



Systematic review-meta-analysis of the effect of chilling on *Campylobacter* spp. during primary processing of broilers



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ARTICLE INFO

Article history:

Received 27 January 2015

Received in revised form

30 March 2015

Accepted 31 March 2015

Available online 8 April 2015

Keywords:

Poultry

Washing

Immersion chilling

Air chilling

Systematic review

Meta-analysis

ABSTRACT

Carcass chilling is a critical control point for *Campylobacter* spp. during the primary processing of broiler chickens. Our objective was to evaluate chilling intervention research that measured the change in *Campylobacter* prevalence and concentration on broiler chicken carcasses during primary processing using systematic review-meta-analysis (SR-MA) methodology. Experimental and observational research published in English that investigated impacts of chilling on *Campylobacter* spp. during primary processing of broiler chicken carcasses were considered. Random-effects MA of air chilling resulted in heterogenous summary effect estimates (mean reduction = 0.74 log₁₀ CFU/carcass, 95% CI: 0.32–1.17, I² = 91.3%; and odds ratio = 7.42, 95% CI: 0.32–174.05, I² = 92.3%). Random-effects MA of immersion chilling with chlorine resulted in heterogenous summary effect estimates (mean reduction = 1.74 log₁₀ CFU/carcass, 95% CI: 1.32–2.16, I² = 86.4%; and odds ratio = 0.50, 95% CI: 0.20–1.28, I² = 90.6%). Effects of immersion chilling with unspecified disinfectants were also determined and varied depending on study design. The SR-MA indicated that air chilling and immersion chilling reduce *Campylobacter* concentrations. Due to conflicting results across studies, the estimated average effect of air chilling on *Campylobacter* prevalence is not informative. Immersion chilling with chlorine demonstrated a trend towards reduced *Campylobacter* prevalence, but this result was not significant; results should be interpreted with caution because the overall methodological soundness of included studies was low. Existing research on the effectiveness of broiler carcass chilling on *Campylobacter* concentration or prevalence is limited and heterogenous. Results generated herein can inform decisions makers and stakeholders on potential effective chilling interventions, and can be used to inform quantitative microbial risk assessment to estimate processing measure impacts on public health.

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1. Introduction

Campylobacter spp. are one of the most common causes of bacterial gastroenteritis worldwide, and are often linked to the consumption of contaminated, undercooked poultry products (CDC, 2012; Kapperud et al., 2003; Parry, Fearnley, & Denehy, 2012). *Campylobacter* is estimated to cause 213,749 domestically acquired illnesses per year in Canada, and 68% of cases are attributed to domestic consumption of contaminated food (Thomas et al., 2013). The organism has been isolated from poultry throughout the farm-to-fork continuum, highlighting the need for process controls to

reduce public health impacts (EFSA, 2006; Hansson, Ederoth, Andersson, Vagsholm, & Olsson, 2005; Herman et al., 2003).

Dressed poultry carcasses are chilled to mitigate bacterial growth and improve product safety and quality (Carroll & Alvarado, 2008). In Canada and the United States, carcasses are chilled to 4.0 °C and 4.4 °C, respectively, within specific time frames (CFIA, 2013 and USDA, 1996). However, as an unintended consequence, chilling can expose carcasses to water sources and aerosols potentially contaminated with *Campylobacter* (Fries & Graw, 1999). Given that this process is one of the last steps during primary processing before packaging and retail distribution, it is an important control point for *Campylobacter* contamination.

In 2009, experts at an international technical meeting concluded that the body of scientific literature to support claims of intervention efficacy on *Campylobacter* during chicken processing

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was lacking, and the mechanisms of potential benefits were not fully understood (FAO/WHO, 2009). Since the technical meeting, many studies have investigated how the physical forces applied to carcasses during immersion chilling and the drying effects during air chilling might contribute to reductions in bacterial contamination (Zhang, Jeong, Janardhanan, Ryser, & Kang, 2011). There is a need to formally evaluate, synthesize and summarize available research to inform stakeholders on effective treatments and identify key knowledge gaps for future work.

Systematic review (SR)-meta-analysis (MA) methodology uses transparent, repeatable steps to identify, appraise and analyze intervention research (Borenstein, Hedges, Higgins, & Rothstein, 2009; Young, Waddell, et al., 2014). The resulting summary effect estimates are more precise and informative for end users, particularly if generated from a reasonable numbers of studies ($n > 20$) and in the absence of statistically significant heterogeneity and publication bias. This approach was used to determine the effects of air and immersion chilling on the concentration and prevalence of *Campylobacter* spp. during primary processing of broiler chickens.

2. Methods

2.1. Systematic review definitions and question

For the purposes of the SR-MA, a study referred to any primary research publication where authors collected, analyzed and reported their own data. Within studies, authors could report any number of trials, defined as the unique treatment-to-control comparisons made within a study, addressing the SR question: “Does chilling reduce *Campylobacter* spp. concentration and/or prevalence during the primary processing of broiler chickens?” Protocol details are available in the [Supplementary Material](#) and followed the PRISMA statement guidelines (Moher et al., 2009).

2.2. Search strategy and information sources

A targeted search strategy comprising the terms (*Campylobacter* OR campylobacteriosis) AND (chicken or chickens OR poultry OR broiler or broilers) AND (immersion OR air OR chlorine or chlorination OR chill or chilling OR acid) was updated from an earlier SR (Guerin et al., 2010) and restricted to citations published in English from 2006 to the present. The search was implemented September 26, 2013 in six electronic databases including: Agricola (2006–present) Commonwealth Agricultural Bureaux International-CAB abstracts (2006–present), Scopus (2006–present), Food Science and Technology Abstracts (2006–present), Biological Sciences (2006–present) and Pubmed (2006–present) ([Supplementary Materials – Section A](#)). The original SR search, conducted in December 2006, was similar, but included additional terms for all processing interventions.

2.3. Relevance screening, risk of bias assessment and data extraction

Two reviewers independently evaluated each citation at relevance screening and each full paper at the risk of bias assessment and data extraction steps. All studies identified as addressing the efficacy of chilling in the 2006 SR were added and screened using the new forms to provide a uniform, consistent dataset. Conflicts between reviewers were resolved by consensus. If consensus could not be reached, a third reviewer resolved the conflict.

Identified articles were screened for relevance to the SR-MA question ([Supplementary Materials – Sections B and C](#), respectively). Abstract-based relevance screening identified primary research investigating changes in concentration of *Campylobacter*

spp. on broiler chickens and/or prevalence of contaminated carcasses during the chilling process, while full paper-based screening confirmed relevance and categorized studies into design types. There were no limitations on study designs, which included experimental (control, challenge, and before-and-after intervention trials) and observational (cohort, case–control and cross-sectional studies) research. Studies investigating chilling conducted under laboratory or pilot plant conditions were eligible for inclusion. Studies reported solely as abstracts, conference proceedings or oral or poster presentations were included provided they contained sufficient information to perform an assessment of their potential risk of bias and data extraction; otherwise, authors were contacted for an updated publication. If contact information was not available or no response was obtained, the study was excluded from the SR-MA.

Study design elements were extracted and evaluated as part of the Risk of Bias assessment (RoB) following the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) approach (Guyatt et al., 2011; Higgins & Green, 2011). Trials were grouped according to outcome, study design and chilling intervention to form a body of evidence for a given intervention. Following the GRADE approach, extracted data from this body of evidence included several fields that contributed to an outcome's within-study risk of bias, directness of evidence, heterogeneity, estimate precision and risk of publication bias ([Supplementary Materials – Section D](#)). Trials were excluded if they did not sufficiently report intervention details to allow replication, did not use a control group in their methodology or did not adequately report data to allow for calculation of summary estimates for MA. Other trial data extracted as part of the SR-MA process included population and intervention details and outcome information ([Supplementary Materials – Section E](#)).

2.4. Meta-analysis

Seven data subsets consisting of ≥ 2 trials were created as a result of the grouping criteria used for the RoB. Separate MA's were conducted at the trial level using STATA 11.2 IC statistical software (StataCorp, College Station, Texas, USA).

Concentration outcomes (mean \log_{10} CFU/ml, CFU/g and CFU/cm²), associated standard deviations (SD) or standard errors (SE) and sample sizes were extracted for treatment and control groups to calculate the raw mean differences (MD). The latter measure was chosen over a single standardized measure of effect to allow more meaningful interpretation (Borenstein et al., 2009). Measures of \log_{10} CFU/ml, CFU/g and CFU/cm² were converted to a CFU/carcass scale following formulas from FAO and WHO (2009–2014). To comply with assumptions of normality for the MA procedures, concentration outcomes were converted to the natural logarithm scale (Higgins, White, & Anzués-Cabrera, 2008) for analysis, then back-transformed to the base ten logarithm for interpretation.

To estimate the efficacy of chilling when using prevalence (binary) data, the number of *Campylobacter*-positive and negative samples in the treatment and control groups of a trial were used to compute odds ratios (OR) with associated SEs, confidence intervals and prediction intervals on the natural logarithm scale to meet assumptions of normality (Borenstein et al., 2009). When zero-cell counts were present, a continuity correction of 0.5 was added to each cell in the 2×2 table (Higgins & Green, 2011). Assumed and comparative risks were calculated to help interpret MA estimates from prevalence data (Schünemann et al., 2011) following:

$$\text{Assumed Risk}(AR) = \frac{\sum n_c}{\sum N_c}$$

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