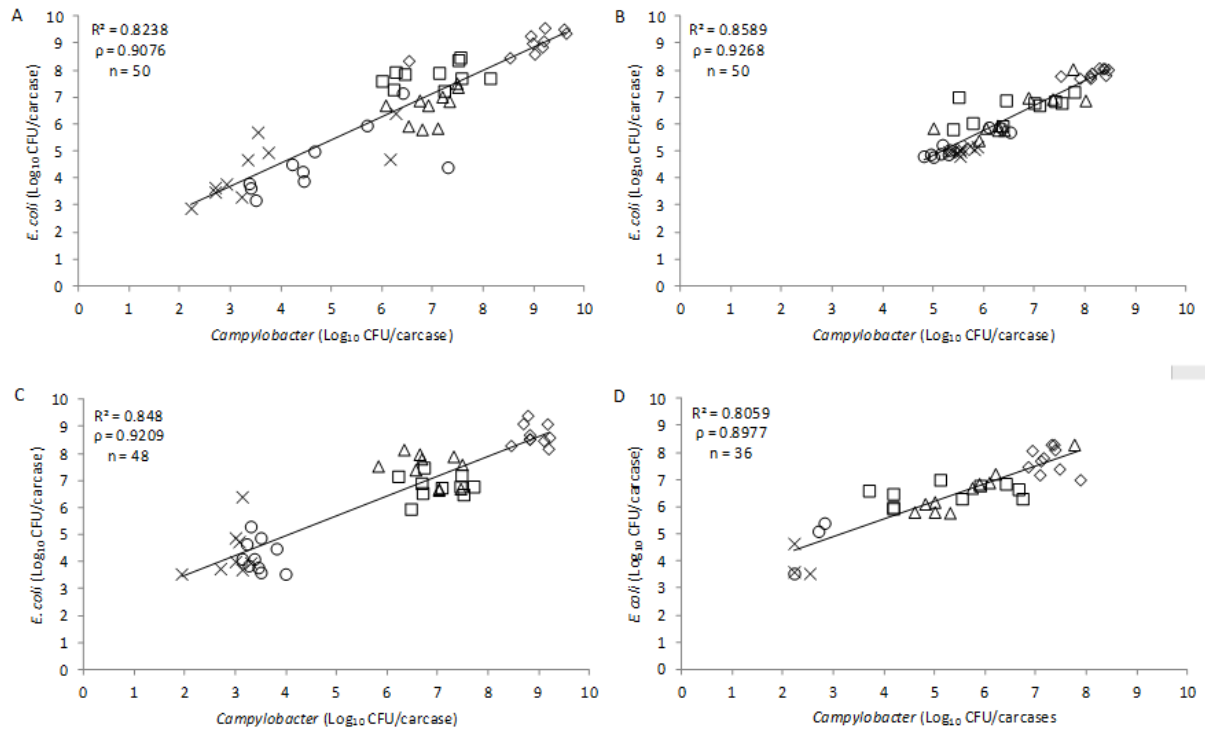
Figure 1

Figure 2

Figure 3

Table 1: Processing conditions at time of slaughter for each flock, as recorded by Quality

Assurance staff.

	Scald temperature/ time	Immersion chiller temperature/ time	Immersion chiller pH	Immersion chiller FAC[a]
Flock 1	55°C/2 min 20 s	4.2°C/30 min	8.00	3.5 ppm
Flock 2	55°C/2 min 30 s	6.9°C/45 min	7.03	1.0 ppm
Flock 3	55°C/2 min 20 s	4.4°C/30 min	7.50	2.7 ppm
Flock 4	55°C/2 min 30 s	7.0°C/45 min	7.05	1.0 ppm

a FAC; free available chlorine

Table 2: Median and mean *Campylobacter* level, for each sampling site from the four tested flocks.

		Caeca (Log_{10} CFU/g)	BS[a] (Log_{10} CFU/carcase)	AS (Log_{10} CFU/carcase)	BC (Log_{10} CFU/carcase)	AC (Log_{10} CFU/carcase)	AP (Log_{10} CFU/carcase)
Flock 1[b]	Median[d]	7.81 (7.74–7.98)^{A}	9.09 (8.84 – 9.32)^{fA}	7.18 (6.26 – 7.56)^{gA}	7.01 (6.69 – 7.37)^{gA}	3.28 (2.70 – 4.35)^{hA}	4.44 (3.48 – 5.88)^{iA}
	Mean ± SE[e]	7.84 ± 0.04	8.88 ± 0.28	7.01 ± 0.23	6.96 ± 0.14	3.68 ± 0.45	4.74 ± 0.42
Flock 2[c]	Median	8.52 (8.13 - 8.72)^{B}	8.21 (8.05 – 8.40)^{fB}	6.72 (5.71 – 7.44)^{gA}	6.34 (6.01 – 7.46)^{gA}	5.36 (5.45 – 5.74)^{hB}	5.24 (4.99 – 6.16)^{hA}
	Mean ± SE	8.36 ± 0.17	8.17 ± 0.09	6.63 ± 0.27	6.59 ± 0.29	5.57 ± 0.06	5.47 ± 0.20
Flock 3[b]	Median	7.00 (6.94 – 7.14)^{C}	8.82 (8.76 – 9.18)^{fA}	6.91 (6.63 – 7.48)^{gA}	6.85 (6.51 – 7.35)^{gA}	3.00 (1.93 – 3.13)^{hA}	3.41 (3.25 – 3.58)^{iB}
	Mean ± SE	7.07 ± 0.06	8.91 ± 0.08	7.00 ± 0.16	6.83 ± 0.17	2.71 ± 0.18	3.45 ± 0.08
Flock 4[c]	Median	6.31 (5.65 – 6.88)^{D}	7.23 (7.04 – 7.40)^{fC}	5.32 (4.18 – 6.47)^{gB}	5.52 (4.95 – 6.11)^{gB}	1.93 (1.93 – 2.22)^{hC}	1.93 (1.93 – 2.34)^{hC}
	Mean ± SE	6.31 ± 0.23	7.25 ± 0.09	5.26 ± 0.36	5.64 ± 0.29	2.05 ± 0.06	2.13 ± 0.11

[a] BS, before scald; AS after scald; BC, before chill; AC, after chill; AP, after packaging

[b] Flocks slaughtered at Plant A

[c] Flocks slaughtered at Plant B

[d] Median (25th percentile – 75th percentile)

[e] Mean ± standard error of the mean (SE)

All analysis was conducted using median values only. Levels of *Campylobacter* that have the same superscript lower case letter following are not significantly different from each other across rows (excluding caeca). Levels of *Campylobacter* that have the same superscript upper case following are not significantly different from each other in columns.

Table 3: Median and mean *E. coli* concentration, for each sampling site from the four tested flocks.

		Caeca (Log$_{10}$ CFU/g)	BS[a] (Log$_{10}$ CFU/carcase)	AS (Log$_{10}$ CFU/carcase)	BC (Log$_{10}$ CFU/carcase)	AC (Log$_{10}$ CFU/carcase)	AP (Log$_{10}$ CFU/carcase)
Flock 1[b]	Median[d]	NT	9.04 (8.57 – 9.41)^{fA}	7.78 (7.52 – 8.04)^{gA}	6.78 (5.91 – 7.12)^{h}	4.23 (3.44 – 5.14)^{iA}	4.32 (3.78 – 5.23)^{jAB}
	Mean ± SE[e]	NT	9.01 ± 0.14	7.81 ± 0.13	6.62 ± 0.19	4.335 ± 0.36	4.57 ± 0.38
Flock 2[c]	Median	4.83 (4.57 – 5.35)^{A}	7.85 (7.76 – 8.06)^{fB}	6.78 (6.01 – 6.91)^{gB}	5.90 (5.78 – 6.94)^{g}	5.03 (4.97 – 5.12)^{hB}	4.97 (4.86 – 5.74)^{hA}
	Mean ± SE	4.95 ± 0.19	7.89 ± 0.05	6.60 ± 0.15	6.34 ± 0.26	5.03 ± 0.04	5.20 ± 0.14
Flock 3[b]	Median	4.80 (4.60 – 5.39)^{A}	8.57 (8.44 – 9.10)^{fA}	6.78 (6.53 – 7.17)^{gB}	7.57 (6.70 – 7.92)^{h}	3.98 (3.66 – 4.96)^{iA}	4.10 (3.74 – 4.71)^{iB}
	Mean ± SE	4.95 ± 0.19	8.70 ± 0.12	6.80 ± 0.14	7.44 ± 0.18	4.37 ± 0.29	4.23 ± 0.18
Flock 4[c]	Median	5.78 (5.47 – 5.85)^{A}	7.23 (7.04 – 7.40)^{fB}	5.33 (4.18 – 6.47)^{gB}	5.52 (4.95 – 6.11)^{g}	1.93 (1.93 – 2.22)^{hA}	1.93 (1.93 – 2.34)^{hB}
	Mean ± SE	5.59 ± 0.19	7.25 ± 0.09	5.26 ± 0.36	5.64 ± 0.29	2.05 ± 0.07	2.13 ± 0.11

[a] BS, before scald; AS after scald; BC, before chill; AC, after chill; AP, after packaging

[b] Flocks slaughtered at Plant A

[c] Flocks slaughtered at Plant B

[d] Median (25th percentile – 75th percentile)

[e] Mean ± standard error of the mean (SE)

All analysis was conducted using median values only. Levels of *E. coli* that have the same superscript lower case letter following are not significantly different from each other across rows (excluding caeca). Levels of *E. coli* that have the same superscript upper case letter following are not significantly different from each other in columns.

NT – Not tested

Highlights

Manuscript Title: Quantitative effects of in-line operations on *Campylobacter* and *E. coli* through two Australian broiler processing plants

Highlights:

- Quantitative data on *Campylobacter* and *E. coli* in broilers through processing
- Use of *E. coli* as an indicator of process effects on *Campylobacter*
- Scalding and chilling decrease concentration of organisms
- Caecal concentration potentially not as important as processing control
- Potential different survival of *C. jejuni* and *C. coli* through processing