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

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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 *Campylobacter coli* and two flocks dominated by *Campylobacter 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 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

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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 *Escherichia 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 × 30 cm; Amyl Media, Victoria, Australia), for a total of 240 samples across all flocks. Caecal samples were held on ice, to ensure they were chilled but not frozen before processing at the Brisbane laboratory within 24 h of collection. Whole birds were placed into individual large stomacher bags (38 × 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 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™ (3 M, 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.