

*CAMPYLOBACTER* CONTROL IN POULTRY PROCESSING OPERATIONS IN THE  
UNITED STATES

by

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ABSTRACT

*Campylobacter* is an important zoonotic bacterial pathogen that is commonly associated with poultry products and can cause gastrointestinal illness. The regulatory approach has mainly focused on reducing pathogen prevalence at the poultry processing operations using performance standards. A survey of broiler processing establishments was conducted to evaluate *Campylobacter* post-harvest interventions and control strategies for cut-up parts adopted by the U.S. broiler industry. A majority (62%) of processing establishments were monitoring for *Campylobacter* and 74% have an established control program for *Campylobacter* control for chicken parts. Establishments met the *Campylobacter* performance standard for whole carcasses (62%) and parts (49%) exceeding USDA-FSIS estimates. *Campylobacter* prevalence was not influenced by processing plant processing capacity and antimicrobial interventions to meet performance standards are being used. *Campylobacter* quantitative microbial risk assessments (QMRA) show that concentrating efforts to lower *Campylobacter* population may help reduce illness risk to the public. Chicken parts and comminuted poultry products have not been part of exposure assessments even though these products constitute a higher share of the total poultry consumption in the U.S. A systematic review and meta-analysis (SR-MA) were performed to

develop *Campylobacter* contamination estimates in poultry parts and comminuted product with and without interventions. These estimates were then used to evaluate intervention data in its ability to control *Campylobacter* contamination. The initial prevalence (82%) and concentration (4.81 log<sub>10</sub> CFU/mL) were calculated from literature data. Odds ratios (OR) and Log changes (LC) were calculated to indicate changes in *Campylobacter* population. The scalding stage had an OR of 0.15 and an LC of -2.86 log<sub>10</sub> CFU/mL, and the chilling stage had an OR of 0.32 and an LC of -1.48 log<sub>10</sub> CFU/mL providing significant reductions from the initial contamination (P < 0.05). A baseline model representing *Campylobacter* population without interventions was constructed. *Campylobacter* was present in 18.4 % of whole birds at 1.38 log<sub>10</sub> CFU/mL, 62.5% of cut-up parts at 0.79 log<sub>10</sub> CFU/mL, and 25.2% comminuted product at 0.45 log<sub>10</sub> CFU/mL. Post-chill immersion after chill reduced prevalence by 96% and concentrations by 94%. *Campylobacter* population in cut-up parts can be used to assess risk of exposure.

INDEX WORDS: *Campylobacter*, Survey, Performance standard, Exposure assessment,  
Poultry parts, Comminuted poultry, Interventions

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## DEDICATION

To the Rivera Andújar family. Brenda, for always supporting me and believing that whatever we decide is to make us better. Alexander and Gabriela, I just want you to understand that in life you must sacrifice to provide a better life for you and the ones you love.

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## CHAPTER 1

### INTRODUCTION AND LITERATURE REVIEW

#### *Campylobacter* and Public Health

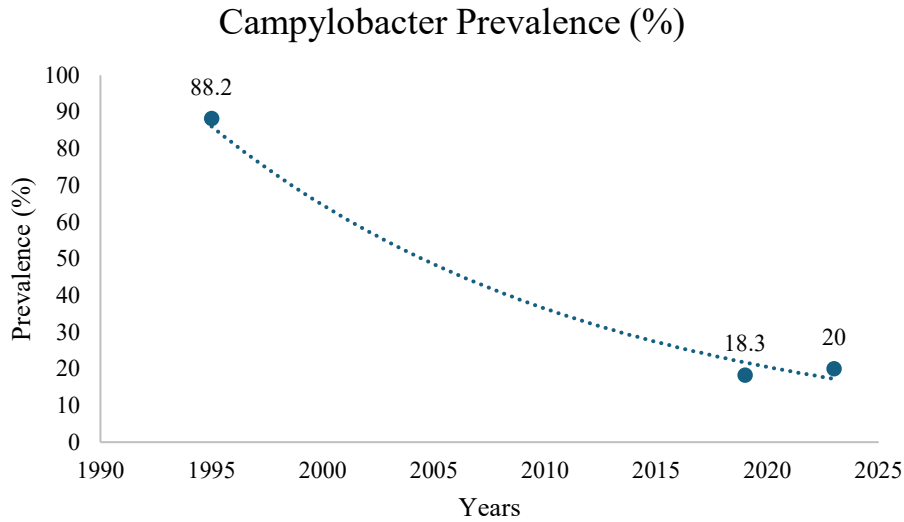
*Campylobacter* cells are slender, spiral-shaped rods, 0.2 to 0.8  $\mu\text{m}$  wide and 0.5 to 5.0  $\mu\text{m}$  long. Cells are motile, with a characteristic cork-screw-like motion performed by means of a single polar unsheathed flagella at one or both ends of the cell. *Campylobacter* species grow in microaerobic environment with hydrogen, formate, or succinate as electron sources (Debruyne et al., 2008).

*Campylobacter*, particularly *C. jejuni* and *C. coli*, is one of the leading cause of gastrointestinal zoonotic bacterial infection in the western world (Ellis-Iversen et al., 2012). Symptoms associated with *Campylobacter* infection include diarrhea, fever, stomach cramps, nausea, and vomiting (CDC, 2022a). These symptoms usually start 2 to 5 days post-ingestion of *Campylobacter* and last about one week (CDC, 2022a). The disease is usually self-limiting, and antimicrobial treatment is only indicated in severe cases (Wagenaar et al., 2013). *Campylobacter* infections can lead to complications, such as irritable bowel syndrome, temporary paralysis, and arthritis (CDC, 2022a). In immunocompromised individuals, such as those with a blood disorder, with AIDS, or receiving chemotherapy, *Campylobacter* occasionally spreads to the bloodstream and causes a life-threatening infection (CDC, 2022a). There is also a link between campylobacteriosis and Guillain-Barre syndrome (Williams et al., 2008).

The Foodborne Diseases Active Surveillance Network (FoodNet) indicates that about 20 cases of campylobacteriosis are diagnosed per 100,000 people annually (CDC, 2022b). Most *Campylobacter* cases go undiagnosed or unreported. The Center for Disease Control and Prevention (CDC) estimates *Campylobacter* infections affect 1.5 million U.S. residents every year (CDC, 2022a). *Campylobacter*

infection is commonly associated with the consumption of poultry products (Berghaus et al., 2013; Ellis-Iversen et al., 2012; Overesch et al., 2020). In 2019, the Interagency Food Safety Analytics Collaboration (IFSAC) reported that over 80% of non-dairy foodborne illnesses linked to *Campylobacter* were attributed to poultry products, especially chicken, other seafood (such as shellfish) and turkey (IFSAC, 2021). As a result, there is an economic burden estimated at \$6.9 billion annually in the U.S. due to medical costs and loss of quality of life (Scharff, 2020).

The U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) develops and monitor food safety regulatory standards for poultry. The USDA-FSIS introduced the Pathogen Reduction; Hazard Analysis and Critical Control Points rule (PR: HACCP Rule) in 1996. The USDA-FSIS has relied on controlling pathogens by developing performance standards aimed at reducing prevalence at the processing plant (USDA-FSIS, 1996). Since the introduction of the PR: HACCP rule, USDA-FSIS conducted several baseline surveys to determine *Salmonella* and *Campylobacter* prevalence on broiler carcasses in poultry processing establishments and revised the performance standards. The USDA-FSIS baseline survey, conducted in 1995 (Figure 1.1), reported a *Campylobacter* prevalence of 88.2% on chicken carcasses, and a following survey in 2019 reported a lower prevalence of 18.3% (Williams et al., 2021). Since the publication of the 2019 USDA-FSIS exploratory *Campylobacter* survey, there has not been a significant change in prevalence as reported by the agency. The USDA-FSIS raw chicken carcass sampling dataset resulted in 20.94% of positive samples for *Campylobacter* for 2022, an increase from 18.80% of positive samples reported in 2021 (USDA-FSIS, 2023a). The USDA-FSIS raw chicken parts sampling dataset resulted in 16.75% of positive samples for *Campylobacter* for 2022, an increase from 15.16% of positive samples reported in 2021 (USDA-FSIS, 2023a). The USDA-FSIS raw comminuted chicken sampling dataset resulted in 5.93% of positive samples for *Campylobacter* for 2022, a decrease from 6.28% of positive samples reported in 2021 (USDA-FSIS, 2023a). Despite a reduction in overall *Campylobacter* prevalence since 1995, the reported *Campylobacter* illnesses have remained steady for many years subsequent to the implementation of HACCP (CDC, 2022b).



**Figure 1.1:** Trends in *Campylobacter* Prevalence (%) from 1995-2023

The USDA-FSIS announced the *Campylobacter* performance standard for chicken and turkey carcasses in 2010, followed by performance standards for parts and comminuted poultry in 2016 (USDA-FSIS, 2010, 2015a; Williams et al., 2021). Microbiological verification testing results were used to categorize poultry processing establishments in categories based on *Campylobacter* prevalence levels. Subsequently, a modified sampling method using Neutralizing Buffered Peptone Water resulted in a marked reduction in *Campylobacter* recovery in chicken parts using direct plating. However, the 30 mL enrichment method resulted in a higher prevalence of *Campylobacter* in chicken parts (USDA-FSIS, 2019). As a result, the USDA-FSIS revised the *Campylobacter* performance standards and discontinued reporting on testing results and categories until the microbiological verification testing were revalidated (USDA-FSIS, 2019). The regulatory approach has mainly focused on reducing and/or eliminating foodborne pathogens at the processing establishment level. Currently, *Campylobacter* reduction is heavily addressed through antimicrobial chemical interventions (Wideman et al., 2016). Efforts at reducing *Campylobacter* throughout the poultry production chain have also received attention. Further control of *Campylobacter* requires an understanding of its behavior throughout the poultry production chain and novel interventions to reduce its incidence.

### *Campylobacter* Contamination in Chicken

Many species of poultry, especially commercial broilers and turkeys carry high levels of *Campylobacter* (*C. jejuni* and *C. coli*) in their gastrointestinal tract as part of their normal microbial flora (Sahin et al., 2015). *Campylobacter* prevalence ranges from 23-100% and with concentrations between 3-7 log CFU (Alter et al., 2005; Berrang et al., 2007; Potturi-Venkata et al., 2007; Stern et al., 2001). Other studies have observed concentrations as high as 8-9 log CFU/g (Keener et al., 2004). Flock prevalence is highly seasonal, reaching its peak occurrence during the summer months, correlating with the peak in human campylobacteriosis cases (Ellis-Iversen et al., 2012; Oakley et al., 2018; Wagenaar et al., 2006).

There are several routes for *Campylobacter* to infect chickens and introduce the pathogen into the poultry supply chain. *Campylobacter* colonization in chickens is affected by several factors such as bird age, genetic susceptibility, stress and underlying disease, level of pathogen exposure, and its ability to evade host defenses and competition of gut microbiota that facilitate colonization in the gut (Alali & Hofacre, 2016). Colonization in chickens is commensal and occurs between 2-3 weeks of age (Sahin et al., 2015). Once *Campylobacter* is introduced into a flock, it spreads very rapidly with virtually all birds becoming colonized and shedding up to 8 log CFU/g of cecal contents (Wagenaar et al., 2013).

Its capacity to withstand the hostile environment of the gastrointestinal tract *Campylobacter* requires a multifactorial approach that involves many molecular processes (Bolton, 2015; Hermans et al., 2011). The colonization mechanisms studies report that *Campylobacter* is very inefficient at establishing itself in the intestinal epithelial cells due to a weak host immune response (Hermans et al., 2011). There is evidence of internalization to organs such as the liver and spleen, but its internalization mechanism in birds and a weak immune response from the host keeps colonization limited to the mucosal layer in the gut, particularly in the cecal mucosal crypts (Hermans et al., 2011). *Campylobacter* counts on carcasses are associated with the level of colonization in the ceca (Seliwiorstow, Bare, Berkvens, et al., 2016).

### Pre-Harvest *Campylobacter* Control

The most common routes of pathogen colonization occur via vertical or horizontal transmission. Vertical transmission occurs through the movement of *Campylobacter* parent flocks to hatching eggs through the reproductive tract. Vertical transmission is not well understood, and it is deemed to be of low risk due to the low recovery of *Campylobacter* in hatching eggs (Alali & Hofacre, 2016; Berkics et al., 2021; Newell et al., 2011). Studies show that *Campylobacter* in hatching eggs come from fecal contamination of the egg surface, either at the time of lay, or mismanagement by placing eggs on contaminated egg trays and surfaces, or poor egg temperature management that cause condensation on the shell surface (Alali & Hofacre, 2016; Berkics et al., 2021). *Campylobacter* has been isolated from hatchery tray liners and hatchery fluff at low levels. The egg transmission rate is deemed to be as low as 0.75% to 1.6% (Cox et al., 2012).

Newly hatched chicks typically start *Campylobacter* free and continue until 2-3 weeks into the production cycle (Sahin et al., 2015). Horizontal transmission is likely caused by the introduction of the pathogen from the environment via the fecal-oral route (Alali & Hofacre, 2016; Amalaradjou, 2019; Cox et al., 2012; Newell et al., 2011; Sahin et al., 2015; Wagenaar et al., 2013). Potential environmental sources that contribute to *Campylobacter* colonization in chickens include contaminated poultry litter, feed, drinking water, rodents, insects, and poor biosecurity practices (Golden & Mishra, 2020; Sahin et al., 2015; Wagenaar et al., 2013).

Active carriage of *Campylobacter* from the environment into a poultry house is an important factor in the exposure of this pathogen to broilers. Poor biosecurity practices can exacerbate the presence of *Campylobacter* in the environment surrounding birds. *Campylobacter* has been detected in puddles, other farm animals and the surrounding environment like poultry house anteroom surfaces (Ellis-Iversen et al., 2012). Theoretically, a high level of biosecurity on farm level should prevent the introduction of *Campylobacter* into a flock (Wagenaar et al., 2013). The implementation of strict biosecurity measures can

delay or reduce the rate of *Campylobacter* colonization in birds (Newell et al., 2011; Sahin et al., 2015; Sibanda et al., 2018). However, strict biosecurity measures are not a guarantee to eliminate *Campylobacter*.

Some of the barriers include proper hygienic practices of farm workers, such as hand washing procedures, providing dedicated clothing and footwear, boot and glove disinfection, preventing carriage into other houses (Alali & Hofacre, 2016; Amalaradjou, 2019; Newell et al., 2011; Wagenaar et al., 2013). Cleaning and disinfection of vehicles and equipment and proper entry and exit procedures are also important since these reduce potential spread to other surfaces (Alali & Hofacre, 2016; Amalaradjou, 2019; Newell et al., 2011; Wagenaar et al., 2013).

Insects like darkling beetles and flies have been shown to carry *Campylobacter*. Rodents can also contribute to the introduction of *Campylobacter* into a poultry house (Alali & Hofacre, 2016; Amalaradjou, 2019; Newell et al., 2011; Wagenaar et al., 2013). The implementation of a pest control program can reduce the numbers of pests around a poultry house.

Litter is the absorbent material used to cover the floor of a chicken house and the material is commonly made of wood, pine straw, peanut hulls, or any other dry absorbent materials (Alali & Hofacre, 2016). Fecal contamination and high moisture content provide an ideal environment for *Campylobacter* and several other pathogens to grow (Amalaradjou, 2019). The reuse of litter for several flock cycles in the U.S. contributes to contamination of broiler flocks if the litter is not managed properly (Alali & Hofacre, 2016). Litter amendments that modify the pH of the litter have been tested. Amendments such as alum, and sodium bisulfate have helped delay the onset of *Campylobacter* colonization in broilers (Line, 2002; Line & Bailey, 2006).

Feed and water can be a vehicle of transmission of foodborne pathogens. The goal of pathogen control in feed and water should be to ensure that contamination is below a threshold that poses minimal risk to bird and human health (Alali & Hofacre, 2016). Feed is mostly free of *Campylobacter* since feed is dried and pelleted under high temperature and survival of this organism is highly unlikely (Doyle & Roman,

1982; Humphery et al., 1993). Acidification of drinking water using organic acids is reported to reduce the risk of colonization (Allain et al., 2014; Sahin et al., 2015).

Feed and water are also used as a vehicle to apply controls that modulate the gut environment in broilers. Nutritional strategies include the application of short chain (formic, acetic, propionic, butyric acid) and medium chain organic acids (caprylic, capric, caproic, and lauric acid) to help in the acidification of feed or water. The acids help lower intracellular pH in pathogens affecting cell functions that allow the establishment of *Campylobacter* in the gut (Amalaradjou, 2019). The use of nutritional strategies such as prebiotics, probiotics, and essential oils has also been shown that it can affect *Campylobacter* populations. Amalaradjou (2019) discussed that these additives show that their antimicrobial properties or its capacity to promote specific gut microbes that can compete with *Campylobacter* colonization and reduce the possibility of establishment in the ceca. However, the questions remain as to what quantities and combinations are optimal to promote competitive exclusion.

Bacteriocins are antimicrobial peptides produced by commensal bacteria, and their use or the infusion of bacteriocin- producing bacteria, has been shown to modulate the gut bacterial community (Svetoch & Stern, 2010). Bacteriophages are viruses that are predatory to bacteria and occur naturally in the environment. *Campylobacter* specific bacteriophages have been isolated and evaluated for their ability to inactivate the pathogen. Several cocktails have been studied and reductions greater than 2 log have been achieved, but the effects are short-lived, and original bacterial levels are observed after a few days (Amalaradjou, 2019; Sahin et al., 2015).

Immunization through the development of vaccines has also been studied. However, there is no effective vaccine currently available against *Campylobacter*. This is due to lack of success in identifying immunogenic and protective antigens in *Campylobacter* (Sahin et al., 2015). The need for effective novel adjuvants, vaccination methods, and the short life of broiler chicken that further limits a response against *Campylobacter* (Cloutier & Gauthier, 2020).

Additional factors that can limit and/ or minimize the shedding and cross-contamination of *Campylobacter* are careful management of feed withdrawal and transportation. Feed withdrawal prior to shipping to the processing plant reduces the amount of feces in the gut and the crop therefore reduces the potential risk of carcass contamination. Poor management of feed withdrawal causes birds to start searching for feed and litter consumption might increase during this period (Alali & Hofacre, 2016; Seliwiorstow, Bare, Berkvens, et al., 2016). Overall, there is no significant change in *Campylobacter* levels in the ceca due to feed withdrawal (Northcutt, Berrang, et al., 2003; Northcutt, Buhr, et al., 2003). Transportation can also contribute to cross-contamination of feathers and skin (Sahin et al., 2015; Seliwiorstow, Bare, Berkvens, et al., 2016; Wagenaar et al., 2013). Berrang et al. (2004) sampled new transport cages used for *Campylobacter* positive flocks at several intervals after birds were unloaded and found that *Campylobacter* was still present after 24 and up to 48 hours of storage. Hastings et al. (2011) tested washing and disinfection of crates and observed that washing alone increased *Campylobacter* prevalence and applying a sanitizer reduced prevalence. Regardless of the procedure, *Campylobacter* was still present, and transport cages can be a source of cross contamination while birds are being transported.

In general, providing a *Campylobacter*-free environment is ideal to reduce the risk of human illness. Pathogen transmission is extremely complex and requires a comprehensive control and prevention program that can address all the factors that contribute to the colonization of *Campylobacter* in poultry (Alali & Hofacre, 2016). There are no effective and technically implementable tools that keep the environment free from the pathogen (Sahin et al., 2015). The unpredictable effectiveness of pre-harvest interventions to reduce *Campylobacter* prevalence and concentration causes a heavy reliance on post -harvest interventions at the processing plant.

#### Post-harvest *Campylobacter* Control

The prevalence and bacterial loads at the farm level has a strong correlation to the *Campylobacter* found at the processing plant (Berghaus et al., 2013). *Campylobacter* is mainly found in the ceca and colon at high levels ranging from 6.9-7.3 log CFU/g of sample (Berrang, Buhr, et al., 2000) and 4.59-7.57 log

CFU/mL, respectively (Dubovitskaya et al., 2023). *Campylobacter* can also be found on feathers, skin, and breast (Berrang, Buhr, et al., 2000; Kotula & Pandya, 1995; Seliwiorstow, Bare, Berkvens, et al., 2016). *Campylobacter* has also been isolated in the liver through either internalization of the pathogen or through cross contamination during processing (Berrang, Buhr, et al., 2000).

The poultry processing plant is a site where *Campylobacter* is spread through cross contamination, particularly in the scalding, defeathering, and evisceration process (McCarthy et al., 2019). *Campylobacter* has also been found to attach to surfaces on processing equipment, belts, and surrounding non-contact surfaces (Garcia-Sanchez et al., 2017). In addition, the bacteria can freely move, and its genetic makeup and cellular mechanisms allow for defense against physical and chemical treatments that can injure or inactivate them (Garcia-Sanchez et al., 2017; Nguyen et al., 2012). Contaminated surfaces can spread *Campylobacter* to poultry meat regardless of the meat's *Campylobacter* status (Garcia-Sanchez et al., 2017). Pre-operational sanitation practices, and sanitary equipment design must take this into account.

Processing plant implements interventions at various processing steps to remove and inactivate *Campylobacter* from chicken carcasses (DeVillena et al., 2022; Hwang & Singer, 2020; Williams et al., 2021). The scalding is the first step where *Campylobacter* reduction can be achieved. The scalding is the processing step where feathers are loosened with hot water to facilitate removal. Scalding can partially inactivate *Campylobacter* by removing cells through debris removal and through partial inactivation due to the high temperature of the water. *Campylobacter* can be present in scalding water and become a source of cross contamination (Reiter et al., 2005). Overall, scalding lowers the concentration and prevalence of *Campylobacter* on the external surface of broiler carcasses (Berrang et al., 2003). The effectiveness of scalding inactivation is dependent on incoming microbial load, water quality and temperature and, scalding design (McCarthy et al., 2019; McCarthy et al., 2018).

The positive effects of *Campylobacter* removal are reversed by the subsequent steps of feather removal and evisceration. During feather removal, the remaining intestinal content is extruded out and quickly contaminates equipment and birds that passed through the defeathering machine (Berrang et al.,

2006a; Musgrove et al., 1997). Leakage of intestinal and crop contents is exacerbated during the evisceration process where vent cutting, and viscera removal equipment can further spread *Campylobacter* to additional birds (Seliwiorstow, Bare, Van Damme, et al., 2016).

Pre-chill wash, chill, and post chill interventions provide the greatest reduction in *Campylobacter* prevalence (Berrang et al., 2007). The poultry industry has traditionally used immersion chillers to cool down and reduce microbial growth. The immersion chiller is also a step where interventions are applied. Immersion chillers have been shown to slightly reduce *Campylobacter* levels either by injuring or washing off cells from the carcass surface. The immersion chiller without intervention can reduce *Campylobacter* concentration by 1 log CFU without much change in prevalence (Demirok et al., 2013; Fluckey et al., 2003; Huezo et al., 2007; Zhang et al., 2011). Water inside the chiller can also be a source of cross contamination since washed off cells may persist after birds are chilled. The volume of water does not influence the removal of *Campylobacter* from the system (Northcutt et al., 2006). Air chillers are another alternative to reduce meat temperature. The *Campylobacter* changes in air chillers have been studied and it can also provide slight reduction of less than 1 log CFU (Demirok et al., 2013; Fluckey et al., 2003; Huezo et al., 2007; Zhang et al., 2011). Both types of chillers cannot provide significant reductions on their own and chemical interventions before or after chilling, or during chilling provide additional reduction in *Campylobacter* prevalence and concentrations (Fluckey et al., 2003; Huezo et al., 2007).

Additional decontamination methods before and after chill have been applied mostly through spray or immersion systems. The inside-outside bird washer (IOBW) is a wash system designed to remove visible contamination from carcasses. The IOBW does not show significant reductions in *Campylobacter* presence (Kemp et al., 2001; Oyarzabal et al., 2004). Additional steps like on-line reprocessing with an antimicrobial and other post IOBW, pre chill steps have shown to be more effective in reducing pathogen concentration and presence by adding antimicrobials (Gonzalez et al., 2021; Kemp et al., 2001). Antimicrobial treatments through spray can reduce *Campylobacter* concentrations but evenness of exposure to surface and contact time is limited (Gonzalez et al., 2021). Dip tanks have been shown to be more effective at reducing

*Campylobacter* than spray cabinets due to their ability to increase contact time and surface coverage (Chen et al., 2014; Gonzalez et al., 2021; Laranja et al., 2023; Nagel et al., 2013; Zhang et al., 2018).

Refrigerated storage is used to control bacterial growth in foods. Refrigeration and freezing have been shown to also have an effect on *Campylobacter* growth. Refrigeration temperatures at 4° C from 3 to 7 days show reductions between 0.31 to 0.81 log CFU/g in ground chicken (Bhaduri & Cottrell, 2004). *Campylobacter* appears to be more sensitive to freezing since reductions of up to 1.57 log CFU/g were observed (Bhaduri & Cottrell, 2004). Refrigeration and freezing can also show a reduction in prevalence. The refrigerated and frozen storage tests at 4°C for 7 days and -20°C for 28 days demonstrated a prevalence reduction from 93% of samples from fresh chicken meat down to 53% and 36%, respectively (Maziero & Oliveira, 2010). *Campylobacter* reductions through refrigeration and freezing can be achieved during the first 7 days of treatment, but prolonged storage did not result in further reduction. (Bhaduri & Cottrell, 2004; Byrd et al., 2011; Georgsson et al., 2006; Gunther et al., 2015; Meredith et al., 2014). Even though some reduction is achieved through refrigeration and freezing, *Campylobacter* can still survive in this environment. This intervention should be a part of an overall control strategy of preserving reductions achieved through processing interventions, packaging, or heat treatments to ensure food safety (Byrd et al., 2011; Meredith et al., 2014; Sampers et al., 2010).

### Chemical Interventions

Currently, *Campylobacter* reduction is heavily addressed through antimicrobial chemical interventions. Poultry processors are constantly adjusting interventions to meet performance standards (Wideman et al., 2016). The key processing steps where *Campylobacter* reduction can be achieved are scalding, evisceration, carcass washers, and immersion chillers (Oyarzabal, 2005). Each step requires observations and adjustments to clean and eliminate fecal contamination (Wideman et al., 2016). Multiple antimicrobial interventions for raw poultry products have been studied. Antimicrobials approved for industry should have documented efficacy at a level and contact time for the step, be cost effective, and have minimal adverse effects on product quality (Cano et al., 2021).

Chlorine has long been used as a processing aid to control pathogens, but its effectiveness is very limited. Chlorine's disinfection capacity is highly dependent on pH changes, and the amount of organic matter in chillers (Chen et al., 2020). Chlorine oxidizes cell components resulting in cell death (Oyarzabal, 2005). Several studies have shown that chlorine's effectiveness is very inconsistent and it's not much different than washing carcasses with water. Zhang (Zhang et al., 2018) showed that 30 ppm Chlorine had less than 1 log CFU/sample reduction. Similar results were shown by Chen et al. (2014). Nagel et al. (2013) tested levels of 40 ppm and showed the reduction of less than 1-log CFU/mL. Kameyama et al. (2012) tested up to 70 ppm of chlorine at different chiller temperatures and concluded that although there was a reduction in *Campylobacter*, it could not be established whether it was an effect of the chemical or the chiller temperatures. Chlorine, typically used as sodium hypochlorite, has seen a decrease in its use due to more attention towards Generally Regarded As Safe (GRAS) status of peroxyacetic acid (PAA) (DeVillena et al., 2022; Kataria et al., 2020).

Other chlorine-based compounds like acidified sodium chloride (ASC) and chlorine dioxide (ClO<sub>2</sub>) have been approved for use (Cano et al., 2021). ASC oxidizes sulfide and disulfide bonds on cell membrane surfaces (Oyarzabal et al., 2004). ASC can be an effective antimicrobial at different concentrations if applied as a spray or dip solution. ASC has been tested at IOBW and as an on-line reprocessing application. Prevalence reductions of over 50% and 2 log CFU concentration reductions can be observed in pre-chill treatments (Kemp et al., 2001; McWhorter et al., 2022). Reductions in IOBW and sprays are dependent on water volume and pressure. Its use may not be applicable in processing establishments where water use reduction programs are in use (Oyarzabal et al., 2004). ASC post-chill dip treatment may be the best application as it achieves the best reductions. Post-chill concentrations of 400 ppm and 900 ppm can have concentration reductions ranging from 1.5 to 3.5 log CFU (McWhorter et al., 2022; Sexton et al., 2007). Negative samples and over a 2 log CFU concentration reduction can be achieved through a post-chill dip intervention and allow to reduce overall water use in a processing plant (Oyarzabal et al., 2004; Sexton et al., 2007). Another factor limiting ASC use is that it must be used with an acidifying agent, such as citric

acid to maintain a pH of 2.5 (Oyarzabal et al., 2004). High temperature and organic matter levels may also limit its effectiveness (Cano et al., 2021). Similar limitations can be observed with ClO<sub>2</sub>. ClO<sub>2</sub> oxidizes the cellular membrane and other cell components disrupting the permeability of the membrane and protein synthesis. It can be 2.5 times more effective than chlorine. Its limitations include pH changes, the presence of organic material in water, and its instability. Therefore, making it difficult to maintain its effectiveness for long periods of time and limits its storage (Cano et al., 2021; Oyarzabal, 2005).

PAA provides a strong oxidizing function that disrupts the permeability of cell membranes and alters protein synthesis (Oyarzabal, 2005). PAA has emerged as a popular choice due to its overall effectiveness in reducing *Campylobacter*. When used at high concentrations, PAA can provide over 2.0 log CFU/mL reduction in *Campylobacter* counts (Kumar et al., 2020; Vaddu et al., 2021; Wideman et al., 2016). Concentrations as low as 50ppm can have similar effects (Kataria et al., 2020). Solutions of 700 ppm of PAA had the ability to lower *Campylobacter* by 1.5 log CFU/g with residual effect in protecting poultry during storage without affecting organoleptic qualities (Chen et al., 2014; Laranja et al., 2023). PAA does not seem to be affected by pH levels. Gonzalez et al. (2021) showed 550ppm of PAA had similar reductions when the solution was acidified versus solutions without acidifiers. Kataria et al. (2020) showed that a pH of 8.2, 10, and 11 did not affect PAA effectiveness in *Campylobacter* reductions. PAA is also effective with different application methods. Both immersion tanks and spray cabinets can be effective applicators of PAA. Immersion tanks are more effective because they provide uniform coverage and an increased contact time (Gonzalez et al., 2021; Laranja et al., 2023).

Cetylpyridinium chloride (CPC) integrates into the lipid membrane of bacterial cells, interfering with osmoregulation (Cano et al., 2021). It can be applied as a spray solution at 0.3g of CPC per pound of raw poultry or as an immersion solution up to 0.8% CPC by weight (USDA-FSIS, 2023c). CPC can be effective in reducing *Campylobacter* concentrations and can inhibit overall bacterial growth for a longer period compared to PAA (Cano et al., 2021). CPC concentrations of 0.35% or 0.60% can achieve 4 log and 5 log reductions, respectively (Zhang et al., 2018). Similarly, 0.15% CPC with a spray cabinet application

reduced *Campylobacter* to below detectable levels (Wideman et al., 2016). CPC is required to be rinsed after application, its application has a short contact time, and specialized equipment is needed for application and recapture. This makes implementation difficult and expensive (Cano et al., 2021; Wideman et al., 2016).

There are additional chemical interventions that have been approved for use by the commercial poultry industry at varying levels. Two common ones are trisodium phosphate (TSP), and lactic acid (LA). TSP disrupts the cell membrane causing leakage of intra cellular fluid (Oyarzabal, 2005). TSP has a pH of 10-12 and has surfactant properties (Cano et al., 2021). TSP is used as a carcass wash that can reduce *Campylobacter* up to 1.26 log. It can also be used in combination with chlorine washes and ASC (Bashor et al., 2004). One major limitation is that TSP increases phosphates concentration in wastewater and this has prevented widespread adoption (Oyarzabal, 2005). Lactic acid (LA) can also be an effective antimicrobial by lowering the intracellular pH of bacteria and causing damage to the cytoplasmic membrane (Cano et al., 2021). The advantage of LA is that it is naturally occurring, and it is an acceptable antimicrobial for organic production (Cano et al., 2021) 1.0% - 3.0% LA solutions can reduce *Campylobacter* from 0.36 log-1.98log (Coşansu & Ayhan, 2010). LA of up to 10% can achieve up to 2 log reduction (Lecompte et al., 2009). One major limitation is that LA can cause off colors and odors when used at concentrations above 3.0% (Cano et al., 2021). Other organic acids have been evaluated with varying levels of effectiveness, but they do not improve prevalence or concentration reductions as the most common chemical interventions (Molatová et al., 2010). Table 1.1 summarizes the comparison of chemical interventions for *Campylobacter* reduction.

**Table 1.1: Comparison of Chemical Interventions for *Campylobacter* Reduction**

<b>Intervention</b>	<b>Concentration</b>	<b>Application Method</b>	<b>Reduction (Log CFU)</b>	<b>Limitations</b>
Peroxyacetic Acid (PAA)	50-700 ppm	Spray, Immersion	1.5-2.0	Cost, potential impact on product quality
Acidified Sodium Chlorite (ASC)	400-900 ppm	Dip, Spray	1.5-3.5	Requires acidifying agent, limited by organic matter
Chlorine	30-70 ppm	Spray, Immersion	<1.0	Ineffective at high organic matter, pH dependent
Cetylpyridinium Chloride (CPC)	0.15-0.60%	Spray, Immersion	4.0-5.0	Requires rinsing, specialized equipment
Lactic Acid (LA)	1-10%	Spray, Dip	0.36-1.98	Off- odors and discoloration at high concentrations
Trisodium Phosphate (TSP)	10-12%	Spray	1.26	Increase phosphate levels in wastewater

### Physical Interventions

There are interventions in development that are being tested for *Campylobacter* like packaging modifications, high pressure inactivation, ultra-violet light, pulse electric field, chilling and freezing interventions, and steam and hot water treatments (Clemente et al., 2020; Dogan et al., 2022; Haughton et al., 2011). Further investments are needed to scale up those interventions that have shown potential to significantly reduce *Campylobacter* in raw poultry products. There are several non-thermal and non-chemical interventions that are increasing their body of work and are providing evidence of additional alternatives to *Campylobacter* reduction.

Packaging modifications are increasingly considered, primarily through modifications in the gas mixture within the packaging. *Campylobacter* requires a microaerobic environment of 5% O<sub>2</sub> to thrive so various combinations of O<sub>2</sub> and CO<sub>2</sub> have been tested. Achieving an optimal gas mixture may be able to

reduce *Campylobacter* concentrations and improve quality aspects like extending shelf life. Trials where there is 100% CO<sub>2</sub> do not show an effect on decreasing *Campylobacter* (Wesley & Stadelman, 1985). Vacuum packaging may not achieve *Campylobacter* reductions due to the lack of O<sub>2</sub> (Byrd et al., 2011). High CO<sub>2</sub> levels may provide an environment where spoilage bacteria decrease its growth, but the lack of oxygen encourages *Campylobacter* growth (Meredith et al., 2014). Storage with higher oxygen levels show decreased *Campylobacter* growth (Byrd et al., 2011; Meredith et al., 2014). The most effective methods to reduce *Campylobacter* through atmospheric packaging modifications may come with the aid of additional interventions like irradiation (Kudra et al., 2012), novel technologies like the incorporation of nanoparticles in packaging (Hakeem et al., 2020), or cold plasma treatments (Rothrock et al., 2017). Packaging treatments serve several purposes by not only reducing pathogenic bacterial growth, but also extending shelf life and maintaining organoleptic qualities of poultry meat (Meredith et al., 2014).

Ultraviolet (UV) treatments have been used for the treatment of surfaces and other food products (Soro et al., 2021). The germicidal effects may come by the effect of UV light on DNA (Haughton, Grau, et al., 2012). UV light treatments can inactivate *Campylobacter*, but its effect is very limited. Isohanni and Lyhs (2009) tested UV light on skinless chicken parts, skin, and whole chicken. The observed reductions in *Campylobacter* were up to 0.4 log CFU. A similar result was observed by Haughton, (Haughton et al., 2012), where similar UV doses achieved up to 0.76 log CFU reduction. These reductions are lower than the common chemical antimicrobial interventions. Using higher doses of 405 nm near visible light at 184-186 J/cm<sup>2</sup> can achieve 1.7 log to 2.1 log reductions but needs long exposure times that are impractical for a poultry processing operation (Gunther et al., 2016).

*Campylobacter* is susceptible to UV treatments when directly exposed to treatments in liquid media, on exposed surfaces and on packaging. *Campylobacter* can be completely inactivated in liquid media using a dose of 0.192 J/cm<sup>2</sup> and reductions of up to 3.97 log CFU/cm<sup>2</sup> can be observed on packaging and other surface materials (Haughton et al., 2011). In another study, 1.1 log and up to 4.9 log reductions were obtained with exposure of near visible light (Gunther et al., 2016). One possible explanation is that

flat and even surfaces are evenly exposed to the UV treatments achieving complete area treatment. The uneven surfaces of poultry meat can create shadows and the UV light may not be able to cover the entire area of the product (Isohanni & Lyhs, 2009).

Irradiation using a Cobalt 60 source or electron beams are approved for use in doses of 1.5 to 3.0 kGy (Lewis et al., 2002; Patterson, 1995). Both types of sources can significantly inactivate microorganisms with effective penetration of product surfaces (Lewis et al., 2002). The effectiveness of irradiation against pathogens is mainly due to hydrogen peroxide production that results from the generation of free radicals during irradiation. Hydrogen peroxide acts as a potent antimicrobial and can eventually result in the production of long-lived hypochlorite, which is very toxic to pathogens (Kuby, 1997). Gamma irradiation with Cobalt 60 is very effective, producing 3 log CFU/g reductions *Campylobacter* with 1 kGy (Patterson, 1995). Similarly, Lewis et al. (2002) showed that electron beam irradiation with doses of 1.0 or 1.8 kGy produce undetectable levels of *Campylobacter* in poultry samples. Irradiation is very effective in combination with modified packaging interventions. Testing has been done, combining irradiation with modified atmospheric packaging where a 3 log CFU/g reduction can be achieved and prolonged storage does not have an effect in outgrowth of surviving bacteria (Kudra et al., 2012).

High pressure applications to inactivate *Campylobacter* are being tested and have the potential to become an option for specific products like ground poultry. Inactivation using high pressure is mostly used to extend the shelf life of juices, ready to eat meats, ground beef products, and guacamole (Liu et al., 2012). High pressure has become an alternative to temperature pasteurization because it can maintain the freshness required for minimally processed products while extending shelf life without thermal treatments (Bièche et al., 2012). Recent studies aim to find optimal applications to poultry products to reduce spoilage microorganisms to extend shelf life and reduce human pathogens. *Campylobacter* is sensitive to high pressure treatments and adequate high-pressure treatments in combination with time and temperature achieve significant cell count reductions. Studies find that 400 MPa is optimal for *Campylobacter* inactivation, but that is dependent on strain, food matrix, and growth phase (Bièche et al., 2010). Liu et al.

(2012) showed that pressure treatment of 300 MPa for 3 minutes on inoculated poultry meat obtained reductions of 0.5 to 1 log CFU/g. Vegetative bacteria are sensitive to pressures ranging from 300 to 800 MPa (Bièche et al., 2012). Treatments of 400 MPa at 40 °C for 30 minutes reduced cell counts by more than 6 log CFU/g (Liu et al., 2012). Solomon and Hoover (2004) demonstrated pressure ranges between 425-580 MPa were sufficient to inactivate *Campylobacter* in different food mediums including chicken puree. 300 MPa is capable of inactivating *Campylobacter* but its effect is dependent on the amount of time poultry meat is exposed to this pressure. The higher the pressure, the less time it requires to inactivate *Campylobacter* (Jackowska-Tracz & Tracz, 2015). Pressures around 200 MPa are not sufficient to inactivate *Campylobacter* and times in this pressure level may be too long to include as a combination with other microbial intervention and may be impractical in a processing setting (Bechstein et al., 2019; Jackowska-Tracz & Tracz, 2015). It is possible that high pressure treatments may induce protein denaturation and may change organoleptic properties in poultry depending on the pressure amount, product temperature, and exposure time. The pressure may need to be adjusted depending on the type of final product desired (Bièche et al., 2012; Liu et al., 2012).

#### Food Safety Assessment Methods

Controlling *Campylobacter* requires evaluation of controls throughout the poultry production chain. Several assessments can be performed on a systematic scale by evaluating published data through Systematic Reviews and Meta-Analysis (SR-MA), mapping processes to provide visuals on performance, or utilizing statistical approaches like Monte Carlo distributions to analyze large amounts of data and scenarios. As a result, recommendations can be developed to improve interventions, efficacy, applications, or suggest future research into areas where more information is needed.

SR-MA provides a methodological framework for collecting scientific evidence that offer maximum transparency and help identifying knowledge gaps to optimize intervention decisions which can eventually improve food processing and public health (Dogan et al., 2022). Systematic reviews follow specific steps to ensure that all literature is being reviewed and analyzed. The steps include development of

a review protocol, followed by formulation of the systematic review question, conducting a comprehensive search of the literature to identify primary (original) research studies, selecting studies for inclusion in the systematic review, collecting data from the relevant studies, assessing the risk of bias in the primary studies, synthesizing results from the primary studies, presentation of results, interpreting results and drawing conclusions (Sargeant & O'Connor, 2014). SR-MAs are becoming prevalent in the medical and veterinary sciences (Ahn & Kang, 2018; Sargeant & O'Connor, 2014).

The benefits of SR-MA are that it can be used for a variety of question types, including those related to the evaluation of interventions, evaluations of risk exposures or risk, prevalence, and diagnostic test accuracy (EFSA, 2010). Conducting systematic reviews requires planification and the careful design of the search items to be studied. A SR-MA will include studies of varying qualities so a standardized method should be used to define which studies will be included, how will variability within studies and between studies be evaluated, and how these variations will impact the final analysis (Higgins et al., 2019; Sargeant & O'Connor, 2014).

Several review articles have gathered evidence about the colonization and tracing of *Campylobacter* at the farm and processing levels. Most evidence identifies that *Campylobacter* contamination occurs at the farm and gets introduced into the processing plant environment from the chickens that are processed (Keener et al., 2004). There are several SR-MAs that evaluate different stages where *Campylobacter* contamination occurs and how it changes throughout the supply chain. Several studies analyze the effect that interventions may have in reducing *Campylobacter* and reduce the risk of exposure to consumers. The reviews have identified as major cross contamination factors in transport modules, receiving areas, scalding, feather picking, and visceral pack rupture during evisceration (Berkics et al., 2021; Seliwiorstow, Bare, Berkvens, et al., 2016). Yet the processing plant is also capable of having an overall reduction in *Campylobacter* concentrations in subsequent steps especially by the application of physical and chemical decontamination methods (Dogan et al., 2022; Gichure et al., 2022). Gichure et al. (2022) discussed that physical interventions such as high temperature, vent/cloacal plugs, ultrasound, etc.

can be effective prior to the carcass wash step and are more effective in reducing prevalence. Bucher et al. (2015) evaluated the efficacy of air and immersion chilling and discussed the limited effectiveness in reducing *Campylobacter* prevalence without the use of additional interventions. Chilling time and the antimicrobial used during processing influence the effectiveness of pathogen prevalence or concentration (Leone et al., 2024). Chemical interventions can be more effective at reducing *Campylobacter* concentration in steps where immersion or spray applications can be implemented (Gichure et al., 2022). Dogan (2022) also reported similar patterns in interventions. In addition, Dogan (2019) mapped concentration and prevalence changes throughout the processing plant demonstrating that significant reductions in prevalence and concentrations can be achieved after scalding and chilling processes. These areas may be adequate to implement interventions (Dogan et al., 2022).

There is a growing need for current microbiological baselines in the post-processing interventions so processors can measure performance and compare reference data (Vargas et al., 2023). SR-MA are tools that help map the areas where additional efforts can be implemented to reduce *Campylobacter* contamination. In addition to SR-MA, process mapping studies, or bio-mapping studies, offer a standardized methodology to produce the evidence that help determine the best use of current and potential interventions (Chavez-Velado et al., 2024). SR-MA can offer researchers and poultry processors similar view of where to optimize the use of interventions and identify gaps where new interventions could be developed. These methods are part of the growing field of predictive microbiology and the emerging data can help food manufacturers reduce the risk of foodborne illness (Membré & Lambert, 2008). Predictive microbiology can also help in other aspects of food production such as reducing spoilage and improving quality (Membré & Lambert, 2008). Future work will include streamlining mapping studies and developing data collection systems in real time (Feye et al., 2020). In the meantime, the current methods like bio-mapping studies and SR-MA can offer enough information for decision making despite data that is focused on past processes.

*Campylobacter* bio-maps demonstrate that the processing plant can reduce overall prevalence and overall concentrations for poultry carcasses. The chilling and post-chill phases of the process are shown to achieve the best results, especially when PAA is applied at pre-chill and/or post-chill applications (DeVillena et al., 2022; Kingsbury et al., 2023). Cut-up parts demonstrate an increase in prevalence and concentration, due to cross contamination during the additional processing steps (DeVillena et al., 2022; Kingsbury et al., 2023; Vargas et al., 2023). *Campylobacter* concentrations can remain low if flocks with low concentrations enter the processing establishment. Concentration varies from processing plant to processing plant, therefore bio-mapping exercises must be done to study the influence of the quantity of *Campylobacter* that enters the processing facility, how it persists in the environment, its cross-contamination of negative flocks, and how process control methods explain the effectiveness of the processing establishment in reducing the pathogen (Chavez-Velado et al., 2024; Melero et al., 2012). More studies are needed to observe the effectiveness of a processing establishment's capability in reducing *Campylobacter* prevalence and concentrations. The additional data can help develop better analysis in reducing exposure risk to consumers.

The information generated from SR-MAs or bio-mapping studies can be part of broader risk assessments that aim to find the critical points where public health can be affected by specific pathogens and develop recommendations to improve controls at these points. The USDA-FSIS conducted a risk assessment to estimate the effects of the proposed raw chicken parts and comminuted performance standards on public health (USDA-FSIS, 2015b). The risk assessment estimated that 46% of U.S. chicken processing plants will not meet the new performance standards for parts. The USDA-FSIS risk assessment estimated that a 30% percent reduction in *Campylobacter* illnesses can be achieved if 40% of chicken processing establishments failing the *Campylobacter* performance standards for Not-Ready-To-Eat Comminuted Poultry become compliant. And a 32% reduction in *Campylobacter* illnesses can be achieved if 50% of chicken processing establishments failing the *Campylobacter* parts performance standards become compliant (USDA-FSIS, 2015b). The new and revised performance standards are designed to

achieve the Healthy People 2020 goal of 33% reduction in *Campylobacter* related illnesses attributed to poultry products. Quantitative microbial risk assessments (QMRA) have developed as an important tool to support food safety control and has been promoted by international bodies such as the World Trade Organization., the Codex Alimentarius, the World Health Organization, and the Food and Agricultural Organization (Nauta et al., 2009). Nauta et al. (2009) compared several QMRAs performed in Europe. The studies evaluating the risk associated with *Campylobacter* in the food chain differ in the processing stages and in the applied methodology. Nevertheless, all evaluations provide valuable insight that help in the decision-making process (Berkics et al., 2021).

The different models analyze different parts of the production chain and several types of products including whole carcasses and cut-up parts. The similarities between the QMRAs include that *Campylobacter* is introduced at the farm level and that within-flock prevalence increases significantly in a matter of days (Nauta et al., 2009). The factors that contribute to cross contamination include fecal contamination and transportation. Once *Campylobacter* is introduced at the processing establishment, cross-contamination factors, such as intestinal leakage, contribute to between-flock proliferation (Nauta et al., 2007). Most risk assessments determine that *Campylobacter* reductions are achieved at the processing plant by applying interventions in several critical points in the process (Berkics et al., 2021; Chapman et al., 2016; Nauta et al., 2009). Chemical interventions show promise, but recent evaluations estimate that additional interventions, such as cloacal plugging, may be needed to prevent fecal contaminations from spreading *Campylobacter* between flocks (Dogan et al., 2019). In addition, most QMRAs are focused on the effect of pre-chill processing interventions with a focus on microbial concentrations after post-chill carcasses (Chapman et al., 2016). There is a lack of data about the effects of *Campylobacter* reduction intervention efforts on cut-up parts and comminuted products. Therefore, QMRA evaluation of the exposure to *Campylobacter* from these products is limited.

In the United States, chicken is mostly purchased in parts instead of whole. Ground poultry is mostly diverted to further processed products that are formed and pre-cooked (NCC, 2023). Nevertheless,

there are some ready to cook products that are available creating an additional avenue for exposure to *Campylobacter*. It may be necessary to include cut-up parts and comminuted poultry products that need a cooking step in future QMRAs. The objective of this study is to survey the U.S. chicken processors to observe the status of parts performance standards and to observe what practices are implemented to reduce *Campylobacter* prevalence and meet standards despite the pause in reporting. In addition, this study analyzes current and experimental interventions based on their efficacy in reducing *Campylobacter* prevalence and concentration in cut-up parts and comminuted chicken meat in the United States, thus reducing the risk of exposure to the consumer.

CHAPTER 2  
SURVEY OF U.S. BROILER ESTABLISHMENTS ON *CAMPYLOBACTER* PERFORMANCE  
STANDARDS FOR PARTS<sup>1</sup>

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<sup>1</sup> Rivera, R.E.; Thippareddi, H.; and Singh, M. To be submitted to Poultry Science

### Abstract

*Campylobacter* is a significant foodborne pathogen associated with consumption of poultry and poultry products and a cause of gastrointestinal illness. The U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) has relied on controlling pathogens by developing performance standards aimed at reducing prevalence at the processing operations. FSIS revised the performance standards for *Campylobacter* in 2019 and subsequently discontinued reporting on testing results and categories. A survey of broiler processing establishments was conducted to evaluate *Campylobacter* post-harvest interventions and control strategies for cut-up parts adopted by the U.S. broiler industry. A majority (62%) of processing establishments were monitoring for *Campylobacter* and 74% have an established control program for *Campylobacter* control for chicken parts. In 2021, 49% and 68% of establishments would have met the *Campylobacter* performance standards for chicken parts based on USDA-FSIS and internal establishment sampling, respectively. 38% reported prevalence under 10% in chicken parts based on USDA-FSIS sampling, whereas 60% of establishments reported prevalence in parts of under 10% resulting from internal establishment sampling. As the USDA-FSIS *Campylobacter* performance standard for parts is 7.7%, establishments that reported prevalence below 10% likely met the *Campylobacter* performance standard for parts. *Campylobacter* prevalence was not influenced by processing plant processing capacity. Over 90% of establishments used peroxyacetic acid as an antimicrobial intervention for chicken parts. The poultry processing (chicken) industry invested in *Campylobacter* reduction interventions since the implementation of the *Campylobacter* performance standards for chicken parts.

KEYWORDS: *Campylobacter*, Survey, Performance Standard, Prevalence

## Introduction

*Campylobacter* is an important cause of gastrointestinal zoonotic bacterial infection in the United States. Symptoms associated with *Campylobacter* infection include diarrhea, fever, stomach cramps, nausea, and vomiting (CDC, 2022a). The disease is usually self-limiting and antimicrobial treatment is only indicated in severe cases (Wagenaar et al., 2013). *Campylobacter* illnesses can lead to complications such as irritable bowel syndrome, temporary paralysis, and arthritis (CDC, 2022a). There is also a link between campylobacteriosis and Guillain-Barre syndrome (Williams et al., 2021).

The Foodborne Diseases Active Surveillance Network (FoodNet) indicates that about 20 cases of campylobacteriosis are diagnosed each year for every 100,000 people. Most *Campylobacter* cases go undiagnosed or unreported. The Center for Disease Control and Prevention (CDC) estimates *Campylobacter* infections affect 1.5 million U.S. residents every year (CDC, 2022a). *Campylobacter* is commonly associated with the consumption of poultry products (Berghaus et al., 2013; Ellis-Iversen et al., 2012; Overesch et al., 2020). In 2019, the Interagency Food Safety Analytics Collaboration (IFSAC) reported that over 80% of non-dairy foodborne illnesses were attributed to chicken, other seafood (such as shellfish) and turkey, with *Campylobacter* illnesses most often linked to chicken (IFSAC, 2021). *Campylobacter* illnesses from poultry products alone cost an estimated \$6.9 billion a year in medical costs and loss of quality of life (Scharff, 2020).

Many species of poultry, especially commercial broilers and turkeys carry high levels of *Campylobacter* (*C. jejuni* and *C. coli*) in their gastrointestinal tract as part of their normal microbial flora (Sahin et al., 2015). The *Campylobacter* prevalence can range from 23.6-60.6% of the flock, with concentration ranges from 3.63-5.89 log MPN (Berghaus et al., 2013). *Campylobacter* prevalence ranging from 45-100% and with concentrations between 3-7 log CFU were reported in literature (Alter et al., 2005; Berrang et al., 2007; Potturi-Venkata et al., 2007; Stern et al., 2001). Potential environmental sources that contribute to *Campylobacter* colonization in chickens include contaminated poultry litter, feed, drinking water, rodents, insects, and poor worker hygiene (Golden & Mishra, 2020; Sahin et al., 2015; Wagenaar et

al., 2013). Transportation can also contribute to transfer of *Campylobacter* to the feathers and skin (Sahin et al., 2015; Seliwiorstow, Bare, Berkvens, et al., 2016; Wagenaar et al., 2013). The *Campylobacter* prevalence and concentration on the broilers at the farm had a strong correlation to the *Campylobacter* found at the processing plant (Berghaus et al., 2013). The effectiveness of pre-harvest interventions to reduce *Campylobacter* prevalence and concentrations are unpredictable and reductions are reliant on post-harvest interventions at the processing plant. Processing plants implement interventions at various processing steps to remove *Campylobacter* on chicken carcasses (DeVillena et al., 2022; Hwang & Singer, 2020; Williams et al., 2021).

The U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) develops and monitor food safety regulatory standards for poultry. The USDA-FSIS published the Pathogen Reduction: Hazard Analysis and Critical Control Points rule (PR: HACCP Rule) in 1996. The USDA-FSIS has relied on controlling pathogens by developing performance standards aimed at reducing prevalence at the processing plant (USDA-FSIS, 1996). Since the introduction of the PR: HACCP Rule, USDA-FSIS conducted several baseline surveys to determine *Salmonella* and *Campylobacter* prevalence on broiler carcasses in poultry processing establishments and revised the performance standards. The USDA-FSIS baseline surveys conducted in 1995 and 2019 reported *Campylobacter* prevalence of 88.2% and 18.3% on chicken carcasses (Williams et al., 2021). Despite a reduction in overall *Campylobacter* prevalence, the reported *Campylobacter* illnesses remained steady for several years subsequent to the implementation of HACCP (CDC, 2022b).

The USDA-FSIS announced the *Campylobacter* performance standard for chicken and turkey carcasses in 2010, followed by performance standards for parts and comminuted poultry in 2016 (USDA-FSIS, 2010, 2015a; Williams et al., 2021). Microbiological verification testing results were used to categorize poultry processing establishments in categories based on *Campylobacter* prevalence levels. Subsequently, a modified sampling method using neutralizing buffered peptone water (nBPW) for rinsing of the whole carcasses resulted in a marked reduction in *Campylobacter* recovery in chicken parts using the

direct plating method. Use of the enrichment method (30 mL of nBPW) resulted in a higher prevalence of *Campylobacter* in chicken parts (USDA-FSIS, 2019). The USDA-FSIS revised the *Campylobacter* performance standards and discontinued reporting on testing results and categories until the microbiological verification testing were revalidated (USDA-FSIS, 2019).

The regulatory approach has mainly focused on reducing and/or eliminating foodborne pathogens at the processing establishment level. Currently, *Campylobacter* reduction is heavily addressed through antimicrobial chemical interventions. Poultry processors adjust antimicrobial interventions to meet performance standards (Wideman et al., 2016). Post-chill dip tanks and sprayers have been evaluated for their efficacy as antimicrobial interventions in chicken parts. Immersion of whole chicken or parts was shown to be more effective at reducing *Campylobacter* population compared to spray application resulting from longer contact time and improved surface coverage (Gonzalez et al., 2021; Laranja et al., 2023). Chlorine has long been used as a processing aid in poultry processing, but its effectiveness is very limited. Chlorine's disinfection capacity is highly dependent on chill water pH and the amount of organic matter in chillers (Chen et al., 2020). Chlorine, typically used as sodium hypochlorite, has seen a decrease in use in favor of peroxyacetic acid (PAA; (DeVillena et al., 2022; Kataria et al., 2020). PAA provides a strong oxidizing function that disrupts the permeability of cell membranes and alters protein synthesis (Oyarzabal, 2005). PAA has emerged as a popular choice due to its overall effectiveness in reducing *Campylobacter*. When used at high concentrations, PAA can provide over 2.0 log CFU/mL reduction in *Campylobacter* counts (Vaddu et al., 2021; Wideman et al., 2016). Chen et al. (2014) reported that PAA solutions as low as 700 ppm can lower *Campylobacter* by 1.5 log CFU/g with residual effect during storage without affecting organoleptic qualities. Other approved antimicrobials that are used to a lesser degree are cetylpyridinium chloride (CPC), trisodium phosphate (TSP), acidified sodium chlorite (ASC), and chlorine dioxide (ClO<sub>2</sub>) (Chen et al., 2014; Oyarzabal, 2005; Zhang et al., 2018).

The USDA-FSIS conducted a risk assessment to estimate the effects of the proposed raw chicken parts and comminuted performance standards on public health ((USDA-FSIS, 2015b) and reported that

46% of U.S. chicken processing plants will not meet the new performance standards for parts. Further, a 30% percent reduction in *Campylobacter* illnesses can be achieved if 40% of chicken processing establishments failing the *Campylobacter* performance standards for Not-Ready-To-Eat Comminuted Poultry become compliant and a 32% reduction in *Campylobacter* illnesses can be achieved if 50% of chicken processing establishments failing the *Campylobacter* parts performance standards come compliant (USDA-FSIS, 2015b). The new and revised performance standards are designed to achieve the Healthy People 2020 goal of 33% reduction in *Campylobacter* related illnesses attributed to poultry products.

The objective of the survey of the U.S. chicken processors was to evaluate the status of parts performance standards and identify practices implemented at poultry processing operations to reduce *Campylobacter* prevalence and meet performance standards.

## Materials and Methods

### Survey

A cross-sectional study was conducted between March and April 2022 following the methods described by Hwang and Singer (2020). The survey was conducted targeting key stakeholders in the U.S. broiler industry using a web-based survey software (Qualtrics, Provo, UT). The stakeholders included broiler processing plants dedicated to producing ready-to-cook parts.

The main objective of the survey was to understand *Campylobacter* post-harvest interventions and control strategies for chicken parts adopted by the U.S. broiler industry. The survey was divided into three sections: the first section focused on collecting basic information about the processing facility at which each respondent is currently employed. The food safety managers within the poultry processing establishments were asked to record data on production capacity such as the number of birds slaughtered per week and average live bird weight. Participants were also asked to record data on outputs, such as the average daily volume in pounds of whole carcasses and parts for the 2021 calendar year. Average daily volume ranges were obtained from the USDA-FSIS Public Health Information System (PHIS; FSIS, 2016).

In the second section, respondents were asked to provide information on whole carcasses and parts *Campylobacter* performance standards results and prevalence for the 2021 calendar year. The last section of the survey focused on post-chill mitigation strategies adopted by the establishments. Respondents were asked to record the antimicrobial interventions used and application methods at second processing.

The questionnaire was reviewed by the National Chicken Council and industry food safety professionals. The online survey was distributed through the U.S. Poultry & Egg Association. Expert elicitation was used to gather additional information on *Campylobacter* microbiological verification testing methods. Participation was voluntary and compensation was not provided, and personal or company-specific information was not collected.

### Statistical Analysis

Responses are the equivalent of broiler processing establishments that produce Not-Ready-to-Eat, raw intact, and raw non-intact poultry. Responses were presented as percentages or responses of each category. Chi-squared tests and Fisher's exact tests were used to compare the association of production parameters on *Campylobacter* performance standards results and prevalence. All statistical analyses were performed using JMP software (*JMP® Pro 17*, 2023)

### Results

A total of 62 responses were collected. The 62 processing establishments represent 30% of registered Not-Ready-to-Eat, raw intact, and raw non-intact poultry processing establishments in the U.S. (USDA-FSIS, 2023b). It also represents 30% of total chicken slaughtered per week and average live weight when compared to the National Agricultural Statistical Service (NASS, 2022). A majority of respondents (62%) have established a company testing program for *Campylobacter*. A majority of respondents (74%) indicated that their operation has an established *Campylobacter* control program for cut-up parts. (Table 2.1).

### Campylobacter Performance Standards

USDA-FSIS samples determine establishment *Campylobacter* performance standard by using a 52-week sample window where a whole carcass and/or a 4 lb. of chicken parts sample is collected per week to be tested for *Campylobacter*. The current *Campylobacter* performance standard categories for chicken carcasses and parts is summarized in Table 2.2 (FSIS, 2016). Establishments in category 1 met 50 percent or less of the maximum allowable percent positive during the most recently completed 52-week moving window. Establishments in category 2 met the maximum allowable percent positive but results are greater than 50 percent of the maximum allowable percent positive during the most recently completed 52-week moving window. Establishments in category 3 exceeded the maximum allowable percent positive during the most recently completed 52-week moving window. Establishments that fall in Categories 1 and 2 meet the *Campylobacter* performance standards for whole carcasses and chicken parts.

Survey responses indicate that 40% and 22% of establishments were in Categories 1 and 2, respectively based on USDA-FSIS whole carcass sampling; whereas 60% and 11% of establishments were in Categories 1 and 2 respectively based on internal establishment sampling. For chicken parts, 38% and 11% of establishments were in Categories 1 and 2 respectively based on USDA-FSIS sampling and 56% and 13% based on internal establishment sampling (Table 2.3). 19% of responses did not provide results for USDA-FSIS Carcass Sampling, 25% for establishment carcass sampling, 22% for USDA-FSIS parts sampling, and 24% for establishment parts sampling. Establishments that did not provide whole carcass or chicken parts *Campylobacter* performance standards results were not used for statistical analysis.

Chi-square tests of independence were performed to examine the relation between whole carcass performance standards and chicken parts performance standards responses (Table 2.4 A and B). The relation between whole carcass and chicken parts performance standards category is significant ( $P \leq 0.05$ ) for responses based on USDA-FSIS and internal establishment sampling. Establishments that met the *Campylobacter* performance standards for whole carcasses were likely to meet the *Campylobacter* performance standards for chicken parts.

During the conversion of the whole chicken carcasses into chicken parts (as reported from USDA-FSIS testing), the majority of the Category 1 (92%) and Category 3 (91%) whole carcasses corresponded to the same categories in the chicken parts for *Campylobacter*. However, the majority of Category 2 whole chicken carcasses resulted in chicken parts in Category 3. This may be due to an increase in the potential for cross-contamination of the product during cut-up. Similarly, further processing of whole chicken carcasses into chicken parts from Categories 1 and 3 corresponded to similar Categories as reported from establishment sampling/testing for *Campylobacter*. Chicken carcasses that were in Category 2 resulted in chicken parts in Category 1 when the carcasses and the parts were sampled and tested by the establishment, with a significant proportion of the samples in Category 1 (33%). These changes in categories for whole chicken carcasses and the chicken parts may be due to sampling protocols that may result from differences in the chicken parts from different whole carcasses and potential co-mingling of the product. Further, individual carcasses may have different *Campylobacter* load and further processing (cut up) may result in cross-contamination from chicken parts from other whole carcasses. Regardless of the sample size for the chicken parts (4 lbs.), the chicken parts sampling may not represent the whole carcasses in terms of *Campylobacter* prevalence rates and also highlights the variability in *Campylobacter* prevalence and/or populations on the whole carcasses.

Tables 2.4 C and D examine the relation between the performance category designation based on USDA-FSIS sampling and the likelihood that the performance standard category based on the establishment's internal sampling would yield the same result. Tests between USDA-FSIS sampling and internal establishment sampling indicated that the relation between the *Campylobacter* performance standards results for whole carcasses and chicken parts based on USDA-FSIS sampling and internal establishment sampling was significant ( $P \leq 0.05$ ). Establishments passing the USDA-FSIS performance standard for whole birds and parts (Category 1 and 2) are likely to pass the performance standard using internal results. If the establishment does not pass the USDA-FSIS performance standard for whole birds and parts (Category 3), there is a higher chance that some establishments do not pass the performance

standard using internal establishment results. Yet, most establishments in category 3 under USDA-FSIS performance standards still pass the performance standards using internal establishment results. In this case, an establishment in USDA-FSIS category 3 indicates that it is likely that internal sampling will result in higher *Campylobacter* prevalence. The internal sampling results cannot predict whether the establishment will comply with the USDA-FSIS *Campylobacter* performance standard for whole birds and parts.

*Campylobacter* performance standards categories were analyzed based on the number of birds slaughtered per week, the average live bird weight, average daily volume in pounds (lbs.) of whole birds without giblets (WOG) and/or chicken parts per day. These production characteristics are common to design an adequate processing establishment, therefore they were used to find if establishment capacity and output has an influence over performance standard results. The poultry slaughter capacity (birds slaughtered per week), the average daily volume (lbs. of WOG or parts produced per day) or the live bird weight were not correlated ( $P \geq 0.05$ ) with the *Campylobacter* prevalence on the whole carcasses or the chicken parts as determined by USDA FSIS testing or the internal establishment testing (Tables 2.5, 2.6, 2.7). The establishment slaughter capacity, average daily volumes and the live bird weight were not indicators of *Campylobacter* performance standard results.

#### *Campylobacter* Prevalence

A total of 73% of establishments (n = 45) provided *Campylobacter* prevalence results from USDA-FSIS carcass sampling. 76% (n = 47) of establishments provided results for establishment carcass sampling. 75% (n = 46) of establishments provided results from USDA-FSIS prevalence results for parts. 77% (n = 47) of establishments provided results from establishment sampling for parts (Table 2.8).

A total of 27% of establishments (n = 16) did not provide *Campylobacter* prevalence results from USDA-FSIS carcass sampling. 24% of establishments did not provide results for establishment carcass sampling. 25% (n = 15) of establishments did not provide results from USDA-FSIS prevalence results for parts. 23% (n = 14) of establishments provided results from establishment sampling for parts.

Establishments that did not provide whole carcass or chicken parts *Campylobacter* prevalence results were not used for statistical analysis (Table 2.8).

A majority of establishments reported prevalence in carcasses of under 20% based on USDA-FSIS (50%) and internal establishment sampling (66%). As the USDA-FSIS *Campylobacter* performance standard for whole carcasses is 15.7%, establishments that reported prevalence below 20% are likely to meet the *Campylobacter* performance standard for whole carcasses.

38% reported prevalence under 10% in chicken parts based on USDA-FSIS sampling, whereas 60% of establishments reported prevalence in parts of under 10% resulting from internal establishment sampling. As the USDA-FSIS *Campylobacter* performance standard for parts is 7.7%, establishments that reported prevalences below 10% likely met the *Campylobacter* performance standard for parts.

Chi-square tests of independence were performed to examine the relation between whole carcass prevalence and chicken parts prevalence (Table 2.9 A and B). A correlation ( $P \leq 0.05$ ) between whole carcass and chicken parts prevalence was observed, based on both the USDA-FSIS and internal establishment sampling. *Campylobacter* prevalence on whole carcasses serves as an indicator of *Campylobacter* prevalence for chicken parts. Additionally, a correlation ( $P \leq 0.05$ ) between USDA-FSIS and establishment prevalence was observed, based on both whole carcass and chicken sampling.

Internal establishment testing is performed mostly for verification and correlation purposes. Low prevalence of 0-20% reported by USDA-FSIS likely results in low prevalence during internal establishment testing (100%). However, as prevalence increases beyond 20%, establishments increase in their internal testing above 20% of samples. 24% of establishments where USDA-FSIS reported *Campylobacter* in 21-30% of samples resulted in internal results of up to 40% of samples. This percentage increases to 75-100% of responses for establishments where USDA-FSIS samples result in *Campylobacter* positive samples above 31%. The survey included one response where USDA-FSIS samples resulted in 41-50% positive

samples and the internal sampling was at 10% as an outlier and obtaining more responses from establishments at this prevalence level should provide more information on the testing result trends.

A similar pattern can be observed when comparing USDA-FSIS prevalence for parts and establishment testing results. Higher prevalences of over 10 % of internal establishment samples were reported when USDA-FSIS samples started reaching results of up to 10%. The outlier is a response from a processing establishment reporting USDA-FSIS parts samples ranging between 31-40% resulting in internal results between 1-10%.

These changes in prevalence for whole chicken carcasses and chicken parts may be due to sampling protocols that may result from differences in the chicken parts from different whole carcasses and potential co-mingling of the product. The rinse samples may come from the same carcass and part sample or from different carcasses and parts samples obtained during different sampling events. Furthermore, different methods of plating may be used.

*Campylobacter* prevalence was analyzed based on the number of birds slaughtered per week, the average live bird weight, average daily volume in pounds (lbs.) of whole birds without giblets (WOG) and/or chicken parts per day. These production characteristics are common to design an adequate processing establishment, therefore they were used to find if establishment capacity and output has an influence over prevalence. The poultry slaughter capacity (birds slaughtered per week), the average daily volume (lbs. of WOG or parts produced per day) or the live bird weight were not correlated ( $P \geq 0.05$ ) with the *Campylobacter* prevalence on the whole carcasses or the chicken parts as determined by USDA FSIS testing or the internal establishment testing (Tables 2.10, 2.11, 2.12). The establishment slaughter capacity, average daily volumes and the live bird weight were not indicators of *Campylobacter* prevalence.

### Campylobacter Controls

Respondents were asked to identify all antimicrobials used in the processing operation, including peroxyacetic acid (PAA), chlorine as sodium hypochlorite and measured as free available chlorine, cetylpyridium chloride (CPC), trisodium phosphate (TSP), acidified sodium chlorite (ASC), chlorine dioxide, ozone, and ultraviolet light (UV). The majority (90%) of the establishments used PAA as the main antimicrobial intervention (Table 2.13). PAA is commonly applied as a combination of immersion (dip tanks) and sprays (spray cabinets; 60.7%). Some (20%) of establishments used PAA for whole carcasses and parts as a combination immersion (dip tanks), spray (spray cabinets), and for disinfection of conveyor belts . Some establishments (9%) relied on immersion (dip tanks) only or a combination of immersion and spraying of conveyor belts (7%) and spray application for carcasses and conveyor belts (2%).

Chlorine is being used by 50% of the establishment, but mostly in spray cabinets (30%) or in a combination of spray cabinets and on conveyor belts (18.2%). A few establishments (9%) use chlorine only on belts and fewer (3%) use chlorine in a combination of dip tanks, spray cabinets, and conveyor belts. 3.2% of establishments use CPC and it is applied using spray cabinets. There was no reported use of ASC, TSP, chlorine dioxide, ozone, or UV.

PAA is the most commonly used antimicrobial (90%) in the chicken processing establishments among participants. The relationship between the type of antimicrobial used (PAA or chlorine) and *Campylobacter* prevalence was not observed for whole carcasses or chicken parts based on USDA-FSIS sampling or the internal establishment sampling ( $P \geq 0.05$ ) (data not shown). The responses collected from *Campylobacter* interventions were insufficient to evaluate its effect on prevalence.

### Discussion

This survey was designed to assess the readiness of the poultry processors to address *Campylobacter* performance standards for chicken parts. Responses were collected to evaluate the effect of the *Campylobacter* performance standards for whole carcass on chicken parts results. The overall findings

indicate that most processing establishments monitor *Campylobacter* prevalence in addition to ongoing USDA-FSIS sampling and testing for *Campylobacter* as part of their control program and implemented control measures.

The USDA-FSIS reported that 52% of establishments would meet the chicken parts performance standard upon implementation (USDA-FSIS (2015a)). Survey responses indicated that chicken processing establishments would not have met the USDA-FSIS 2015 estimates of overall processing establishments for passing the *Campylobacter* performance standards for parts for the 2021 calendar year. However, the industry exceeded the risk assessment's estimates when using the internal establishment microbial verification testing. *Campylobacter* prevalence was low, with a majority of responses (60%) indicating *Campylobacter* prevalence up to 10% of chicken parts samples. Results from the *Campylobacter* performance standard and prevalence results trended lower on establishment samples compared to USDA-FSIS sampling.

It is likely for an establishment that meets the whole carcass *Campylobacter* performance standards would meet the performance standard for chicken parts. Similarly, lower *Campylobacter* prevalence in whole carcasses was related to lower prevalence in parts. Production parameters such as number of birds slaughtered per week, average live bird weight, and average daily volume did not influence *Campylobacter* prevalence. Processing establishments were mostly able to meet *Campylobacter* performance standards for whole carcasses and parts regardless of production capacity. The correlation suggests that poultry processing establishments could maintain consistent *Campylobacter* prevalence throughout the production chain through process improvements implemented since the establishment of the *Campylobacter* performance standards.

The differences between USDA-FSIS and internal establishment testing results (prevalence) were discussed with industry food safety experts. The main cause might be that poultry processing establishments may collect samples more frequently than USDA-FSIS as part of their food safety program verification activities. USDA-FSIS collects a sample based on a schedule and the frequency may vary depending on the

size of the establishment. Processing establishments may have a larger sample size allowing for increased precision. *Campylobacter* recovery may be dependent on the microbiological method employed. Some establishments utilize the same protocols as the USDA-FSIS, while others may utilize PCR or different types of enrichment and culture methods. Some processing establishments may have a diagnostic laboratory on site, some may have a corporate laboratory where samples can be shipped or may need to send samples to an accredited off-site laboratory. Shipping can affect *Campylobacter* recovery if there are delivery delays, temperature deviations, or sample contamination in route. Consistency in sampling and detection protocols and correlation between the agency and the establishments are needed to get reliable data.

The survey collected responses to evaluate post-chill interventions in chicken parts. A majority of establishments (90.3%) use PAA as an antimicrobial intervention for *Campylobacter*. In most establishments, PAA (60.7%) is applied as an immersion treatment (dip tanks) or in combination with a spray treatment (spray cabinets). Immersion treatment is more effective in applying antimicrobials because it allows increased coverage and contact time of the antimicrobial (Gonzalez et al., 2021). Survey responses did not establish a relation between *Campylobacter* prevalence and PAA use in chicken parts. PAA use has increased after the implementation of the *Salmonella* parts performance standards in parts and comminuted poultry. Studies have shown that PAA is more effective at reducing *Campylobacter* compared to chlorine (Chen et al., 2014; Laranja et al., 2023; Zhang et al., 2018). Its increased use over chlorine may be due to PAA's effect not being diminished by the organic matter present on tank water and surfaces while chlorine's effectiveness is diminished by the presence of organic matter, thus retaining its effectiveness over time (Zhang et al., 2018).

Survey responses indicated that 50% of establishments use chlorine as an antimicrobial, but its application is mostly by spray (30.3%), or in combination with application on belts (18.2%). The survey could not establish a relationship between *Campylobacter* and chlorine use in chicken parts. CPC use in the establishments was low (3.2%) although it is also effective at reducing *Campylobacter* but additional

equipment is needed to recover and neutralize the antimicrobial due to carryover effects in the process downstream, making its adoption costly (Zhang et al., 2018).

Many of the antimicrobials that are used for meeting the *Campylobacter* performance standards are similar to those used in *Salmonella* control programs and processing establishments (Zhang et al., 2018). All survey responses indicated that there is a *Salmonella* control program in conjunction with the *Campylobacter* control program. Current interventions allow most processing establishments to meet the *Campylobacter* performance standards for chicken parts, but *Campylobacter* specific interventions need to be evaluated separately from *Salmonella* control interventions.

The survey presented several limitations. The survey took longer than the estimated 30 minutes, causing incomplete questionnaires that could not be included in the analysis. A future survey may ask for the total number of samples and number of positive results for the year and per season. This will allow for detailed assessments of interventions, adjustments throughout the year, and evaluations of seasonal effects.

This study presented an overview of the U.S. broiler industry's efforts in reducing *Campylobacter* on whole chicken carcasses and chicken parts. The chicken industry invested in *Campylobacter* reduction interventions since the implementation of the *Campylobacter* performance standards for chicken parts in 2015. *Campylobacter* reduction was achieved through the implementation of antimicrobial interventions, along with monitoring and microbial verification, but did not achieve the goal of USDA-FSIS 2015 estimate of processing establishments meeting the *Campylobacter* performance standard for chicken parts. There is a need for refining the antimicrobial interventions, along with additional microbial verification testing to meet the USDA-FSIS goals. Further, additional interventions and measures may be necessary at preharvest to reduce the *Campylobacter* prevalence and or population on birds arriving at the processing establishment to achieve the USDA-FSIS goal.

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**Table 2.1: Survey Responses on Establishments with an Established *Campylobacter* Control Program**

Preliminary Survey Responses	% Response
<hr/>	
Broiler processing plants (n=63)	
<hr/>	
Plants with established company testing program for <i>Campylobacter</i> in second and further processing alongside USDA-FSIS sampling	62%
Plants with an established <i>Campylobacter</i> control program for parts, ground, and other comminuted poultry	74%
<hr/>	

**Table 2.2: Current and Proposed USDA-FSIS *Campylobacter* Performance Standards**

**(A) *Campylobacter* Performance Standard**

Product	Maximum Acceptable %Positive	Performance Standard
Broiler Carcasses	15.7 %	8/51
Chicken Parts	7.7 %	4/52
Comminuted Chicken	9.6 %	5/52

**(B) *Campylobacter* Performance Standard Category**

**Definitions**

Category	Definition
1	Establishments that have achieved 50 percent or less of the maximum allowable percent positive during the most recently completed 52-week moving window.
2	Establishments that meet the maximum allowable percent positive but have results greater than 50 percent of the maximum allowable percent positive during the most recently completed 52-week moving window.
3	Establishments that have exceeded the maximum allowable percent positive during the most recently completed 52-week moving window.

**Table 2.3: *Campylobacter* Performance Standard Responses**

USDA FSIS Category	USDA-FSIS Carcass Sampling	Establishment Carcass Sampling	USDA-FSIS Parts Sampling	Establishment Parts Sampling
Category 1	40%	60%	38%	56%
Category 2	22%	11%	11%	13%
Category 3	19%	3%	29%	8%
NA	19%	25%	22%	24%

Total, Responses (n=62)

NA = No response

**Table 2.4: Association Between Chicken Carcass *Campylobacter* Performance Standards and Parts Performance Standards**

(A) USDA-FSIS Performance Standard Samples

USDA-FSIS Carcass Performance Standard	USDA-FSIS Parts Performance Standard		
	Category 1	Category 2	Category 3
Category 1	92%	8%	0%
Category 2	8%	33%	58%
Category 3	0%	9%	91%

n= 47,  $\chi^2= 41.485$ , DF= 4, P-Value  $\leq 0.0001$

(B) Establishment Performance Standard Samples

Establishment Carcass Performance Standard	Establishment Parts Performance Standard		
	Category 1	Category 2	Category 3
Category 1	81%	11%	8%
Category 2	33%	67%	0%
Category 3	0%	0%	100%

n= 45,  $\chi^2= 27.765$ , DF= 4, P-Value  $\leq 0.0001$

(C) Carcass *Campylobacter* Performance Standard

USDA-FSIS Carcass Performance Standard	Establishment Carcass Performance Standard		
	Category 1	Category 2	Category 3
Category 1	95%	5%	0%
Category 2	58%	33%	8%
Category 3	75%	17%	8%

n= 46,  $\chi^2=7.474$ , DF= 4, P-Value  $\leq 0.0406$

(D) Parts *Campylobacter* Performance Standard

USDA-FSIS Parts Performance Standard	Establishment Parts Performance Standard		
	Category 1	Category 2	Category 3
Category 1	91%	9%	0%
Category 2	50%	50%	0%
Category 3	56%	17%	28%

n= 46,  $\chi^2= 14.503$ , DF= 4, P-Value  $\leq 0.0058$

**Table 2.5: Association Between the Amount of Birds Slaughtered/Week and *Campylobacter* Performance Standard**

(A) USDA-FSIS Carcass *Campylobacter* Performance Standard

USDA-FSIS Carcass <i>Campylobacter</i> Performance Standard			
Birds Slaughtered/Week	Category 1	Category 2	Category 3
0-250000	60%	40%	0%
25001-500000	67%	33%	0%
500001-750000	43%	29%	29%
750001-1000000	55%	27%	18%
1000001-1250000	31%	25%	44%
1250001-1500000	71%	14%	14%
1500501-1750000	100%	0%	0%

n= 50,  $\chi^2= 9.002$ , DF= 12, P-Value  $\leq 0.7857$

(B) Establishment Carcass *Campylobacter* Performance Standard

Establishment Carcass <i>Campylobacter</i> Performance Standard			
Birds Slaughtered/Week	Category 1	Category 2	Category 3
0-250000	75%	25%	0%
25001-500000	75%	0%	25%
500001-750000	86%	14%	0%
750001-1000000	73%	18%	9%
1000001-1250000	92%	8%	0%
1250001-1500000	71%	29%	0%
1500501-1750000	100%	0%	0%

n= 47,  $\chi^2= 8.921$ , DF= 12, P-Value  $\leq 0.7237$

(C) USDA-FSIS Parts *Campylobacter* Performance Standard

USDA-FSIS Parts <i>Campylobacter</i> Performance Standard			
Birds Slaughtered/Week	Category 1	Category 2	Category 3
0-250000	57%	14%	29%
25001-500000	67%	0%	33%
500001-750000	43%	14%	43%
750001-1000000	44%	33%	22%
1000001-1250000	33%	13%	53%
1250001-1500000	71%	0%	29%
1500501-1750000	100%	0%	0%

n= 49,  $\chi^2= 8.590$ , DF= 12, P-Value  $\leq 0.8307$

**Table 2.5 (Continued): Association Between the Amount of Birds Slaughtered/Week and *Campylobacter* Performance Standard**

(D) Establishment Parts *Campylobacter* Performance Standard

Birds Slaughtered/Week	Establishment Parts <i>Campylobacter</i> Performance Standard		
	Category 1	Category 2	Category 3
0-250000	67%	33%	0%
25001-500000	75%	0%	25%
500001-750000	57%	14%	29%
750001-1000000	56%	33%	11%
1000001-1250000	93%	0%	7%
1250001-1500000	71%	29%	0%
1500501-1750000	100%	0%	0%

n= 48,  $\chi^2= 12.435$ , DF= 12, P-Value  $\leq 0.2407$

**Table 2.6: Association Between Average Live Bird Weight in Pounds (LBS) and *Campylobacter* Performance Standard**

(A) USDA-FSIS Carcass *Campylobacter* Performance Standard

Live Bird Weight	USDA-FSIS Carcass <i>Campylobacter</i> Performance Standard		
	Category 1	Category 2	Category 3
< 5 LBS	45%	18%	36%
5-7 LBS	44%	38%	19%
7-9 LBS	45%	9%	45%
> 9 LBS	60%	40%	0%

n= 48,  $\chi^2= 8.443$ , DF= 6, P-Value  $\leq 0.1938$

(B) Establishment Carcass *Campylobacter* Performance Standard

Live Bird Weight	Establishment Carcass <i>Campylobacter</i> Performance Standard		
	Category 1	Category 2	Category 3
< 5 LBS	80%	20%	0%
5-7 LBS	67%	27%	7%
7-9 LBS	100%	0%	0%
> 9 LBS	80%	10%	10%

n= 45,  $\chi^2= 5.655$ , DF= 6, P-Value  $\leq 0.4716$

(C) USDA-FSIS Parts *Campylobacter* Performance Standard

Live Bird Weight	USDA-FSIS Carcass <i>Campylobacter</i> Performance Standard		
	Category 1	Category 2	Category 3
< 5 LBS	44%	0%	56%
5-7 LBS	47%	20%	33%
7-9 LBS	45%	18%	36%
> 9 LBS	60%	10%	30%

n= 45,  $\chi^2= 3.305$ , DF= 6, P-Value  $\leq 0.8467$

(D) Establishment Parts *Campylobacter* Performance Standard

Live Bird Weight	Establishment Carcass <i>Campylobacter</i> Performance Standard		
	Category 1	Category 2	Category 3
< 5 LBS	80%	20%	0%
5-7 LBS	71%	14%	14%
7-9 LBS	70%	10%	20%
> 9 LBS	70%	20%	10%

n= 44,  $\chi^2= 2.464$ , DF= 6, P-Value  $\leq 0.9173$

**Table 2.7: Association Between WOG and Parts Average Daily Volume in Pounds (LBS) and *Campylobacter* Performance Standard**

(A) USDA-FSIS Carcass *Campylobacter* Performance Standard

LBS. WOG/Day	USDA-FSIS Carcass <i>Campylobacter</i> Performance Standard		
	Category 1	Category 2	Category 3
1001-3000 LBS	25%	50%	25%
50001-250000 LBS	31%	31%	38%
250001-600000 LBS	86%	0%	14%
600001-1000000 LBS	43%	29%	29%
>1000000 LBS	33%	33%	33%

n= 37,  $\chi^2= 7.621$ , DF= 8, P-Value  $\leq 0.5140$

(B) Establishment Carcass *Campylobacter* Performance Standard

LBS. WOG/Day	Establishment Carcass <i>Campylobacter</i> Performance Standard		
	Category 1	Category 2	Category 3
1001-3000 LBS	75%	25%	0%
50001-250000 LBS	77%	23%	0%
250001-600000 LBS	100%	0%	0%
600001-1000000 LBS	71%	29%	0%
>1000000 LBS	83%	0%	17%

n= 38,  $\chi^2= 9.400$ , DF= 8, P-Value  $\leq 0.3067$

(C) USDA-FSIS Chicken Parts *Campylobacter* Performance Standard

LBS. Parts/Day	USDA-FSIS Parts <i>Campylobacter</i> Performance Standard		
	Category 1	Category 2	Category 3
6001-50000 LBS	100%	0%	0%
50001-250000 LBS	50%	13%	38%
250001-600000 LBS	50%	17%	33%
600001-1000000 LBS	11%	22%	67%
>1000000 LBS	78%	0%	22%

n= 45,  $\chi^2= 9.762$ , DF= 8, P-Value  $\leq 0.1931$

(D) Establishment Chicken Parts *Campylobacter* Performance Standard

LBS. WOG/Day	Establishment Parts <i>Campylobacter</i> Performance Standard		
	Category 1	Category 2	Category 3
6001-50000 LBS	100%	0%	0%
50001-250000 LBS	75%	13%	13%
250001-600000 LBS	89%	11%	0%
600001-1000000 LBS	33%	33%	33%
>1000000 LBS	75%	13%	13%

n= 45,  $\chi^2= 11.472$ , DF= 8, P-Value  $\leq 0.1058$

**Table 2.8: *Campylobacter* Prevalence Responses**

<b>Campylobacter prevalence (%)</b>	<b>USDA FSIS Carcass Sampling</b>	<b>Establishment Carcass Sampling</b>	<b>USDA FSIS Parts Sampling</b>	<b>Establishment Parts Sampling</b>
0	2%	24%	3%	21%
1-10	32%	40%	35%	39%
11-20	16%	2%	19%	6%
21-30	14%	3%	8%	5%
31-40	2%	2%	2%	2%
41-50	2%	3%	3%	5%
>50	6%	2%	5%	0%
NA	27%	24%	25%	23%

Total, Responses (n=62)

NA = No Response

**Table 2.9: Association Between Chicken Carcass and Parts *Campylobacter* Prevalence**

(A) USDA-FSIS Sampling Prevalence

USDA-FSIS Carcass Prevalence	USDA-FSIS Parts Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
0%	0%	100%	0%	0%	0%	0%	0%
1-10%	11%	89%	0%	0%	0%	0%	0%
11-20%	0%	20%	70%	10%	0%	0%	0%
21-30%	0%	0%	38%	38%	13%	0%	13%
31-40%	0%	0%	0%	0%	0%	0%	100%
41-50%	0%	0%	0%	0%	0%	100%	0%
>50%	0%	25%	25%	0%	0%	25%	25%

n= 44,  $\chi^2= 87.70$ , DF= 36, P-Value  $\leq 0.0001$

(B) Establishment Sampling Prevalence

Establishment Carcass Prevalence	Establishment Parts Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
0%	80%	20%	0%	0%	0%	0%	0%
1-10%	4%	75%	13%	8%	0%	0%	0%
11-20%	0%	100%	0%	0%	0%	0%	0%
21-30%	0%	0%	0%	50%	50%	0%	0%
31-40%	0%	0%	0%	0%	0%	100%	0%
41-50%	0%	0%	0%	0%	0%	100%	0%
>50%	0%	0%	100%	0%	0%	0%	0%

n= 46,  $\chi^2= 114.724$ , DF= 30, P-Value  $\leq 0.0001$

(C) Carcass Sampling Prevalence

USDA-FSIS Carcass Prevalence	Establishment Carcass Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
0%	100%	0%	0%	0%	0%	0%	0%
1-10%	28%	72%	0%	0%	0%	0%	0%
11-20%	40%	50%	10%	0%	0%	0%	0%
21-30%	22%	56%	0%	11%	11%	0%	0%
31-40%	0%	0%	0%	100%	0%	0%	0%
41-50%	0%	100%	0%	0%	0%	0%	0%
>50%	25%	0%	0%	0%	0%	50%	25%

n= 44,  $\chi^2= 67.307$ , DF= 36, P-Value  $\leq 0.0067$

**Table 2.9 (Continued): Association Between Chicken Carcass and Parts Campylobacter Prevalence**

(D) Parts Sampling Prevalence

USDA-FSIS Parts Prevalence	Establishment Parts Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
0%	100%	0%	0%	0%	0%	0%	0%
1-10%	19%	71%	5%	0%	0%	5%	0%
11-20%	42%	25%	17%	17%	0%	0%	0%
21-30%	20%	60%	0%	20%	0%	0%	0%
31-40%	0%	100%	0%	0%	0%	0%	0%
41-50%	0%	50%	0%	0%	0%	50%	0%
>50%	33%	0%	0%	0%	33%	33%	0%

n= 45,  $\chi^2= 42.235$ , DF= 30, P-Value  $\leq 0.0317$

**Table 2.10: Association Between the Amount of Birds Slaughtered/Week and *Campylobacter* Prevalence**

(A) USDA-FSIS Carcass *Campylobacter* Prevalence

Birds Slaughtered/Week	USDA-FSIS Carcass <i>Campylobacter</i> Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
0-250000	0%	100%	0%	0%	0%	0%	0%
25001-500000	0%	67%	33%	0%	0%	0%	0%
500001-750000	0%	29%	14%	43%	0%	0%	14%
750001-1000000	0%	55%	9%	18%	9%	0%	9%
1000001-1250000	0%	15%	54%	15%	0%	8%	8%
1250001-1500000	0%	67%	0%	17%	0%	0%	17%
1500501-1750000	50%	0%	0%	50%	0%	0%	0%

n= 46,  $\chi^2= 51.516$ , DF= 36, P-Value  $\leq 0.1084$

(B) Establishment Carcass *Campylobacter* Prevalence

Birds Slaughtered/Week	Establishment Carcass <i>Campylobacter</i> Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
0-250000	0%	100%	0%	0%	0%	0%	0%
25001-500000	50%	25%	25%	0%	0%	0%	0%
500001-750000	14%	71%	0%	0%	0%	0%	14%
750001-1000000	27%	55%	0%	9%	0%	9%	0%
1000001-1250000	46%	54%	0%	0%	0%	0%	0%
1250001-1500000	33%	33%	0%	0%	17%	17%	0%
1500501-1750000	50%	0%	0%	50%	0%	0%	0%

n= 47,  $\chi^2= 46.911$ , DF= 36, P-Value  $\leq 0.1621$

(C) USDA-FSIS Parts *Campylobacter* Prevalence

Birds Slaughtered/Week	USDA-FSIS Parts <i>Campylobacter</i> Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
0-250000	0%	83%	17%	0%	0%	0%	0%
25001-500000	0%	75%	0%	25%	0%	0%	0%
500001-750000	0%	29%	57%	14%	0%	0%	0%
750001-1000000	10%	50%	20%	0%	10%	0%	10%
1000001-1250000	8%	17%	42%	17%	0%	8%	8%
1250001-1500000	0%	67%	0%	0%	0%	17%	17%
1500501-1750000	0%	50%	0%	50%	0%	0%	0%

n= 47,  $\chi^2= 31.972$ , DF= 36, P-Value  $\leq 0.3545$

**Table 2.10 (Continued): Association Between the Amount of Birds Slaughtered/Week and *Campylobacter* Prevalence**

(D) Establishment Parts *Campylobacter* Prevalence

Birds Slaughtered/Week	Establishment Parts <i>Campylobacter</i> Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
0-250000	0%	100%	0%	0%	0%	0%	0%
25001-500000	25%	75%	0%	0%	0%	0%	0%
500001-750000	14%	43%	14%	29%	0%	0%	0%
750001-1000000	30%	40%	10%	0%	10%	10%	0%
1000001-1250000	46%	38%	15%	0%	0%	0%	0%
1250001-1500000	33%	33%	0%	0%	0%	33%	0%
1500501-1750000	0%	50%	0%	50%	0%	0%	0%

n= 47,  $\chi^2= 46.911$ , DF= 36, P-Value  $\leq 0.1621$

**Table 2.11: Association Between Average Live Bird Weight in Pounds (LBS) and *Campylobacter* Prevalence**

(A) USDA-FSIS Carcass *Campylobacter* Prevalence

Live Bird Weight	USDA-FSIS Chicken Carcass <i>Campylobacter</i> Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
< 5 LBS	0%	50%	20%	20%	0%	0%	10%
5-7 LBS	0%	29%	18%	29%	6%	6%	12%
7-9 LBS	10%	30%	30%	20%	0%	0%	10%
> 9 LBS	0%	75%	25%	0%	0%	0%	0%

n= 45,  $\chi^2= 13.720$ , DF= 18, P-Value  $\leq 0.8226$

(B) Establishment Carcass *Campylobacter* Prevalence

Live Bird Weight	Establishment Carcass <i>Campylobacter</i> Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
< 5 LBS	36%	45%	0%	0%	9%	9%	0%
5-7 LBS	31%	44%	0%	13%	0%	6%	6%
7-9 LBS	40%	60%	0%	0%	0%	0%	0%
> 9 LBS	22%	67%	11%	0%	0%	0%	0%

n= 46,  $\chi^2= 15.761$ , DF= 18, P-Value  $\leq 0.9016$

(C) USDA-FSIS Parts *Campylobacter* Prevalence

Live Bird Weight	USDA-FSIS Parts <i>Campylobacter</i> Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
< 5 LBS	11%	56%	11%	11%	0%	0%	11%
5-7 LBS	0%	25%	38%	13%	6%	13%	6%
7-9 LBS	0%	50%	30%	10%	0%	0%	10%
> 9 LBS	11%	67%	11%	11%	0%	0%	0%

n= 44,  $\chi^2= 14.287$ , DF= 18, P-Value  $\leq 0.7482$

(D) Establishment Parts *Campylobacter* Prevalence

Live Bird Weight	Establishment Parts <i>Campylobacter</i> Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
< 5 LBS	36%	36%	9%	0%	0%	18%	0%
5-7 LBS	33%	33%	7%	13%	7%	7%	0%
7-9 LBS	20%	50%	20%	10%	0%	0%	0%
> 9 LBS	22%	78%	0%	0%	0%	0%	0%

n= 45,  $\chi^2= 13.667$ , DF= 15, P-Value  $\leq 0.6984$

**Table 2.12: Association Between WOG and Parts Average Daily Volume in Pounds (LBS) and *Campylobacter* Prevalence**

(A) USDA-FSIS *Campylobacter* Sampling Prevalence

LBS. WOG/Day	USDA-FSIS <i>Campylobacter</i> Sampling Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
1001-3000 LBS	0%	50%	0%	50%	0%	0%	0%
50001-250000 LBS	0%	15%	31%	38%	0%	5%	15%
250001-600000 LBS	0%	83%	0%	0%	0%	0%	17%
600001-1000000 LBS	0%	43%	14%	29%	0%	14%	0%
>1000000 LBS	0%	33%	50%	0%	17%	0%	0%

n= 36,  $\chi^2= 26.842$ , DF= 20, P-Value  $\leq 0.0810$

(B) Establishment *Campylobacter* Sampling Prevalence

LBS. WOG/Day	Establishment <i>Campylobacter</i> Sampling Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
1001-3000 LBS	0%	75%	0%	0%	25%	0%	0%
50001-250000 LBS	46%	38%	0%	8%	0%	0%	8%
250001-600000 LBS	63%	25%	0%	0%	0%	13%	0%
600001-1000000 LBS	14%	86%	0%	0%	0%	0%	0%
>1000000 LBS	20%	60%	0%	20%	0%	0%	0%

n= 37,  $\chi^2= 25.216$ , DF= 20, P-Value  $\leq 0.1438$

(C) USDA-FSIS Chicken Parts *Campylobacter* Sampling Prevalence

LBS. Parts/Day	USDA-FSIS Parts <i>Campylobacter</i> Sampling Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
6001-50000 LBS	0%	0%	100%	0%	0%	0%	0%
50001-250000 LBS	0%	30%	30%	40%	0%	0%	0%
250001-600000 LBS	0%	63%	19%	6%	0%	6%	6%
600001-1000000 LBS	0%	33%	33%	0%	11%	0%	22%
>1000000 LBS	13%	63%	25%	0%	0%	0%	0%

n= 44,  $\chi^2= 29.357$ , DF= 24, P-Value  $\leq 0.1489$

(D) Establishment Chicken Parts *Campylobacter* Sampling Prevalence

LBS. WOG/Day	Establishment Parts <i>Campylobacter</i> Sampling Prevalence						
	0%	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
6001-50000 LBS	50%	50%	0%	0%	0%	0%	0%
50001-250000 LBS	20%	50%	10%	20%	0%	0%	0%
250001-600000 LBS	35%	53%	6%	0%	0%	6%	0%
600001-1000000 LBS	22%	44%	0%	11%	11%	11%	0%
>1000000 LBS	0%	71%	29%	0%	0%	0%	0%

n= 45,  $\chi^2= 18.457$ , DF= 20, P-Value  $\leq 0.4777$

**Table 2.13: *Campylobacter* Interventions for Post-chill Cut-up Parts**

Intervention	% of Use (n= 62)
Peroxyacetic Acid	90.3
<b>Application Method</b>	
Dip Tank only	8.9
Dip Tank and Spray Cabinet	60.7
Dip Tank and Belts	7.1
Spray Cabinets and Belts	1.8
Dip Tank, Spray Cabinets, and Belts	19.6
<b>Intervention</b>	
Chlorine (sodium hypochlorite)- Measured as Free Available Chlorine	50
<b>Application Method</b>	
Spray Cabinet only	30.3
Belts only	9
Spray Cabinets and Belts	18.2
Dip Tank, Spray Cabinets, and Belts	3
<b>Intervention</b>	
Cetylpyridinium chloride (CPC)	3.2
<b>Application Method</b>	
Spray Cabinet only	100

CHAPTER 3  
EXPOSURE ASSESSMENT MODEL OF PREVALENCE OF *CAMPYLOBACTER* IN U.S.  
PROCESSING PLANTS<sup>2</sup>

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<sup>2</sup> Rivera, R.E.; Wang, J.; Mishra, A.; Thippareddi, H.; and Singh, M. To be submitted to Poultry Science

## Abstract

Poultry processing plants establish controls to reduce *Campylobacter* prevalence to reduce the incidence of gastrointestinal disease caused by this zoonotic pathogen. Quantitative microbial risk assessments (QMRA) analyze changes in *Campylobacter* prevalence in poultry processing and effectiveness of available controls in U.S. poultry processing plants through exposure assessments. Exposure assessments of *Campylobacter* prevalence from parts and comminuted poultry products have not been done although parts and comminuted poultry constitute a higher share of the total poultry consumption in the United States. A systematic review and meta-analysis (SR-MA) was performed to develop *Campylobacter* estimates without the use of interventions and to develop estimates on interventions for *Campylobacter* reduction (prevalence) on carcasses, cut-up parts, and comminuted poultry. The initial prevalence (82%) was calculated from literature data. Odds ratios (OR) were calculated to indicate changes in *Campylobacter* prevalence. The scalding stage had an OR of 0.15 and the chilling stage had an OR of 0.32 providing the greatest reduction from initial prevalence ( $P < 0.05$ ). A baseline model representing *Campylobacter* prevalence at various processing stages without interventions was constructed. *Campylobacter* contamination in whole birds was reduced to 18.4%, cut-up parts was 62.5%, and comminuted product was 25.2%. The model was validated using comparisons with prevalence data obtained from U.S. commercial integrators. The whole bird prevalence from integrators was less than 10%, and parts prevalences were as high as 15%. A multi-hurdle intervention scenario reduced prevalence in whole birds (2.14%), cut-up parts (6.5%), and comminuted (2.62%). Available interventions are effective at reducing prevalence in chicken parts and comminuted product and reduces the variability between parts categories. *Campylobacter* prevalence in parts can help to estimate the exposure spread.

Keywords: *Campylobacter*, QMRA, Poultry parts, Comminuted poultry, Interventions

## Introduction

*Campylobacter* is an important cause of gastrointestinal zoonotic bacterial infection in the United States. Symptoms associated with *Campylobacter* infection include diarrhea, fever, stomach cramps, nausea, vomiting, and death, in severe cases. (CDC, 2022a). The Foodborne Diseases Active Surveillance Network (FoodNet) indicates that about 20 cases of campylobacteriosis are diagnosed each year for every 100,000 people. The Center for Disease Control and Prevention (CDC) estimates *Campylobacter* infections affect 1.5 million U.S. residents every year (CDC, 2022a). *Campylobacter* is commonly associated with the consumption of poultry products (Berghaus et al., 2013; Ellis-Iversen et al., 2012; Overesch et al., 2020). In 2019, the Interagency Food Safety Analytics Collaboration (IFSAC) reported that over 80% of non-dairy foodborne illnesses were attributed to chicken, other seafood (such as shellfish) and turkey, with *Campylobacter* illnesses most often linked to chicken (IFSAC, 2021).

The U.S. Department of Agriculture, Food Safety, and Inspection Service (USDA-FSIS) develops and monitors food safety regulatory standards for poultry. The USDA-FSIS published the Pathogen Reduction; Hazard Analysis and Critical Control Points rule (PR: HACCP Rule) in 1996. The USDA-FSIS has relied on controlling pathogens by developing performance standards aimed at reducing prevalence at the processing plant (FSIS, 1996). Since the introduction of the PR: HACCP rule, USDA-FSIS conducted several baseline surveys to determine *Salmonella* and *Campylobacter* prevalence on broiler carcasses in poultry processing establishments and revised the performance standards. The USDA-FSIS baseline survey, conducted in 1995, reported a *Campylobacter* prevalence of 88.2% on chicken carcasses were *Campylobacter* positive, and a following survey in 2019 reported a lower prevalence of 18.3% (Williams et al., 2021). Despite a reduction in overall *Campylobacter* prevalence, the reported *Campylobacter* illnesses remained steady for several years after the implementation of HACCP (CDC, 2022b).

*Campylobacter* prevalence has remained consistent since the publication of the latest baseline survey. USDA-FSIS raw chicken carcass sampling dataset shows that 20.94% of samples were positive for *Campylobacter* for the 2022 calendar year, up from 18.8% in 2021 (USDA-FSIS, 2023a). The FSIS raw

chicken parts sampling dataset shows that 16.75% of samples were positive for *Campylobacter* for the 2022 calendar year, up from 15.16% in 2021 (USDA-FSIS, 2023a). The FSIS raw comminuted chicken sampling dataset shows that 5.93% of samples were positive for *Campylobacter* for the 2022 calendar year, down from 6.28% in 2021 (USDA-FSIS, 2023a). Despite a reduction in overall *Campylobacter* prevalence since 1995, the reported *Campylobacter* illness rate has remained unchanged for many years with no significant reduction since the introduction of the PR: HACCP rule (CDC, 2022b).

The regulatory approach has targeted *Salmonella* and *Campylobacter* prevalence reduction at the processing plants through antimicrobial interventions. Poultry processors are constantly adjusting interventions to meet performance standards (Wideman et al., 2016). Chlorine has long been used as a processing aid to control pathogens, but its effectiveness is limited. Disinfection capacity of chlorine is highly dependent on pH of the solution (chiller water) and amount of organic matter in chillers (Chen et al., 2020). Chlorine, typically used as sodium hypochlorite, has seen a decrease in use in favor of peroxyacetic acid PAA (DeVillena et al., 2022; Kataria et al., 2020). PAA provides a strong oxidizing function that disrupts the permeability of cell membranes and alters protein synthesis (Oyarzabal, 2005). PAA has emerged as a popular choice due to its overall effectiveness in reducing *Campylobacter*. Other approved antimicrobials that are used to a lesser degree are cetylpyridinium chloride (CPC), trisodium phosphate (TSP), acidified sodium chlorite (ASC), and chlorine dioxide (ClO<sub>2</sub>) (Chen et al., 2014; Oyarzabal, 2005; Zhang et al., 2018). Periodic evaluations of the effectiveness of interventions are needed to adjust pathogen reduction strategies.

Quantitative microbial risk assessments (QMRA) allow for risk-based evaluations for controlling microbial contamination and is becoming widely used to analyze food supply chains and their intervention strategies for microbial control (Ntakiyisumba et al., 2024). Several QMRAs that characterize *Campylobacter* contamination throughout the farm-to-fork continuum have been published. QMRA model comparison is ineffective as defined and consistent criteria are not used in developing the QMRAs and there is still a need to incorporate assessments from the different production components to characterize risk

(Chapman et al., 2016). Exposure assessments are components within QMRAs that analyze the contribution of food processing to the spread of pathogens to the final consumer. Exposure assessments can be conducted using systematic reviews and meta-analysis to obtain baseline data to construct processing plant models to evaluate process improvements.

The systematic reviews utilize data from published experimental interventions from literature and can be used to develop models that identify where its use is most effective. It is important to observe the differences between poultry processing without interventions versus processing with interventions on all possible finished products to characterize which may present higher exposure risks to the consumer. Recent risk assessments and systematic reviews are limited to using whole carcass *Campylobacter* population data and do not include comparisons of poultry cut-up parts and comminuted poultry (Chapman et al., 2016; Dogan et al., 2022; Dogan et al., 2019; Golden & Mishra, 2020; Keener et al., 2004; Sahin et al., 2015). Cut-up parts and comminuted poultry are the most consumed raw product in the U.S. and interventions must be evaluated and characterized to reduce the exposure risk (NCC, 2023). Comminuted product includes chicken that has been ground, chopped, shredded, or minced. Therefore, *Campylobacter* prevalence analysis in chicken cut-up parts and comminuted product in the U.S. should be collected and incorporated into future exposure assessments for better QMRAs and improved illness risk calculations.

The objective of this study is to estimate the *Campylobacter* prevalence in chicken parts and comminuted product that can potentially reach consumers through an exposure assessment. Prevalence will be obtained by 1) developing baseline *Campylobacter* prevalence in chicken cut-up parts and comminuted product in U.S. processing plants through a systematic review and meta-analysis and 2) estimating the efficacy of processing interventions in reducing *Campylobacter* prevalence in cut-up chicken parts, and comminuted product through simulation modeling.

## Materials and Methods

### Systematic Review and Meta-Analysis

*Campylobacter* prevalence (% positive samples) at each stage of poultry processing was estimated by developing a flow chart depicting the common processing steps from receiving to grinding in the U.S. (Fig. 3.1). *Campylobacter* prevalence at the receiving stage of an establishment was determined as the initial concentration. The chicken processing model includes scalding, feather picking, rehang, evisceration, carcass washing, immersion chilling, parts cut-up, and grinding (comminuted) as standard operations of processing in the U.S. Final *Campylobacter* prevalence data of cut-up parts, or comminuted chicken were collected to evaluate process and intervention efficacy. Additionally, the changes in *Campylobacter* prevalence in each subsequent processing stage up to the grinding stages were estimated.

Each step was evaluated for its effect on *Campylobacter* prevalence with and without interventions. *Campylobacter* prevalence from published literature was extracted for each processing stage to develop a baseline model using trials without reported use of interventions or reported chlorine use. Chlorine was used as a baseline intervention since it has been historically used for pathogen control in poultry processing. It was assumed that commercial processing plant studies without reported use of interventions in control trials were using chlorine at the time of sampling.

*Campylobacter* prevalence is the percentage of positive samples from the total number of samples during a sampling event. Data for risk assessment inputs were obtained through a systematic review of literature and meta-analysis.

### Literature search strategy

A systematic review was adapted from Golden and Mishra (2020), and Sargeant and O'Connor (2014) to address the following research questions:

1. How does *Campylobacter* prevalence on broiler carcasses change at each stage of processing from receiving to chicken parts and comminuted product in the U.S.?
2. What is the efficacy of chemical intervention and processing equipment on reducing *Campylobacter* prevalence and their interactions in the U.S.?

To address this question, the Web of Science ([www.webofknowledge.com](http://www.webofknowledge.com)) and PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) online databases were searched using the following keywords: (“*Campylobacter*” OR “*Campylobacter jejuni*” OR “*C. jejuni*”), AND (“United States” OR “U.S.”) AND (“Poultry” OR “Broiler” OR “Chicken”) AND “Intervention” AND “Processing” AND (“Prevalence” OR “Isolation”). Literature up to January 2023 that reported the data in the U.S. was retrieved. In the absence of geographic description of the study, the location was inferred by the first and corresponding address. Additional studies were identified by searching review articles or other reference lists by hand. Studies included peer-reviewed journal articles only. All references were managed by the EndNote citation manager (Endnote 20, Clarivate Analytics, Philadelphia, PA). Duplicates were removed from EndNote by using the “find duplicates” function or manually.

#### Inclusion criteria

Abstracts were screened to determine eligibility with the following criteria included: 1) English language; 2) peer-reviewed journal articles; 3) primary research studies, excluding reviews; 4) interventions tested at a processing stage; 5) intervention tested on whole carcasses, cut-up parts, or ground chicken product. The prevalence from before-after studies in U.S. commercial broiler processing environments, and interventions tested in pilot plants needs to be reported for including in the meta-analysis. Commercial processing establishment before-after studies in different languages other than English or regions other than the U.S. were excluded from review. Following the initial screening, full-text articles were obtained for the remaining studies and analyzed for inclusion in model assessment. Studies with uncertain eligibility were reviewed and discussed by the authors until a consensus was reached.

Along with the previously mentioned screening criteria, details on the type of intervention used, application method, and necessary data to perform a meta-analysis (i.e., samples size, mean, standard deviation, confidence intervals, standard error of the mean, number of positive samples for prevalence) were evaluated as additional inclusion criteria.

#### Data extraction

Articles deemed eligible were screened and the extracted data were stored on Microsoft Excel Spreadsheets. Quantitative data extracted from screened literature included the number of initial and final *Campylobacter* positive samples and sample size. Qualitative data included the study type, processing step, type of intervention, intervention application method. The data were directly collected if the table is available, whereas the Plot Digitizer tool (Plot Digitizer, 3.1.5, 2024, <https://plotdigitizer.com>) was used to extract the prevalence values from the figures. Positives were further calculated from sample size.

#### Quality assessment of included studies

Quality assessment was often included in systematic reviews and meta-analysis to determine the quality of the evidence presented by each study. However, selection bias could happen since the types of quality scores can affect the interpretation of meta-analysis (Stone et al., 2019), therefore, quality scores for the included studies were not determined.

#### Data Analysis

All data analysis was performed using R version 4.0.1 (R Core Team). Meta-analyses and forest plot generation were conducted using the meta package (Schwarzer, 2007). A baseline model of *Campylobacter* prevalence at each stage of poultry processing establishments in the U.S. was constructed using a generalized linear mixed model. A logit link was used to stabilize the variance for the model. For each included study, prevalence values at each stage of broiler processing were calculated by dividing the sample size by the number of positive samples. The prevalence of *Campylobacter* was first transformed using the logit transformation:

$$\text{logit } p = \ln \left( \frac{p}{1-p} \right)$$

with variance

$$\text{var}(\text{logit } p) = \frac{1}{Np} + \frac{1}{1-Np}$$

where  $p$  is the prevalence of *Campylobacter* reported in a study at a specific processing stage and  $N$  is the sample size of that study. A post-hoc comparison of all the processing stage were performed using multcomp (Hothorn et al., 2016) with Tukey multiple comparison correction.

For studies reporting changes in prevalence with a binary outcome, the number of positive samples and the total sample size were extracted from both the treatment and control groups. Odds ratios (OR) were calculated and used in the meta-analysis as the effect size based on the following formula:

$$OR = \frac{\left( \frac{p_{\text{treatment}}}{1 - p_{\text{treatment}}} \right)}{\left( \frac{p_{\text{control}}}{1 - p_{\text{control}}} \right)}$$

Where  $p_{\text{treatment}}$  and  $p_{\text{control}}$  is the prevalence in the treatment intervention and control group. An  $OR < 1$  indicates a decrease in prevalence, an  $OR = 1$  indicates no change, and an  $OR > 1$  indicates an increase in prevalence (Dogan et al., 2022).

Inverse variance weighting was used to pool the prevalence studies. In the presence of zero-cell counts in either the treatment or control groups, a continuity correction of 0.5 was applied to all affected cells in the  $2 \times 2$  table (Higgins et al., 2019; J. Sweeting et al., 2004).

Random-effects meta-analyses were performed with subgroup analyses of data based on the groups (i.e., interventions and intervention methods). The between-study variance ( $\tau^2$ ) was estimated using the DerSimonian and Laird method (DerSimonian & Kacker, 2007; Schwarzer et al., 2015). The effect of heterogeneity was quantified on a relative scale using the  $I^2$  value with thresholds for interpretation as follows: 0 to 40% heterogeneity might not be important, 30 to 60% moderate heterogeneity, 50 to 90%

substantial heterogeneity, and 75 to 100% considerable heterogeneity (Higgins et al., 2019; Higgins et al., 2003; Schwarzer et al., 2015).

The estimated prevalence and OR in the forest plot is displayed along with a 95% confidence interval (CI) along with the tau-squared ( $\tau^2$ ) variance that describes the variance between the studies (Higgins et al., 2019) and  $I^2$  as the measure of heterogeneity. The value of  $I^2$  up to 40% was considered low, 30–60% was considered moderate, 50–90% was considered substantial, and beyond 75% was considered high (Deek et al., 2019).

#### Test for publication bias due to small-study effects

The limitations of statistical tests to quantify asymmetry in funnel plots and recommended these tests only be conducted when there is enough studies ( $n > 10$ ) and low heterogeneity ( $I^2 < 50\%$ ). Unfortunately, none of the meta-analyses examined in this study met these criteria, so publication bias could not be appropriately assessed.

#### Exposure Assessment

##### Processing plant module overview

The first objective of this module was to estimate *Campylobacter* prevalence levels in cut-up chicken parts and comminuted poultry from a control group that included trials where chlorine or no reported interventions were recorded during the systematic review and meta-analysis (SR-MA). *Campylobacter* prevalence (% positive) was estimated at each processing step from receiving until the cut-up or grinding stages. The second objective was to estimate the efficacy of interventions in improving the processing capabilities in reducing *Campylobacter* prevalence in whole birds, cut-up chicken parts, and comminuted chicken.

### Campylobacter prevalence baseline model

The processing plant model starts with the initial *Campylobacter* prevalence at the receiving stage before slaughter. Initial *Campylobacter* concentrations were obtained from SR-MA results. The processing model includes scalding, feather picking, rehang, evisceration, carcass washing, carcass chilling, parts cut up and grinding processes as standard processing stages in the U.S. The baseline model inputs were obtained by pooling trials where chlorine or no reported interventions were recorded during the SR-MA. The OR derived from the SR-MA were fit as a pert distribution where the 5<sup>th</sup> and 95<sup>th</sup> percentiles were the minimum and maximum value respectively and the observed mean OR its most likely value. Distributions were simulated using Monte Carlo simulation by Latin Hypercube Sampling with 10,000 iterations using @Risk (version 8.4.1 (Build10), Palisade Company LLC, New York, USA). Baseline *Campylobacter* prevalence for chicken cut-up parts, and comminuted chicken were obtained for intervention efficacy analysis.

### Baseline model validation

*Campylobacter* prevalence routine testing data was obtained from receiving, scalding, feather pick, rehang, evisceration, carcass wash, immersion chill, cut-up parts, and comminuted chicken of 33 commercial processing facilities from two U.S. commercial chicken integrators over the period from 2018 to 2024. *Campylobacter* prevalence was measured using various plating methods with a limit of detection (LOD) of 1 CFU/mL. Quantification samples that were below the LOD were deemed negative. *Campylobacter* detection methodology from commercial processing facilities differed because of efforts of finding reliable testing methods that would help match USDA-FSIS testing methods. LOD may be the same between testing methods, but rinse media and laboratory methods may have been different, depending on sampling dates. *Campylobacter* prevalence variation of across whole carcass and parts at various stages as well as individual cut-up parts categories, including boneless skinless breast, bone-in breast, tenders, leg quarters, thighs, wings, cut- wings, and MDM were analyzed using chi-square test in R (R Core Team, 2024). Significance values were set at 0.05.

## Intervention Efficacy Analysis

Single interventions obtained from the SR-MA were evaluated for its ability to improve a processing plant's ability to reduce *Campylobacter* prevalence in cut-up parts and comminuted chicken. The OR derived from the SR-MA were fit as a pert distribution where the 5<sup>th</sup> and 95<sup>th</sup> percentiles were the minimum and maximum value respectively and the observed mean OR its most likely value. A single intervention OR data was used to replace the input of a processing stage (such as replacing immersion chilling with air chilling), or it was added to the baseline model as an additional step in the processing chain (such as adding a post-chill dip tank) to obtain an adjusted *Campylobacter* prevalence estimate for cut-up parts and comminuted chicken. Results from the interventions scenarios were expressed as 1. Mean *Campylobacter* prevalence (%) with its 95% CI and 2. Intervention efficacy calculated using equation:

$$\text{Intervention efficacy} = \frac{\text{Mean Prevalence}_{\text{baseline}} - \text{Mean Prevalence}_{\text{intervention}}}{\text{Mean Prevalence}_{\text{baseline}}} \times 100, \quad \text{where}$$

$\text{Mean Concentration}_{\text{baseline}}$  refers to the mean *Campylobacter* prevalence for either whole birds, cut-up parts or comminuted chicken and  $\text{Mean Prevalence}_{\text{intervention}}$  refers to the mean *Campylobacter* prevalence for the alternative intervention scenario. In addition, scenarios using multiple pre-chill and post-chill intervention scenarios were developed to observe *Campylobacter* prevalence in cut-up parts and comminuted chicken utilizing multiple interventions.

## Results

### Search results

The initial search criteria produced 2,261 studies. After removing duplicates and screening the titles and abstracts, 181 records were retained for full text screening. A total of 72 records were retained for analysis after full text screening. 47 records were excluded due to missing sample number, variation data, and prevalence. A total of 21 commercial plants before/after studies, 2 pilot plants before/after studies, 2 years of USDA-FSIS performance standard samples for whole carcasses, parts, and comminuted chicken,

totaling 25 studies were included for the meta-analysis and systematic review. The overview of the systematic review process is illustrated in Fig 3.2.

### Characteristics of included studies

The characteristics of the included studies in the meta-analysis and risk assessment model are presented in Table 3.1. Nine studies reporting *Campylobacter* prevalence at receiving were used to determine an initial prevalence. The control group consisted of trials using chlorine or that no intervention was reported. Chlorine was used as part of the baseline since this intervention has historically been used for pathogenic bacterial control. It was assumed that commercial processing plant studies without reported use of interventions in control trials were using chlorine at the time of sampling. 20 studies used chlorine or did not report an intervention in its control group. Baseline model of *Campylobacter* prevalence and prevalence change for each processing stage was constructed from the data of control group.

A few studies (11) reported intervention trials other than the control group to control *Campylobacter* prevalence. The interventions identified from literature and included for meta-analysis were acidified sodium chlorite (ASC, n=3), air chill (AC, n=2), cloacal plug (CP, n=2), cetylpyridinium chloride (CPC, n=2), high scalding pH (n=1), peroxyacetic acid (PAA n=4), and trisodium phosphate (TSP n=1). Chemical applications were applied either through an immersion application in a dip tank (n=18) or a spray application (n=4).

### Meta-analysis for *Campylobacter* prevalence per processing stage for control group

The estimated *Campylobacter* prevalence from the control group (no intervention or chlorine) at the various processing stages is presented in Figure 3.3. The control group estimated a  $\tau^2$  value of 3.74 and an  $I^2$  of 99.1%, indicating significant heterogeneity among the studies. *Campylobacter* prevalence at the receiving stage was 84% (95% CI: 61%–95%). After scalding, prevalence significantly decreased to 52% (95% CI: 39%–65%) compared to the receiving stage ( $P < 0.05$ ). However, prevalence increased to 82% (95% CI: 55%–94%) post-picking and remained at 75% (95% CI: 62%–85%) at rehang. The highest

prevalence was observed after evisceration, reaching 90% (95% CI: 89%–91%). Inside and outside carcass washing slightly reduced prevalence to 83% (95% CI: 69%–91%). A significant reduction was observed post-chill, with prevalence decreasing to 53% (95% CI: 32%–73%) compared to evisceration ( $P < 0.05$ ). Among processed products, *Campylobacter* prevalence was 23% (95% CI: 10%–45%) in cut-up parts, 42% (95% CI: 22%–65%) in mechanically separated chicken (MSC), and 4% (95% CI: 2%–10%) in comminuted chicken. Data to obtain prevalence for parts and comminuted poultry from commercial processing facilities was limited. USDA-FSIS datasets of 2016 and 2023 were included as additional evidence of parts and comminuted sampling to add additional data points. The meta-regression model showed that the *Campylobacter* prevalence was significantly reduced at the scalding and carcass chill stage compared to the prevalence at receiving ( $P < 0.05$ ).

#### Meta-analysis for *Campylobacter* prevalence changes per processing stage for control group

Odds ratio was calculated for the comparison of the *Campylobacter* prevalence change from the control group (no intervention or chlorine) for the various processing stages (Figure 3.4). A significant heterogeneity among the studies with a  $\tau^2$  value of 1.24 and an  $I^2$  of 97% from the meta-analysis model on the prevalence change. Seven stages were identified with Scalding having an OR of 0.15 (95% CI: 0.05 to 0.46), Feather Pick being 2.90 (95% CI: 0.61 to 13.85), Rehang being 0.33 (95% CI: 0.05 to 1.99), Evisceration being 3.45 (95%CI: 0.97 to 12.23), Carcass Wash (IOBW) being 0.72 (95% CI: 0.47 to 1.09), Carcass Chill (Immersion Chiller) being 0.32 (95% CI: 0.20 to 0.53), Cut-Up Parts being 2.89 (95% CI: 0.65 to 12.84). Comminuted poultry and MSC were calculated using only the FSIS performance standard samples with an OR of 0.40 (95% CI: 0.32 to 0.50) and 6.28 (95% CI: 0.99 to 39.98) respectively.

Scalding represents the highest prevalence reduction due to a washing effect and some *Campylobacter* inactivation due to temperature. Post-pick represents the highest increase in *Campylobacter* prevalence due to cross contamination from cloacal extrusion, the spread of *Campylobacter* from feather pickers to carcass, and spread from carcass-to-carcass. High heterogeneity existed for the stage ( $I^2 = 97%$ ,  $p < 0.01$ ), indicating inconsistency in the dataset. Additional reductions are represented by rehang ( $I^2 =$

89%,  $p < 0.01$ ), carcass wash ( $I^2 = 60\%$ ,  $p < 0.03$ ), immersion chilling ( $I^2 = 91\%$ ,  $p < 0.01$ ), due to a washing off effect. Subsequent processes for cut-up or grinding represent increases in *Campylobacter* prevalence due to cross contamination by comingling of product. Due to the substantially high heterogeneity across trials, no definitive conclusions can be made about the effectiveness of each processing stage. Additionally, some subgroups in this meta-analysis included only a single study due to limited available literature, which reduces statistical power and increases the potential for bias, limiting the reliability and interpretability of the results. However, these results were still presented in the forest plot as a reference for the reader's interest, highlighting potential research gaps in *Campylobacter* changes during poultry processing.

#### Meta-analysis for interventions against *Campylobacter*

Several pre-chill and post-chill interventions were compared on *Campylobacter* prevalence change (Figure 3.5). Pre-chill interventions against *Campylobacter* included analysis for scalding and feather picking applications, overall prevalence change from interventions (Figure 3.5), and detailed analyses for pre-chill chemical applications (Figure 3.6 and 3.7). A treatment to increase pH was analyzed for the scalding stage. The OR for high pH treatment being 0.02 (95% CI: 0.00 to 0.10). A cloacal plug (CP) intervention was analyzed for the feather picking stage with an OR of 30.27 (95% CI: 1.78 to 515.23). Only one data point was obtained for each intervention. Between-study tests were not obtained.

The reported pre-chill chemical interventions included CPC with an OR of 0.03 (0.00 to 0.69), PAA with an OR of 0.76 (95% CI: 0.48 to 1.21), TSP with an OR of 0.44 (95% CI: 0.10 to 1.97), and ASC with an OR 0.34 (95% CI: 0.24 to 0.49) (Figure 3.6). CPC application showed high heterogeneity ( $I^2 = 90\%$ ,  $p < 0.01$ ), indicating inconsistency in the dataset. PAA had moderate heterogeneity ( $I^2 = 41\%$ ,  $p = 0.13$ ), there are more included studies that result in a more consistent effect, but some of the studies demonstrate noticeable variations in confidence intervals and increases in odds where caution must be taken when evaluating the results as being effective against *Campylobacter*. TSP spray OR being 0.44 (95% CI: 0.10 to 1.97), ASC spray OR being 0.34 (95% CI: 0.24 to 0.49). ASC had 0% heterogeneity ( $I^2 = 0\%$ ,  $p < 0.69$ ).

A 0% heterogeneity in this case may indicate that the studies are measuring the same underlying effect, but that the observed differences are likely due to sampling error.

A breakdown of the pre-chill PAA treatments resulted in PAA being applied as a pre-chill immersion treatment resulting in an increase in prevalence with an OR of 1.56 (95% CI: 0.47 to 5.15). The included study for the PAA as a pre-chill immersion treatment included a manual rehang stage plus a dip treatment prior to entering an air chiller. Possible cross contamination and the sampling procedure did not adequately represent an automated process that is common in a commercial processing plant. It was decided to keep the treatment in the SR-MA for reference for use in future SR-MA. Typically, PAA applied through immersion results in a prevalence decrease Leone et al. (2024). PAA as a pre-chill spray application resulted in a prevalence decrease with an OR of 0.61 (95%CI: 0.42 to 0.90) (Figure 3.7).

Spray application of CPC was the most effective despite high heterogeneity ( $I^2 = 90\%$ ,  $p < 0.01$ ). PAA spray has the most consistent effect with moderate heterogeneity ( $I^2 = 41\%$ ,  $p = 0.19$ ) suggesting consistency in the quality of studies. PAA immersion represents an increase in prevalence but within study variation being a factor for this increase, and additional studies can reduce variability. TSP and ASC reduce prevalence with only one reported trial. Since only one study reported the incorporation of TSP and ASC, more research is needed for their efficacy and consistency.

The identified chiller and post-chill application studies were all chemical immersion interventions (Figs. 3.8 and 3.9). PAA and ASC applications were identified as immersion treatments. PAA as an immersion treatment had an OR of 0.07 (95% CI: 0.01 to 0.49). There was high heterogeneity between studies ( $I^2 = 85\%$ ,  $p < 0.01$ ). ASC as an immersion treatment had an OR of 0.01 (95% CI: 0.00 to 0.03) with one reported study. Post-chill immersion application of ASC was the most effective despite reporting one study. PAA as an immersion treatment is the most studied application but results had high variability between studies. However, PAA provides a reduction in prevalences when used as an immersion treatment.

Air chilling was identified as an alternative chilling stage (Figure 3.8). The effect of air chilling on *Campylobacter* prevalence had an OR of 0.63 (95% CI: 0.33 to 1.20). There was moderate heterogeneity between studies ( $I^2 = 56\%$ ,  $p < 0.08$ ). Each reported study had a different control or “before” sampling location that caused a significant difference in calculating the effect of air chilling. The effect of air chilling represents no change or a decrease in prevalence. The trials had different objectives in their observations and the processing plant settings and locations where the samples were collected were different. However, air chilling represents a modest decrease in *Campylobacter* prevalence.

A breakdown of the chill and post-chill PAA treatments resulted in PAA being applied as a treatment in immersion chillers and as a post-chill immersion treatment representing decreases in prevalence with an OR of 0.25 (95% CI: 0.01 to 12.11) and 0.03 (95% CI: 0.00 to 0.24) respectively. There was high heterogeneity between studies ( $I^2 = 88\%$ ,  $p < 0.01$ ) (Figure 3.9).

## Exposure Assessment

### Baseline model and model validation

The baseline model is defined as a basic commercial chicken processing plant in the U.S. including scalding, feather picking, rehang, evisceration, carcass washing through Inside-Outside Bird Washers (IOBW), immersion chilling, parts cut-up and comminuted with no reported interventions or reported chlorine use from the SR-MA. Grinding, shredding, and mincing equipment is not available in most chicken processing plants, but comminuted product is available in several further processed products (NCC, 2023). Ground products, such as mechanically separated chicken (MSC) often go to further processes that include a lethality step. Therefore, comminuted products are seldom included in raw ready-to-cook pathogen analysis. The input parameters for the baseline processing model are described in (Table 3.2).

The simulation estimated *Campylobacter* prevalence to be 18.41% (95%CI: 3.49% to 33.02%) for whole birds after chill, 62.49% (95%: 9.82% - 100%) for cut-up parts, and 25.21% (95% CI: 3.96% to 44.01%) for comminuted chicken (Figure 3.10). The model output suggests that a processing plant can

reduce *Campylobacter* prevalence for whole birds, but subsequent cut-up and grinding stages result in an increase in prevalence if additional controls are not implemented. The incoming *Campylobacter* prevalence of this model was 82% (95% CI: 70.95% - 91.41%). The model presents typical areas of prevalence increase, such as feather picking 58.03% (95% CI: 15.05-100%), and evisceration 74.50% (95% CI: 15.27-100%). These stages commonly expose chicken meat and skin surfaces to fecal material through leakage of the intestinal content and contact between contaminated and non-contaminated carcasses and surfaces. A reduction is represented at the rehang stage 32% (95% CI: 4.32% - 85.58%). Subsequent steps like carcass wash 55.11% (95%CI: 11-90%), and immersion chill 24.97% (95%CI: 4.68% to 45.88%) ultimately reduce prevalence and correct previous increases.

The model output represents an increase in *Campylobacter* prevalence in cut-up parts. Cross contamination can vary depending on sanitation practices while handling and the comingling of products during cut-up. Grinding and similar processes such as MSC can further increase prevalence because of comingling of products that are positive for *Campylobacter* with products that are negative. The data reported from the SR-MA for parts was highly variable because it compared one reported study analyzing cut-up parts *Campylobacter* prevalence with USDA-FSIS cut-up parts results from the performance standard sampling program. Including USDA-FSIS datasets does not provide an even comparison of plants due to the differences in sample quantities between processing plants. This analysis resulted in a high OR contributing to significantly high prevalence in the model. The quality of the available data does not allow for an accurate estimation of prevalence change from post-chill whole birds to cut-up parts and comminuted poultry.

Validation of the final *Campylobacter* prevalence for cut up parts and comminuted chicken was done by comparing the baseline model with the *Campylobacter* prevalence estimates obtained from data provided by commercial processing plants in the U.S. Two poultry integrators provided available *Campylobacter* sampling results from a total of 33 processing plants to validate the simulation model.

Prevalence data from 2018-2023 was provided. Both integrators reported using PAA for post-chill and cut-up processes.

The *Campylobacter* prevalence bio-map from the commercial processing plants represents *Campylobacter* testing results for receiving, scalding, feather pick, rehang, post-chill whole birds, several cut-up parts categories, and MSC (Figure 3.11). The prevalence at receiving was 98.15%, for scalding 81.48%, feather pick 96.43%, rehang 63.65%, post-chill whole birds 4%, cut up 3.5%, and MSC 60.74%. The rehang stage represented a significant reduction in prevalence compared to the receiving scalding and feather pick stage ( $P < 0.05$ ). The integrators reported that some processing plants implemented interventions between feather pick and rehang, but the type of intervention was not disclosed. There was a significant reduction from the rehang to post-chill whole bird stage ( $P < 0.05$ ). Prevalence does not change for cut-up parts. MSC represents a significant increase. The integrators provided a sample size of 135 and the results are similar to the USDA-FSIS sampling datasets. MSC is used for further processing that undergoes a lethality step. There are few samples due to exploration sampling. MSC is seldom sampled as raw ready-to-cook product and prevalence results cannot be an indicator of exposure to consumers.

The comparison between the baseline simulation model and the integrator bio-map represents similar change patterns between stages (Figure 3.12). The post-chill prevalence for whole birds and parts is lower than the simulation model. The integrators used PAA at several stages in the process whereas the simulation model includes data without interventions. When a multi-intervention simulation scenario is performed, the prevalences for whole birds and parts are similar to the integrator data (Tables 3.4, 3.5). The baseline model is a representation of a processing plant without the use of interventions. The model input distributions are adequate to compare interventions against the baseline.

The integrators provided cut-up parts data that was subsequently categorized and compared to observed differences between categories (Figure 3.13). The cut-up parts samples were obtained after all PAA interventions were applied. Bone-in breast had a significantly higher prevalence than its boneless derivatives (boneless breast, tenders, and fillets) ( $P < 0.05$ ). This may be a result of bone-in breast product

including skin whereas boneless products are usually skinless. Nuggets are boneless breast trim that is used for whole breast nuggets. The prevalence of nuggets is significantly higher ( $P < 0.05$ ) than other boneless breast products. This may be a result of cross contamination due to additional steps, such as placement in storage bins and additional manipulation. *Campylobacter* prevalence can be influenced by additional cut-up steps. The prevalence of wings does not differ from most cut-up parts with the exception of leg quarters and bone-in parts. The prevalence for cutting wings increases may be due to the additional manipulation needed to transfer wings to a wing splitting machine. Yet, the prevalence for drums is significantly lower ( $P < 0.05$ ) than leg quarters and thighs. Thighs may be where *Campylobacter* is present in leg quarters and distribution is not even between drums and thighs.

There are differences in *Campylobacter* prevalence between cut-up parts. It is not clear whether handling, interventions, processing or storage conditions affect prevalence at this stage. The processing conditions at time of sampling were not provided with the data, therefore definitive conclusions of what causes the difference in prevalence distributions cannot be made from the integrator data.

### Scenario analysis

13 different single intervention scenarios and one multiple intervention scenario were obtained from the SR-MA and analyzed for intervention efficacy. The input parameters for the single intervention model are described in (Table 3.3). Pre-chill interventions included: PAA spray at the IOBW, PAA immersion treatment, and several pre-chill spray applications (CPC, PAA, TSP, ASC), including an overall pre-chill spray analysis. Chill interventions included: Immersion chill with PAA and air chill. Post-chill interventions included: post-chill immersion treatments for whole birds with PAA and ASC, including an overall post-chill immersion analysis. An analysis utilizing post-chill immersion with PAA was simulated for cut-up parts. One scenario utilizing multiple interventions using the input distributions at the IOBW (PAA), pre-chill spray (ASC), immersion chill (PAA), post-chill dip (PAA), and post cut-up dip (PAA) was performed to evaluate a multi-hurdle approach.

Tables 3.4, 3.5, and 3.6 demonstrate the intervention effect on whole bird, cut-up parts, and comminuted product prevalence respectively. PAA was the most reported intervention in the SR-MA. PAA as an intervention in IOBW improves *Campylobacter* reduction by 6.90% in whole birds, 5.15% in cut-up parts, and 5.16% for comminuted products. PAA as a pre-chill immersion application was not effective because only one study reported PAA use as a pre-chill immersion treatment and thus a proper assessment cannot be done. The included study utilized sampling methods that did not reflect a common processing plant. The simulation was performed to obtain data on the effect of a study of this kind on final testing. The simulation serves as a reminder to be cautious during data extraction from a SR-MA and researchers must ensure that the data reflects real-world scenarios for models to be accurate. CPC and ASC also resulted in improvements as pre-chill spray interventions. Most treatments can have a process improvement effect when applied as a pre-chill intervention.

Chilling and post-chill applications were analyzed. Process improvements of 60.95% for whole bird, 53.85% for cut-up parts, and 53.87% for comminuted poultry can be achieved when using PAA in immersion chillers. Only one study was included. Variation in PAA use in the chiller is high due to limited data points to analyze. Nevertheless, PAA use in chillers can improve the process. Air chill was ineffective in *Campylobacter* prevalence for whole birds, cut-up parts, and comminuted product. Air chillers provide a modest decrease in prevalence, but they are not an effective method as an alternative stage when compared to immersion chillers.

Overall post-chill immersion applications of whole birds can improve reductions by 95.82% for whole birds, 94.85% for cut-up parts, and 94.84% for comminuted poultry. Post-chill immersion provides the greatest improvements when compared to all other interventions and *Campylobacter* prevalences are like those provided by the commercial integrators. PAA and ASC are also very effective as chemical interventions when applied to whole birds after chill. PAA improves reductions by 86.96% for whole birds, 83.97% for cut-up parts, and 83.93% for comminuted poultry. ASC improves reductions by 98.86% for whole birds, 98.56% for cut-up, and 98.57% for comminuted poultry. Effectiveness may be diminished

when using PAA as an immersion application only for cut-up parts. When using PAA for parts, reductions can be improved by 14.31% for parts, and 14.36% for comminuted poultry.

Only one scenario with multiple interventions was generated. A simulation was performed using PAA in IOBW, ASC pre-chill spray, PAA in chiller, PAA post-chill immersion, PAA post-cut-up. Prevalence reductions of 88.38% for whole birds, 89.6% for cut-up parts, and 89.61% for comminuted product were achieved. The prevalence estimates for whole birds and cut-up parts were like those provided by the commercial companies. *Campylobacter* prevalence for comminuted products were like those obtained through USDA-FSIS sampling. A breakdown of comminuted categories cannot be achieved due to limited data.

## Discussion

### Meta-analysis for *Campylobacter* baseline prevalence per processing stage

The SR-MA identified that prevalence varies per stage, and all stages impact *Campylobacter* prevalence. *Campylobacter* prevalence for whole bird carcasses and cut-up parts are significantly reduced after the carcass chilling stage when immersion chilling is used. The SR-MA demonstrates that the chilling and subsequent stages have the capability of reducing *Campylobacter* prevalence with just water or chlorine. Prevalence was higher for cut-up parts and comminuted poultry because there are instances in the process of comingling of negative and positive products. Any grinding process can also mix negative and positive products. *Campylobacter* prevalence after grinding or production of products such as MSC or MDM is very high. These types of products are seldomly sampled since many of these products tend to go to a further process that includes a lethality step. Therefore, a separate SR-MA for different grinding processes are needed to observe differences in product types. The *Campylobacter* prevalence per stage SR-MA serves as a reference to comparing these results against the simulation model results and data from commercial processing facilities.

The average OR for each stage was also consistent with the variation pattern observed with the *Campylobacter* prevalence per stage. This pattern is similar to other bio-mapping studies from commercial processing plants performed in the U.S. and abroad (Betancourt-Barszcz et al., 2024; Chavez-Velado et al., 2024; DeVillena et al., 2022; Kingsbury et al., 2023; Vargas et al., 2023). The meta-analysis results have a high heterogeneity between studies. The number of studies per stage is very limited, and although it provides prevalence change patterns like commercial processing facilities, more data can provide more confidence in the prevalence changes and improve the reliability of future exposure assessment modules. Additional factors that contribute to variability between studies include, variation in sampling numbers, rinse type and volume, enrichment and culture method, and processing conditions.

The USDA-FSIS data set from samples collected for the performance standards presented limitations when incorporating into the meta-analysis and the subsequent baseline model. The prevalence from the overall number of samples obtained from whole bird rinses was compared to those obtained from cut-up parts rinses. Both sample sets differed in the number of samples and the comparison was done as an overall result instead of an establishment-to-establishment comparison. The same procedure was applied to calculate the OR for comminuted poultry. This may contribute to a lower OR that may indicate that a stronger prevalence reduction is achieved. In addition, the interventions and conditions at each establishment are not recorded. The samples cannot be distinguished based on processing conditions. The reason to use the 2016 dataset was to have a dataset where the parts performance standard was yet to be implemented and buffered peptone water was the rinse solution. An assumption had to be made that many processing establishments had not yet implemented interventions in the cut-up stage of the process. The 2023 dataset was used to include prevalence results when using the neutralizing buffered peptone water. Yet, the prevalence of cut-up parts obtained from the meta-analysis resulted in a significant increase in prevalence from cut-up parts, when compared to whole birds. The overall post-chill prevalence is higher than the parts prevalence because more studies on chiller effect were identified in the SR-MA, and several of the most recent studies show a pattern of lower post-chill whole bird prevalence compared to older

studies where *Campylobacter* prevalence was higher. This is an indication of improving processing capabilities over time. The OR analysis indicates that processing stages contribute to lower *Campylobacter* prevalence in whole birds, yet an increase in prevalence in cut-up parts is possible.

Comminuted product also resulted in lower prevalence than cut-up parts. This may be because interventions may be applied prior to the grinding process, there are less samples that are obtained in these processes, and there is no distinction of what type of product is ground. Skin-on and skinless products can go through these processes and further analysis on the effect of skin-on ground products can provide the differentiation needed for future prevalence models. The type of part and the initial prevalence of these products can influence final prevalence. The OR calculation relied on the USDA-FSIS dataset and thus the same limitations for cut-up parts were present in this analysis. The prevalence for MSC is significantly higher than ground product overall. The characteristics of the type of product that is being ground and the conditions in which these are being processed can provide context to make a better assessment.

#### Meta-analysis for interventions against *Campylobacter*

The identified interventions were analyzed based on their application method and on intervention category. Categorizing interventions presented two main limitations. One limitation is that the few available studies per category cause high between study variability and caution must be taken when analyzing interventions in predictive models. The limitation in number of studies is observable when analyzing increases in pH in the scald, and in the analysis for the effect of cloacal plugs after feather picking. The comparison in the studies demonstrates that these two interventions can be effective at reducing *Campylobacter* prevalence in carcasses, but the studies are very limited to consider these as effective interventions on a larger scale. The analysis for air chiller provides preliminary information on how this stage can serve as a possible intervention scenario in the U.S. There are few available studies in the U.S., nevertheless tests show a modest decrease to no change in *Campylobacter* prevalence. The way the differences were calculated were dependent on sample locations. Some of the intervention trials were done

on by placing them before or after this stage and it is recommended to consider including a pre- and/or post-chill intervention that is effective against *Campylobacter* when an air chiller is in use.

Another limitation is that some of the parameters tested within the studies cannot be included in the assessment. For example, PAA is one of the most studied interventions in the U.S. It is applied either by spray or by immersing products in a dip tank. Variations such as solution concentration, pH, and contact time can affect the efficacy of the antimicrobial intervention in reducing microbial prevalence or concentrations (Vaddu et al., 2021). Neither of those factors were included in this meta-analysis. A separate analysis should be conducted to investigate how these factors influence *Campylobacter* prevalence. Nevertheless, similar results from other meta-analyses were observed (Dogan et al., 2022; Leone et al., 2024; Ntakiyisumba et al., 2024) in this study. Regardless of these limitations, immersion applications perform better than spray applications at *Campylobacter* prevalence reductions. There are differences in the outcomes when it comes to the location where these interventions are applied. In this study, PAA sprays when applied pre-chill (between IOBW and Chiller) performed better than a pre-chill immersion. Only one study was included as pre-chill PAA immersion and there is uncertainty that the sampling reflects a typical processing plant. When PAA was applied as a post-chill immersion treatment, *Campylobacter* prevalence was reduced at a higher rate than spray applications.

The additional intervention categories included CPC, TSP, and ASC. CPC is very effective as an intervention even with the high variability within studies. Only results for CPC as a pre-chill spray application were obtained for this meta-analysis. CPC adoption has been very limited due to the high cost of equipment and management, and regulatory limitations (Cano et al., 2021; Thames & Theradiyil Sukumaran, 2020). ASC and TSP assessment was limited due to the few included studies. Overall, chemical interventions result in prevalence reductions, but more studies can provide a better understanding of what processing parameters influence the results. It is also important to conduct targeted validation studies with each chemical intervention in a commercial processing location that examines the different application parameters that influence the effectiveness of these.

## Exposure assessment

The simulated prevalence bio-map from Figure 3.10 resulted in similar prevalence patterns observed in Figure 3.3. The prevalence of cut-up parts resulted in an increase in *Campylobacter* prevalence. A significant increase in *Campylobacter* prevalence in cut-up parts occurs without interventions or chlorine. There are uncertainties from the limitations in the availability of trials for cut-up parts and comminuted product in literature, from commercial processing plants, and USDA-FSIS. Access to perform controlled trials in U.S. commercial facilities is limited and only internal testing from integrators was available. The available USDA-FSIS dataset does not allow for a one-to-one comparison of whole bird, parts, and comminuted product within one processing establishments. The availability of such data would allow comparisons of overall change in prevalence within one processing plant and study the variability between processing plants. Additional observations on the effect on *Campylobacter* prevalence of reduced intervention levels or chlorine in cut-up parts, and comminuted product are not available because regulatory limitations do not allow for testing without interventions. The SR-MA was sufficient in providing data for the simulation models. Utilizing processing plant data can strengthen the baseline model.

Two poultry integrators provided available *Campylobacter* sampling results to validate the simulation model. Both integrators reported using PAA for post-chill and cut-up processes. The bio-map from pooled data represent similar prevalence patterns per stage. Prevalence was higher in the early processing stages (Receiving to Feather Pick), but similar reduction patterns resulted when product passed through the entire process. The processing plants are capable of significantly reducing *Campylobacter* prevalence from the rehang stage to post-chill whole birds to cut-up parts. The bio-map results are similar to the prevalence obtained from single and multiple intervention scenarios (Tables 3.4, 3.5, and 3.6). This indicates that the input distributions used in the simulation model are adequate and processing plants can use their own data to model their process and obtain estimates reflecting actual prevalence results for their products.

The cut-up parts category comparison showed that there are differences between categories. The available data was insufficient to define causes for those differences. The available data with the exception of MSC

were from parts samples collected after all interventions were applied. The prevalence was mostly at or below 10% and variation may be attributed to specific processing conditions at the time of sampling. The variation in prevalence is an indication that each cut-up part category should be evaluated individually. The cause of *Campylobacter* contamination has to be determined by observing each processing step for that category. Future validation studies utilizing minimal intervention configurations between the post-chill and cut-up stages in commercial processing plants can increase the reliability of the baseline model. Target trials testing different cut-up parts characteristics (skin vs skinless, bone vs. boneless, before vs after an intervention, etc.) will offer details to determine if certain product characteristics present a higher risk of increased prevalence that will translate into a higher probability of exposure to consumers

Comminuted values obtained from the model simulations incorporating PAA were like those obtained from the USDA-FSIS 2023 data set. The baseline *Campylobacter* prevalence from the model may be an accurate estimate of prevalence without interventions. The available data did not allow for a thorough analysis of each comminuted chicken product category. Comminuted products could be analyzed by developing a separate exposure assessment module that incorporates further processing and its effect in reducing prevalence in this type of product.

In general, the model presents a realistic scenario when interventions are not used, and the estimates are a reference on how a typical U.S. processing plant may look like if these conditions were present. Additional testing with a reduced presence of interventions for cut-up parts and comminuted product can help reduce the variability for these product types and strengthen the model. Prevalence can be influenced by conditions during processing that increase the chance of cross-contamination and bacterial outgrowth, and these conditions can be incorporated in future exposure assessments.

#### Intervention efficacy

All interventions identified in the SR-MA were incorporated into the baseline model and analyzed based on the percent change in the *Campylobacter* prevalence in whole birds, cut-up parts, and comminuted

poultry. Most interventions improve a processing plant's ability to reduce *Campylobacter* prevalence. Air chill resulted in a decrease in prevalence, but immersion chill was more effective in reducing prevalence. PAA as pre-chill immersion was ineffective because the extracted data from the SR-MA did not represent a realistic process scenario. These results may not be accurate due to the few studies that prevented a proper analysis of the effect of these interventions. Incorporation of chemical interventions such as spray or immersion are effective at improving the process. Immersion treatments are more effective than spray applications overall. All types of chemical interventions could not be compared as spray versus immersion. PAA had studies where this effect could be evaluated, and the model demonstrates that applying PAA as an immersion treatment has a higher success rate at reducing *Campylobacter* prevalence. These improvements are best if the immersion treatment is applied to post-chill whole birds. The treatment may not be as effective if the treatment is applied only to parts and not in any other stages upstream.

Only whole bird post-chill immersion could reduce *Campylobacter* prevalence to levels like the ones provided by the integrators. Establishing an intervention at this stage allows for the previous stages to remove all other contamination sources and reduce the presence of *Campylobacter* through physical means, like removing viscera and feathers, and washing. Then the post-chill stage can disinfect a finished product that is free from factors that harbor most of the *Campylobacter* cells. Applying an immersion treatment after parts cut up by itself has a minimal effect, but if used as an additional step, it can maintain the improvements from previous processing stages and reduce the effects of cross contamination.

One scenario with multiple interventions was tested. The simulation incorporating a combination of PAA sprays and immersion treatment with pre-chill ASC after the IOBW is a realistic scenario at a common U.S. processing plant. The prevalence results from incorporation of a multiple hurdle approach resembles a typical U.S. processing plant.

In conclusion, the SR-MA provided sufficient data to build a baseline model that presented a possible scenario of *Campylobacter* prevalence in chicken parts and comminuted product from U.S. poultry processing plants without the use of interventions. This simulation model is an option to observe and

analyze data changes overtime and it can be modified by incorporating testing results from integrators and future SR-MA. The simulation model can be used to compare the efficacy of interventions used in different processing plants to assess its ability to reduce *Campylobacter* prevalence. The model can be improved by obtaining more data of prevalence before and after the cut-up process. The model can also be modified to evaluate what contributes to differences between individual cut-up parts and comminuted product categories. Chicken cut-up parts and comminuted product undergoing a multi-hurdle approach to *Campylobacter* control has similar prevalences to whole birds. Cut-up parts are the most consumed product in the U.S. therefore the likelihood of being exposed to *Campylobacter* may come from this product category instead of whole birds. Prevalences can be used to monitor how widespread exposure can be. Processing plants are capable of reducing the likelihood of widespread exposure to *Campylobacter* when interventions are used but are insufficient to completely eliminate *Campylobacter* in poultry. Interventions at subsequent steps may help to reduce exposure.

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**Table 3.1: Characteristics of Included Studies from Systematic Review**

<b>Reference</b>	<b>Study Type</b>	<b>Equipment</b>	<b>Stage</b>	<b>Treatment</b>	<b>Sample Type</b>	<b>Rinsate</b>	<b>Enrichment Broth</b>	<b>Plating</b>
Bailey et al. (2019)	Commercial plant before/after study		Receiving		Fecal/Ceca PBS /Colon		Bolton Broth	Campy-Cefex
Berghaus et al. (2013)	Commercial plant before/after study		Receiving		Carcass Rinse	BPW	Bolton Broth	Campy-Cefex
Berrang and Dickens (2000)	Commercial plant before/after study		Receiving		Carcass Rinse	Distilled Water	PBS	Campy-Cefex
Berrang, Meinersman n, et al.	Commercial plant before/after study		Receiving		Carcass Rinse	Butterfield's Buffer		Campy-Cefex
DeVillena et al. (2022)	Commercial plant before/after study		Receiving		Carcass Rinse	BPW		Tempo
Kotula and Pandya (1995)	Commercial plant before/after study		Receiving		Carcass Rinse	Lactose Broth		Campy-BAP
Mead et al. (1995)	Commercial plant before/after study		Receiving		Neck Skin	MRD	Preston Broth	mCCDA
Potturi-Venkata et al. (2007)	Commercial plant before/after study		Receiving		Fecal/Ceca /Colon		Preston Broth	mCCDA/ Campy-Cefex
Son et al. (2007)	Commercial plant before/after study		Receiving		Carcass Rinse	Sterile Water	Bolton Broth	Campy-Cefex

**Table 3.1 (Continued): Characteristics of Included Studies from Systematic Review**

Reference	Study Type	Equipment	Stage	Treatment	Sample Type	Rinsate	Enrichment Broth	Plating
Berrang and Dickens (2000)	Commercial plant before/after study	Scalder	Scalding	Chlorine	Carcass Rinse	Distilled Water	PBS	Campy-Cefex
Berrang et al. (2003)	Commercial plant before/after study	Scalder	Scalding	None	Carcass Rinse	PBS	PBS	Campy-Cefex
Berrang, Meinersmann, et al. (2011)	Commercial plant before/after study	Scalder	Scalding	None/High pH (Calcium Hydroxide)	Carcass Rinse	Butterfield's Buffer	PBS	Campy-Cefex
Berrang et al. (2019)	Commercial plant before/after study	Feather Picker	Post-Pick	None	Carcass Rinse	nBPW	Bolton Broth	Campy-Cefex
Berrang et al. (2001)	Pilot plant before/after study	Feather Picker	Post-Pick	None/Chlorine	Sponge	PBS		Campy-Cefex
Berrang et al. (2001)	Pilot plant before/after study	Feather Picker	Post-Pick	Cloacal Plug	Sponge	PBS		Campy-Cefex
Berrang and Dickens (2000)	Commercial plant before/after study	Feather Picker	Post-Pick	Chlorine	Carcass Rinse	Distilled Water	PBS	Campy-Cefex
Berrang, Meinersmann, et al. (2011)	Commercial plant before/after study	Dip Tank	Post-Pick	Chlorine	Carcass Rinse	Butterfield's Buffer	PBS	Campy-Cefex
Musgrove et al. (1997)	Commercial plant before/after study	Feather Picker	Post-Pick	None/Cloacal Plug	Carcass Rinse	PBS		Campy-Cefex

**Table 3.1 (Continued): Characteristics of Included Studies from Systematic Review**

Reference	Study Type	Equipment	Stage	Treatment	Sample Type	Rinsate	Enrichment Broth	Plating
Thames et al. (2022)	Commercial plant before/after study	Feather Picker	Post-Pick	PAA	Carcass Rinse	BPW	Bolton Broth	Campy-Cefex
Berghaus et al. (2013)	Commercial plant before/after study	Rehang	Rehang	None	Carcass Rinse	BPW	Bolton Broth	Campy-Cefex
Berrang et al. (2007)	Commercial plant before/after study	Rehang	Rehang	Chlorine	Carcass rinse		PBS	Campy-Cefex
DeVillena et al. (2022)	Commercial plant before/after study	Rehang	Rehang	Chlorine/PAA	Carcass rinse	BPW	BPW	Tempo
Bailey et al. (2019)	Commercial plant before/after study	Before IOBW	Evisceration	None	Carcass rinse	nBPW	Bolton Broth	Campy-Cefex
Berrang and Dickens (2000)	Commercial plant before/after study	Before IOBW	Evisceration	Chlorine	Carcass rinse	Distilled Water	PBS	Campy-Cefex
Cason et al. (1997)	Commercial plant before/after study	Post-Pick/PreChill	Evisceration	None	Carcass Rinse	PBS	Campylobacter Enrichment Broth	Campy-Cefex
DeVillena et al. (2022)	Commercial plant before/after study	Before IOBW	Evisceration	Chlorine	Carcass rinse	BPW	BPW	Tempo
Bailey et al. (2019)	Commercial plant before/after study	Pre-Chill Spray	Carcass Wash	CPC/PAA	Carcass rinse	nBPW	Bolton Broth	Campy-Cefex

**Table 3.1 (Continued): Characteristics of Included Studies from Systematic Review**

Reference	Study Type	Equipment	Stage	Treatment	Sample Type	Rinsate	Enrichment Broth	Plating
Bashor et al. (2004)	Commercial plant before/after study	IOBW	Carcass Wash	None/TSP/ASC	Carcass rinse	Phosphate BPW		CCDA
Berghaus et al. (2013)	Commercial plant before/after study	IOBW	Carcass Wash	None	Carcass rinse	BPW	Bolton Broth	Campy-Cefex
Berrang and Dickens (2000)	Commercial plant before/after study	IOBW	Carcass Wash	Chlorine	Carcass rinse	Distilled Water	PBS	Campy-Cefex
DeVillena et al. (2022)	Commercial plant before/after study	IOBW	Carcass Wash	Chlorine/PAA	Carcass rinse	BPW	BPW	Tempo
Kemp et al. (2001)	Commercial plant before/after study	Pre-Chill Spray	Carcass Wash	None/ASC	Carcass rinse	Butterfield's Buffer	Hunt Broth	Campy-Line/mCCDA
Oyarzabal et al. (2004)	Commercial plant before/after study	IOBW	Carcass Wash	None	Carcass rinse	BPW	Hunt Broth	Campy-Cefex
Thames et al. (2022)	Commercial plant before/after study	Pre-Chill Spray	Carcass Wash	PAA	Carcass rinse	BPW	Bolton Broth	Campy-Cefex
Zhang et al. (2011)	Commercial plant before/after study	Pre-Chill Spray	Carcass Wash	CPC	Carcass rinse	BPW	Bolton Broth	Campy-Cefex
Bailey et al. (2019)	Commercial plant before/after study	Pre-Chill Immersion	Carcass Wash	PAA	Carcass rinse	nBPW	Bolton Broth	Campy-Cefex

**Table 3.1 (Continued): Characteristics of Included Studies from Systematic Review**

Reference	Study Type	Equipment	Stage	Treatment	Sample Type	Rinsate	Enrichment Broth	Plating
Bailey et al. (2019)	Commercial plant before/after study	Immersion Chill	Carcass Chill	PAA	Carcass rinse	nBPW	Bolton Broth	Campy-Cefex
Bashor et al. (2004)	Commercial plant before/after study	Immersion Chill	Carcass Chill	None	Carcass rinse	Phosphate BPW		CCDA
Bauermeister et al. (2008)	Commercial plant before/after study	Post-Chill Immersion	Carcass Chill	Chlorine/ PAA	Carcass rinse	BPW	Bolton Broth	mCCDA
Berghaus et al. (2013)	Commercial plant before/after study	Immersion Chill	Carcass Chill	Chlorine	Carcass rinse	BPW	Bolton Broth	Campy-Cefex
Berrang et al. (2007)	Commercial plant before/after study	Immersion Chill	Carcass Chill	Chlorine	Carcass rinse		PBS	Campy-Cefex
Berrang, Dickens, et al. (2000)	Commercial plant before/after study	Immersion Chill	Carcass Chill	Chlorine	Carcass rinse	Distilled Water	PBS	Campy-Cefex
Cason et al. (1997)	Commercial plant before/after study	Immersion Chill	Carcass Chill	None	Carcass rinse	PBS	PBS	Campy-Cefex
Demirok et al. (2013)	Commercial plant before/after study	Immersion Chill	Carcass Chill	None/Chlorine	Carcass rinse	BPW	Bolton Broth	Campy-Cefex
DeVillena et al. (2022)	Commercial plant before/after study	Post-Chill Immersion	Carcass Chill	Chlorine/PAA	Carcass rinse	BPW		Tempo

**Table 3.1 (Continued): Characteristics of Included Studies from Systematic Review**

Reference	Study Type	Equipment	Stage	Treatment	Sample Type	Rinsate	Enrichment Broth	Plating
Northcutt, Berrang, et al. (2003)	Pilot plant before/after study	Immersion Chill	Carcass Chill	Chlorine	Carcass rinse	PBS	PBS	Campy-Cefex
Oyarzabal et al. (2004)	Commercial plant before/after study	Immersion Chill	Carcass Chill	None/ASC	Carcass rinse	BPW	Hunt Broth	Campy-Cefex/Campy-Line/Karmali/
Son et al. (2007)	Commercial plant before/after study	Immersion Chill	Carcass Chill	None	Carcass rinse	Sterile Water	Bolton Broth	CVA
Stern et al. (2001)	Commercial plant before/after study	Immersion Chill	Carcass Chill	Chlorine	Carcass rinse		CEB	Campy-Cefex
Thames et al. (2022)	Commercial plant before/after study	Post-Chill Immersion	Carcass Chill	PAA	Carcass rinse	BPW	Bolton Broth	Campy-Cefex
Zhang et al. (2011)	Commercial plant before/after study	Immersion Chill	Carcass Chill	None/Chlorine	Carcass rinse	BPW	Bolton Broth	Campy-Cefex
DeVillena et al. (2022)	Commercial plant before/after study	Immersion Tank	Parts	Chlorine/PAA	Wing rinse	BPW		Tempo
Thames et al. (2022)	Commercial plant before/after study	Immersion Tank	Parts	None/PAA	DrumstickBPW rinse		Bolton Broth	Campy-Cefex

**Table 3.1 (Continued): Characteristics of Included Studies from Systematic Review**

<b>Reference</b>	<b>Study Type</b>	<b>Equipment</b>	<b>Stage</b>	<b>Treatment</b>	<b>Sample Type</b>	<b>Rinsate</b>	<b>Enrichment Broth</b>	<b>Plating</b>
USDA-FSIS (2016)	Commercial Plant Performance Standard samples	NA	Ground	NA	Comminuted	BPW		
USDA-FSIS (2016)	Commercial Plant Performance Standard Samples	NA	Parts	NA	legs/breast/wings	BPW		
(USDA-FSIS, 2016)	Commercial Plant Performance Standard Samples	NA	Ground	NA	MSC	BPW		
(USDA-FSIS, 2023a)	Commercial Plant Performance Standard Samples	NA	Ground	NA	Comminuted	nBPW		
(USDA-FSIS, 2023a)	Commercial Plant Performance Standard Samples	NA	Parts	NA	legs/breast/wings	nBPW		
(USDA-FSIS, 2023a)	Commercial Plant Performance Standard Samples	NA	Ground	NA	MSC	nBPW		

**Table 3.2: Input Parameters for Baseline Model Simulation from the SR-MA**

Processing Stage	Concentration Change Distribution	Unit
Receiving (Initial Prevalence)	PertAlt(5%,0.61,0.84,95%,0.95)	%
Scalding	PertAlt(5%,0.05,0.15,95%,0.46)	OR
Feather Picking	PertAlt(5%,0.61,2.90,95%,13.85)	OR
Rehang	PertAlt(5%,0.05,0.33,95%,1.99)	OR
Evisceration	PertAlt(5%,0.97,3.45,95%,12.23)	OR
Carcass Wash	PertAlt(5%,0.47,0.72,95%,1.09)	OR
Carcass Chill	PertAlt(5%,0.20,0.32,95%,0.53)	OR
Cut Up Parts	PertAlt(5%,0.65,2.89,95%,12.84)	OR
Comminuted	PertAlt(5%,0.32,0.40,95%,0.50)	OR

**Table 3.3: Input Distributions of Processing Interventions from the SR-MA Used for Intervention Efficacy Analysis**

Intervention Type	Concentration Change Distribution	Unit
IOBW-PAA	PertAlt(5%,0.37,0.65,95%,1.16)	OR
Pre-Chill Immersion - PAA	PertAlt(5%,0.47,1.56,95%,5.15)	OR
Pre-Chill Spray	PertAlt(5%,0.07,0.20,95%,0.62)	OR
a. CPC	PertAlt(5%,0.00,0.03,95%,0.69)	OR
b. PAA	PertAlt(5%,0.42,0.61,95%,0.90)	OR
c. TSP	PertAlt(5%,0.10,0.44,95%,1.97)	OR
d. ASC	PertAlt(5%,0.24,0.34,95%,0.49)	OR
Air Chill	PertAlt(5%,0.33,0.63,95%,1.20)	OR
Immersion Chill PAA	PertAlt(5%,0.01,0.25,95%,12.11)	OR
Post Chill Immersion - Whole Bird	PertAlt(5%,0.01,0.03,95%,0.12)	OR
a. PAA	PertAlt(5%,0.01,0.07,95%,0.49)	OR
b. ASC	PertAlt(5%,0.00,0.01,95%,0.03)	OR
Post Chill Immersion - Cut-Up Parts	PertAlt(5%,1.07,2.66,95%,6.64)	OR

**Table 3.4: Intervention Efficacy Analysis for Whole Birds**

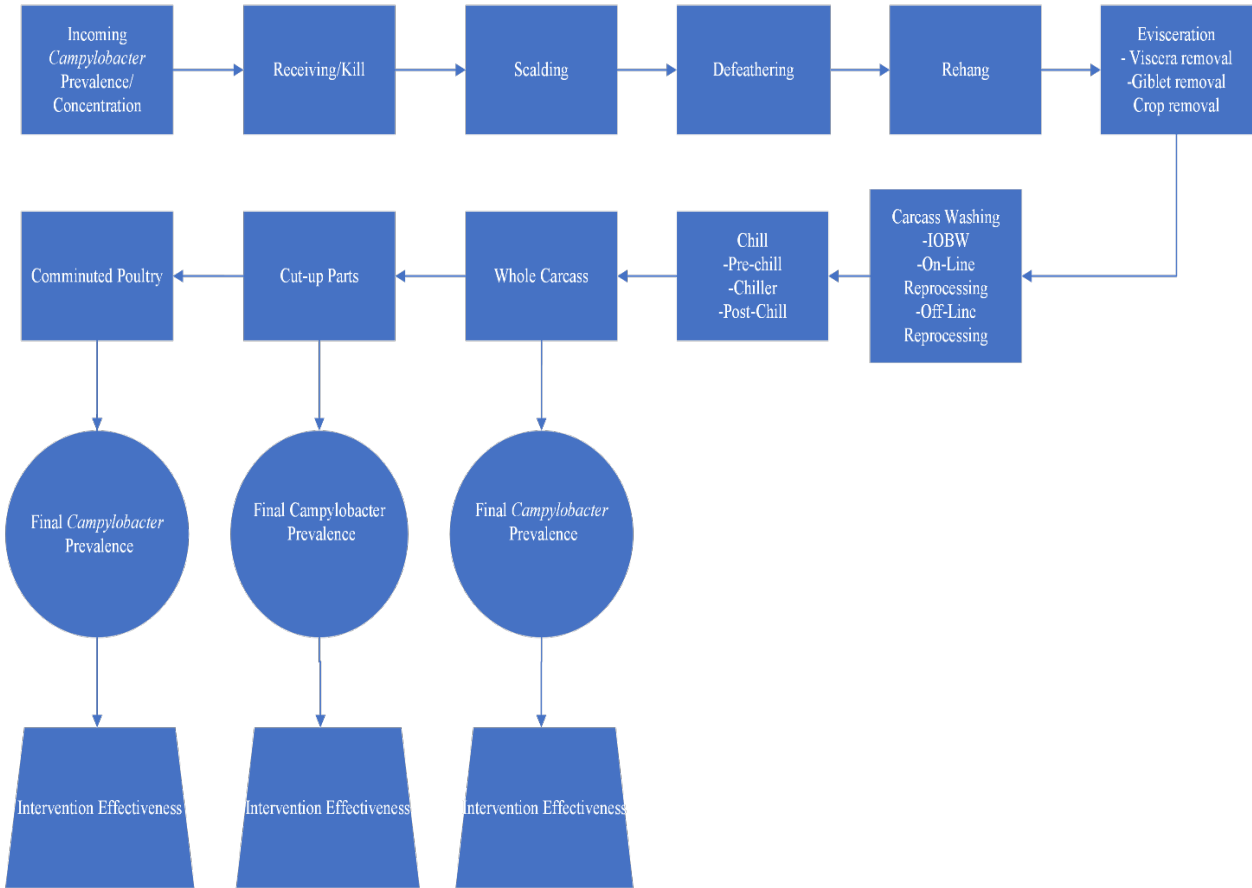
Scenario	Prevalence (%)	Lower 95% CI	Upper 95% CI	Intervention Efficacy (%)
Baseline	18.41	3.49	33.02	-
IOBW-PAA	17.14	3.03	32.01	6.90
Pre-Chill Immersion - PAA	25.59	5.32	42.06	Not Effective
Pre-Chill Spray	4.58	0.7	10.8	75.12
a. CPC	2.49	0.12	7.43	86.47
b. PAA	14.52	2.57	27.89	21.13
c. TSP	11.64	1.57	28.63	36.77
d. ASC	6.42	1.22	11.98	65.13
Air Chill	37.14	6.79	69.66	Not Effective
Immersion Chill PAA	7.19	0.78	18.68	60.95
Post Chill Immersion - Whole Bird	0.77	0.11	1.82	95.82
a. PAA	2.4	0.25	6.47	86.96
b. ASC	0.21	0.02	0.51	98.86
Baseline + IOBW PAA + Pre-Chill ASC Spray + Chiller PAA + Post Chill PAA Dip + Post Cut-Up PAA Dip	2.14	0.05	7.66	88.38

**Table 3.5: Intervention Efficacy Analysis for Cut-up Parts**

Scenario	Prevalence (%)	Lower 95% CI	Upper 95% CI	Intervention Efficacy (%)
Baseline	62.49	9.82	100	-
IOBW-PAA	59.27	9.64	100	5.15
Pre-Chill Immersion - PAA	74.27	15.69	100	Not Effective
Pre-Chill Spray	18.96	2.07	51.66	69.66
a. CPC	10.35	0.38	34.85	83.44
b. PAA	53.23	7.55	100	14.82
c. TSP	42.7	4.49	100	31.67
d. ASC	26.63	3.73	66.06	57.39
Air Chill	81.25	19.54	100	Not Effective
Immersion Chill PAA	28.84	2.29	92.09	53.85
Post Chill Immersion - Whole Bird	3.22	0.32	9.19	94.85
a. PAA	10.02	0.72	31.12	83.97
b. ASC	0.9	0.08	2.66	98.56
Post Chill Immersion - Cut-Up Parts	53.55	8.96	100	14.31
Baseline + IOBW PAA + Pre-Chill ASC Spray + Chiller PAA+ Post Chill PAA Dip + Post Cut-Up PAA Dip	6.5	0.15	24.23	89.60

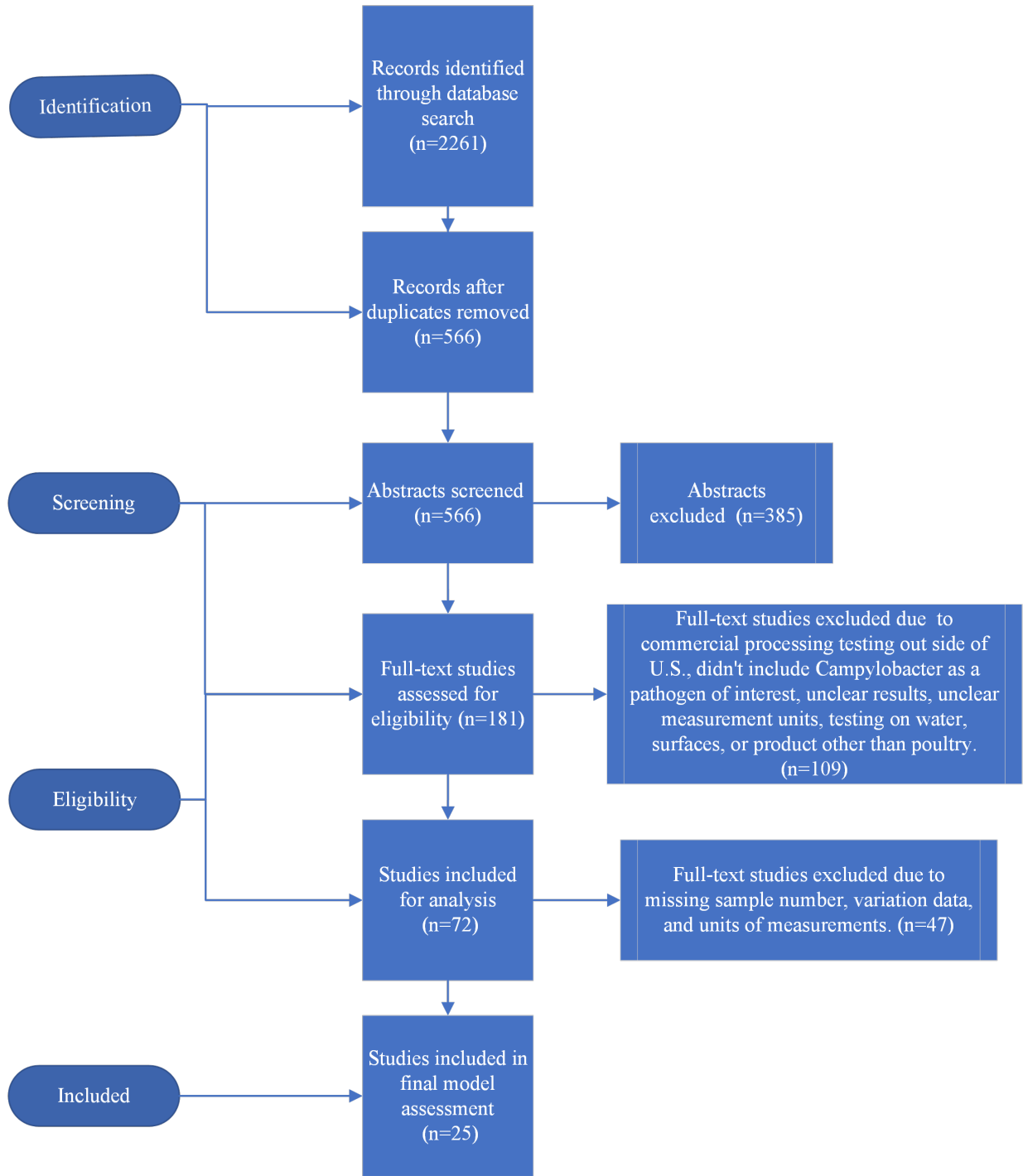
**Table 3.6: Intervention Efficacy Analysis Comminuted Product**

Scenario	Prevalence (%)	Lower 95% CI	Upper 95% CI	Intervention Efficacy (%)
Baseline	25.21	3.96	44.01	-
IOBW-PAA	23.91	3.79	43.61	5.16
Pre-Chill Immersion - PAA	29.96	6.42	44.91	Not Effective
Pre-Chill Spray	7.66	0.85	21.14	69.62
a. CPC	4.17	0.15	14.08	83.46
b. PAA	21.48	3.06	42.78	14.80
c. TSP	17.22	1.8	41.6	31.69
d. ASC	10.75	1.46	26.87	57.36
Air Chill	32.76	7.7	45.26	Not Effective
Immersion Chill PAA	11.63	0.93	36.21	53.87
Post Chill Immersion - Whole Bird	1.3	0.13	3.73	94.84
a. PAA	4.05	0.29	12.51	83.93
b. ASC	0.36	0.03	1.08	98.57
Post Chill Immersion - Cut-Up Parts	21.59	3.62	41.71	14.36
Baseline + IOBW PAA + Pre-Chill ASC Spray + Chiller PAA+ Post Chill PAA Dip + Post Cut-Up PAA Dip	2.62	0.06	9.75	89.61

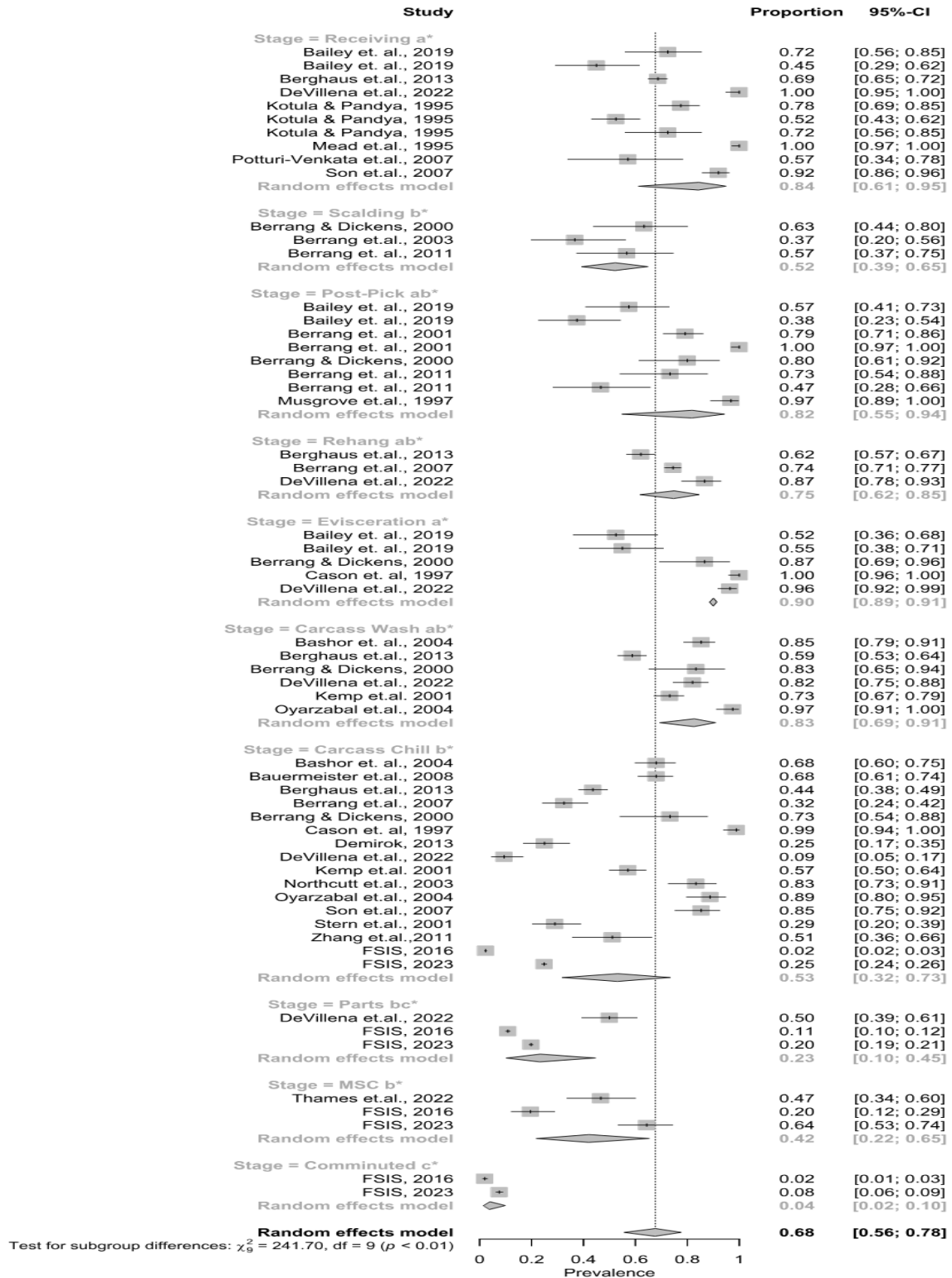


**Figure 3.1:** Flow Diagram of Poultry Processing Stages Used for Exposure Assessment

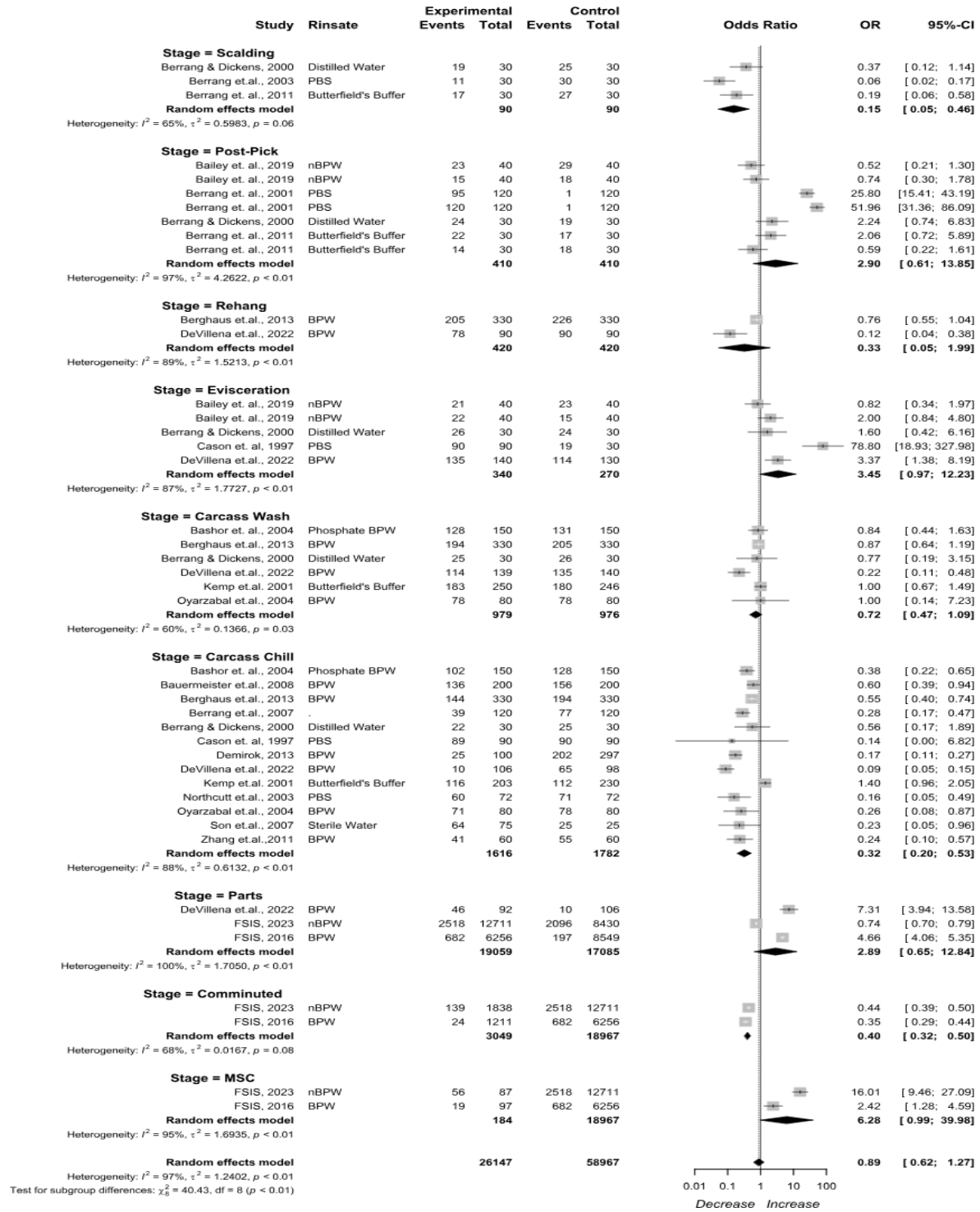
\*IOBW = Inside-Outside Bird Washer



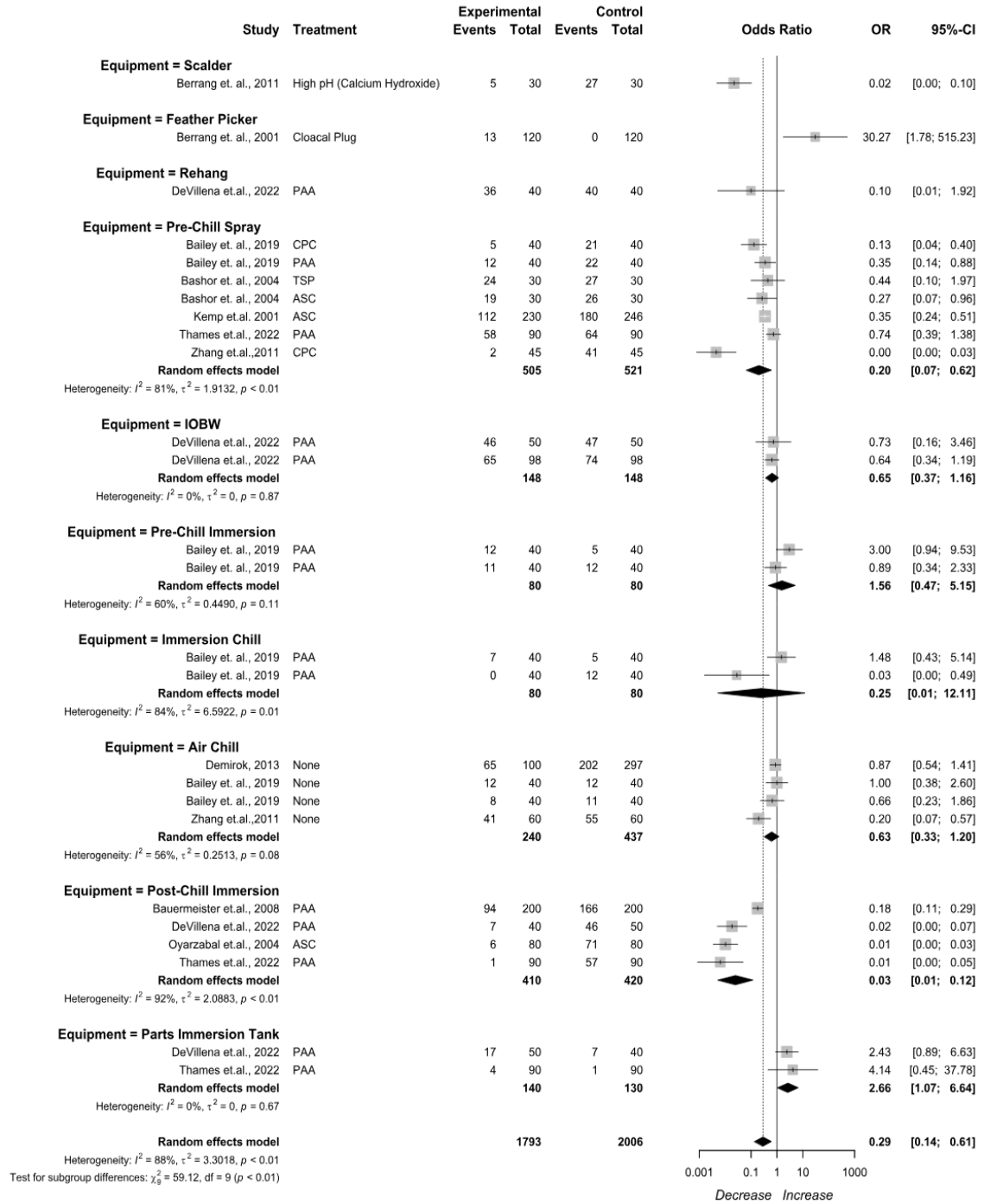
**Figure 3.2:** Flow Chart of Systematic Review Process



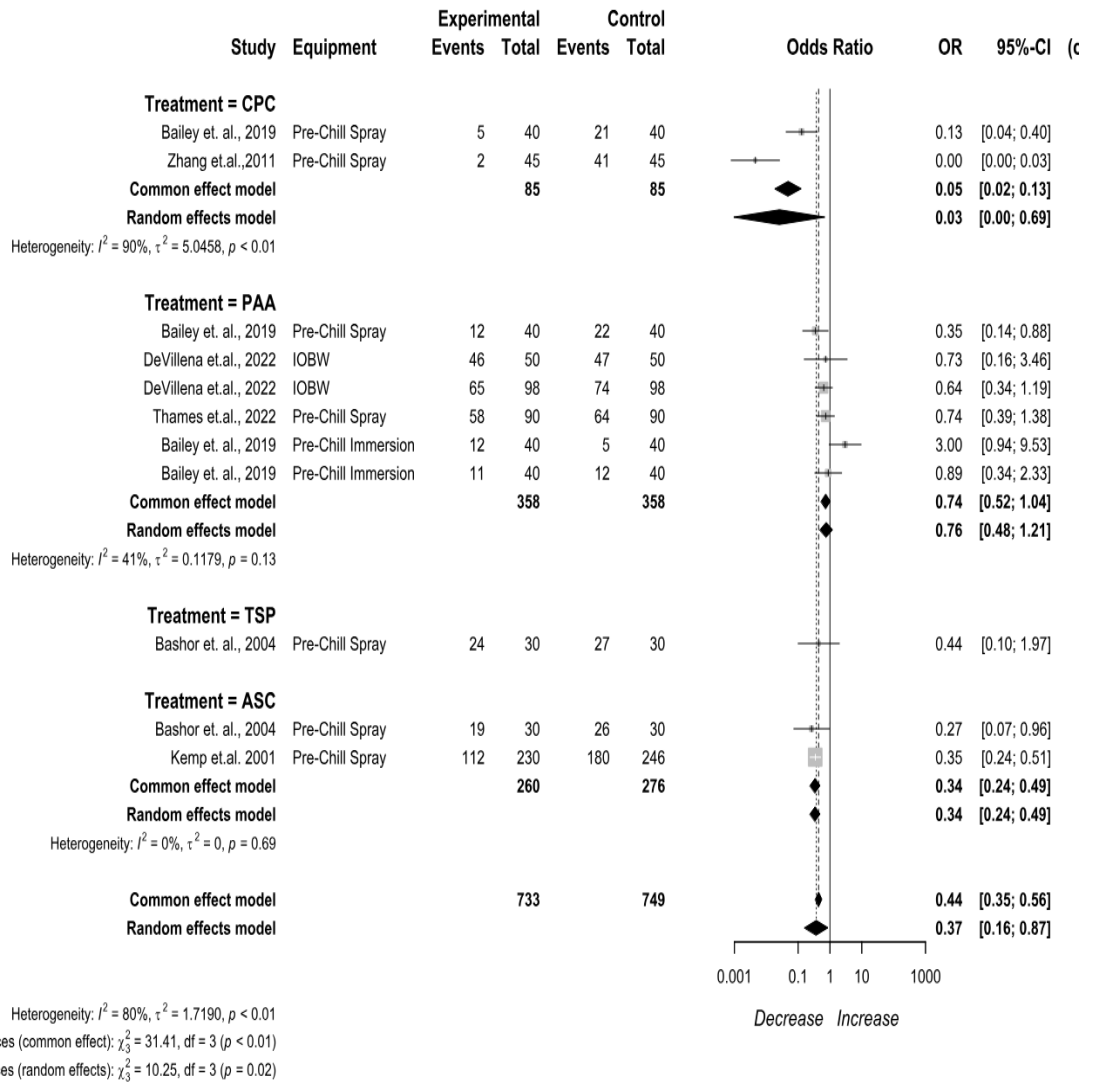
**Figure 3.3:** *Campylobacter* Prevalence per Stage Without Reported Interventions or Chlorine. The random effects model results represent the prevalence per stage for the control group that includes studies without reported interventions or chlorine. Distinct letters next to the stage description indicate statistically significant differences ( $P < 0.05$ ).



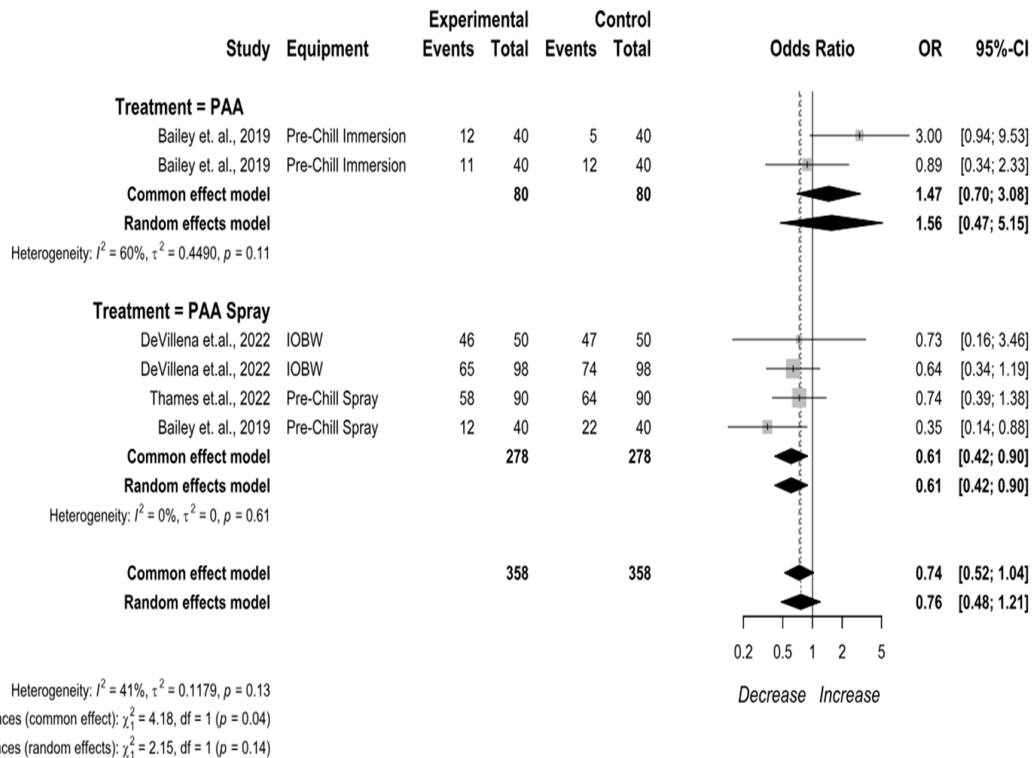
**Figure 3.4:** *Campylobacter* Prevalence Change per Stage Without Reported Interventions or Chlorine. The random effects model results represent the prevalence change per stage for the control group that includes studies without reported interventions or chlorine. Results > 1 indicate an increase in concentration. Results < 1 indicate a decrease. Results = 1 indicate no change.



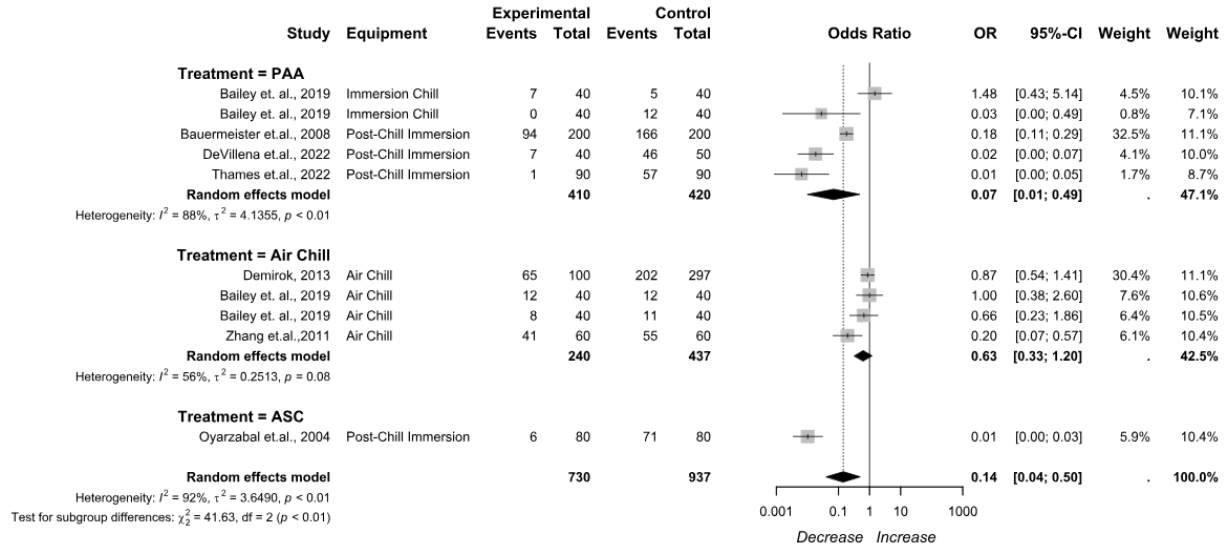
**Figure 3.5: *Campylobacter* Prevalence Change from Intervention Application Method.** The random effects model results represent the prevalence change of the equipment used to apply interventions. Results > 1 indicate an increase in concentration. Results < 1 indicate a decrease. Results = 1 indicate no change.



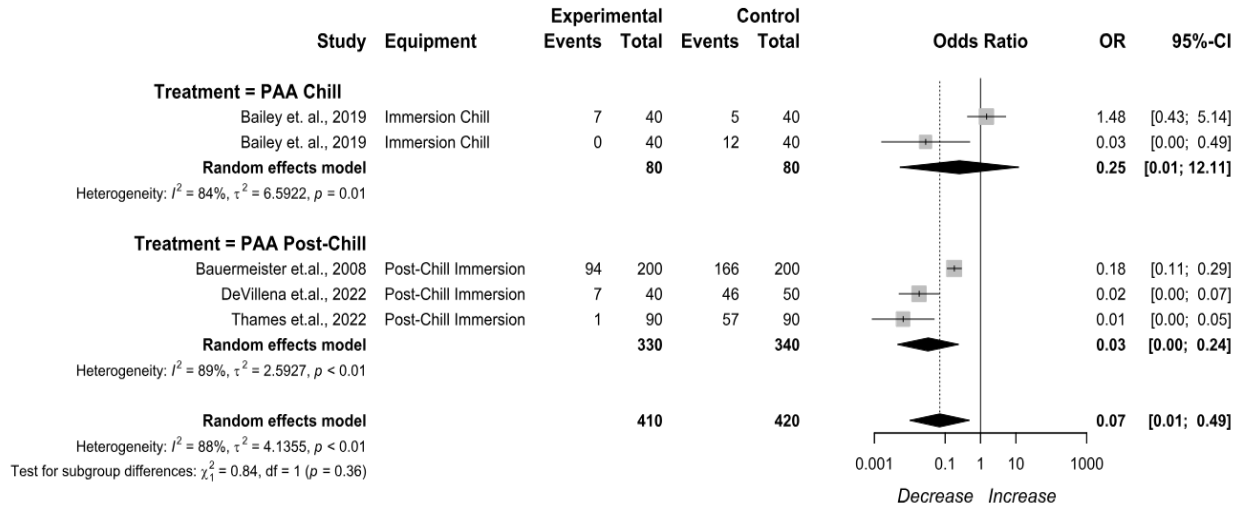
**Figure 3.6:** *Campylobacter* Prevalence Change for Pre-chill Interventions. The random effects model results represent the prevalence change of the interventions applied prior to the chilling process. Results > 1 indicate an increase in concentration. Results < 1 indicate a decrease. Results = 1 indicate no change.



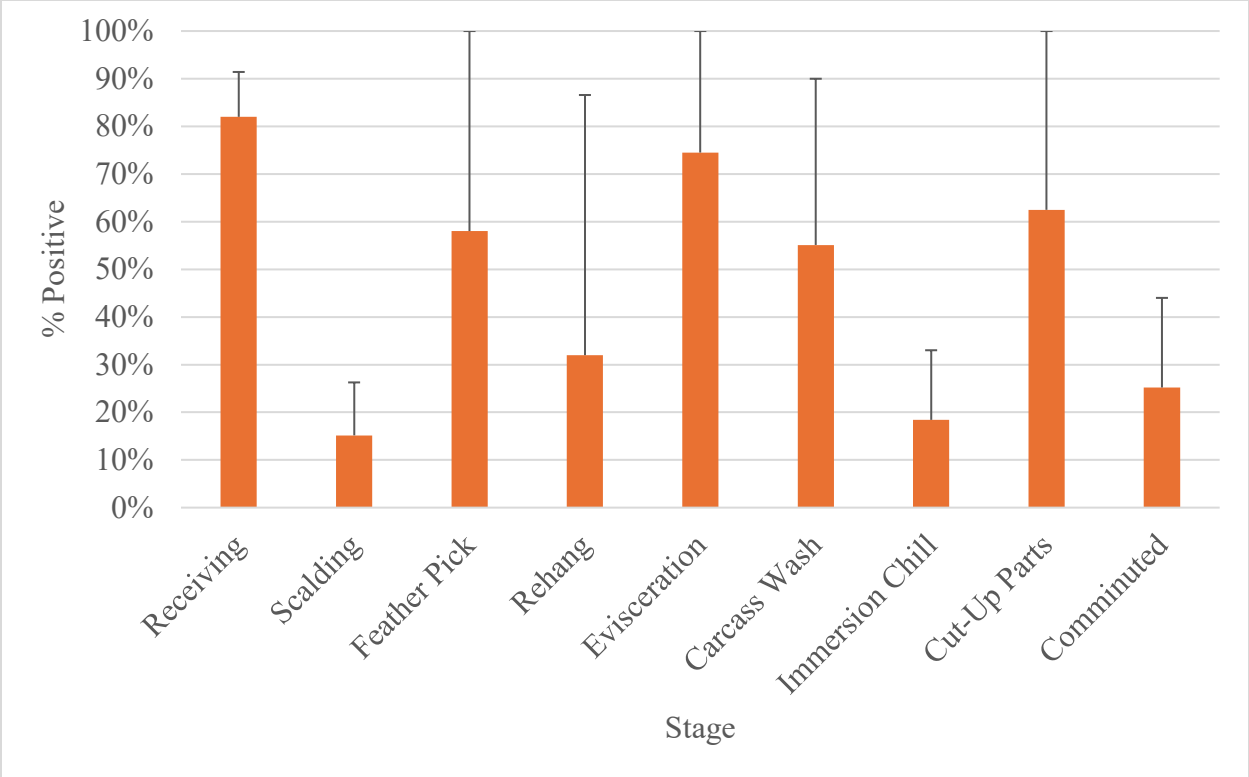
**Figure 3.7:** Pre-chill Peroxyacetic Acid (PAA) *Campylobacter* Prevalence Change. The random effects model results represent the prevalence change of PAA applied prior to the chilling process. Results > 1 indicate an increase in concentration. Results < 1 indicate a decrease. Results = 1 indicate no change.



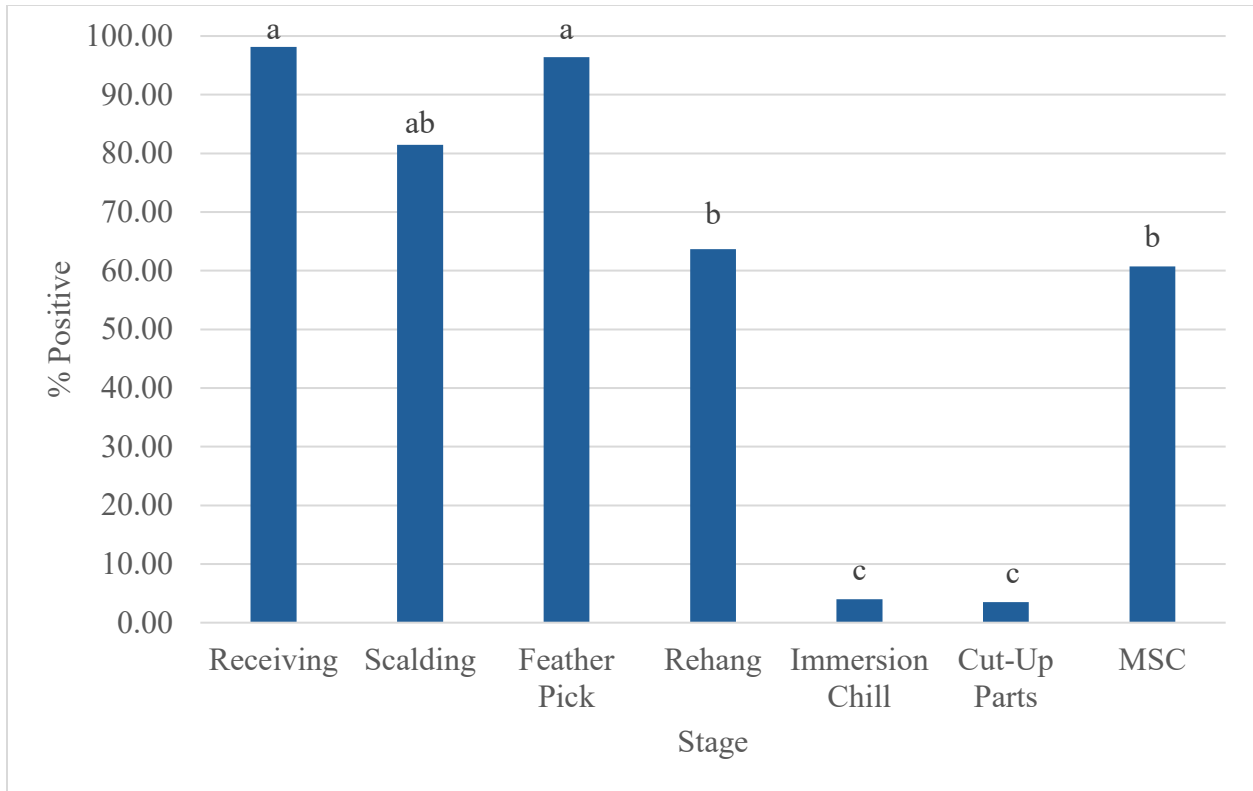
**Figure 3.8:** Chilling and Post-chill Interventions *Campylobacter* Prevalence Change. The random effects model results represent the prevalence change of chilling and post chill interventions. Results  $> 1$  indicate an increase in concentration. Results  $< 1$  indicate a decrease. Results  $= 1$  indicate no change.



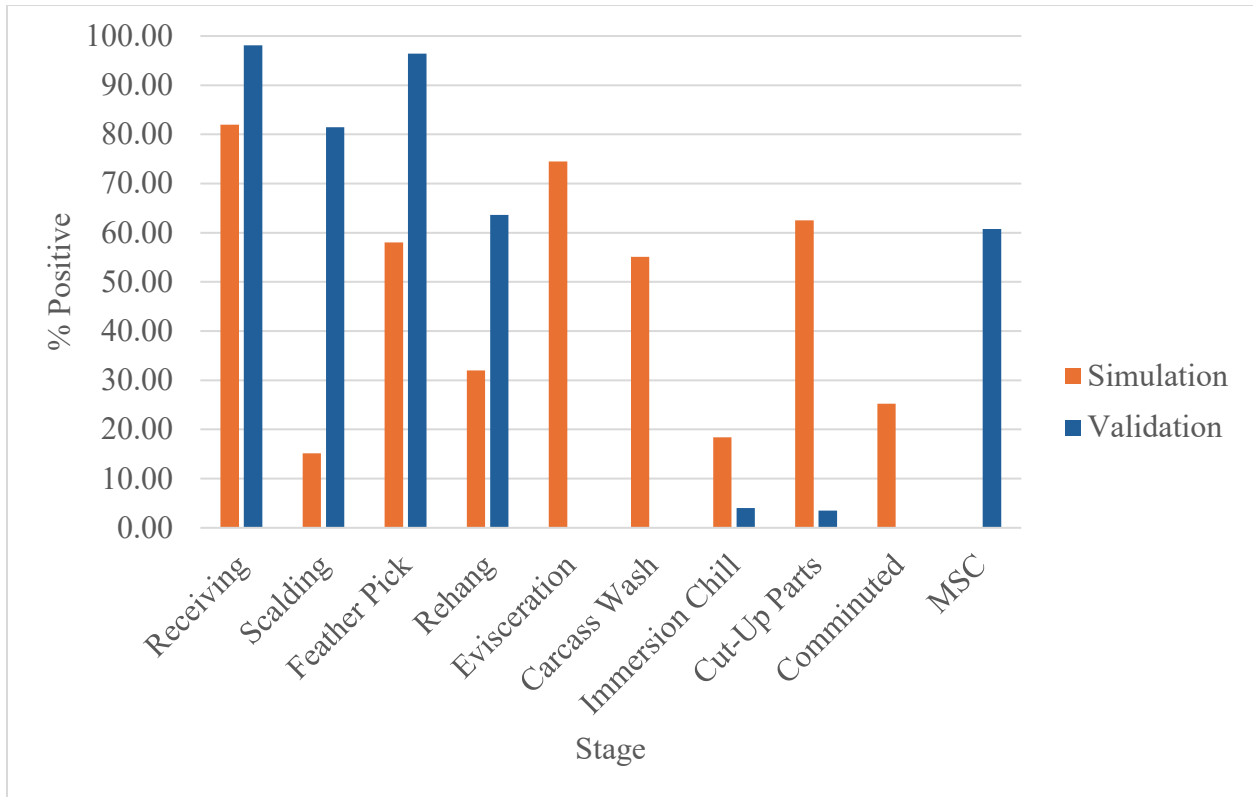
**Figure 3.9:** PAA Post-chill Treatments Prevalence Change. The random effects model results represent the prevalence change of PAA applied during and after the chilling process. Results  $> 1$  indicate an increase in concentration. Results  $< 1$  indicate a decrease. Results  $= 1$  indicate no change.



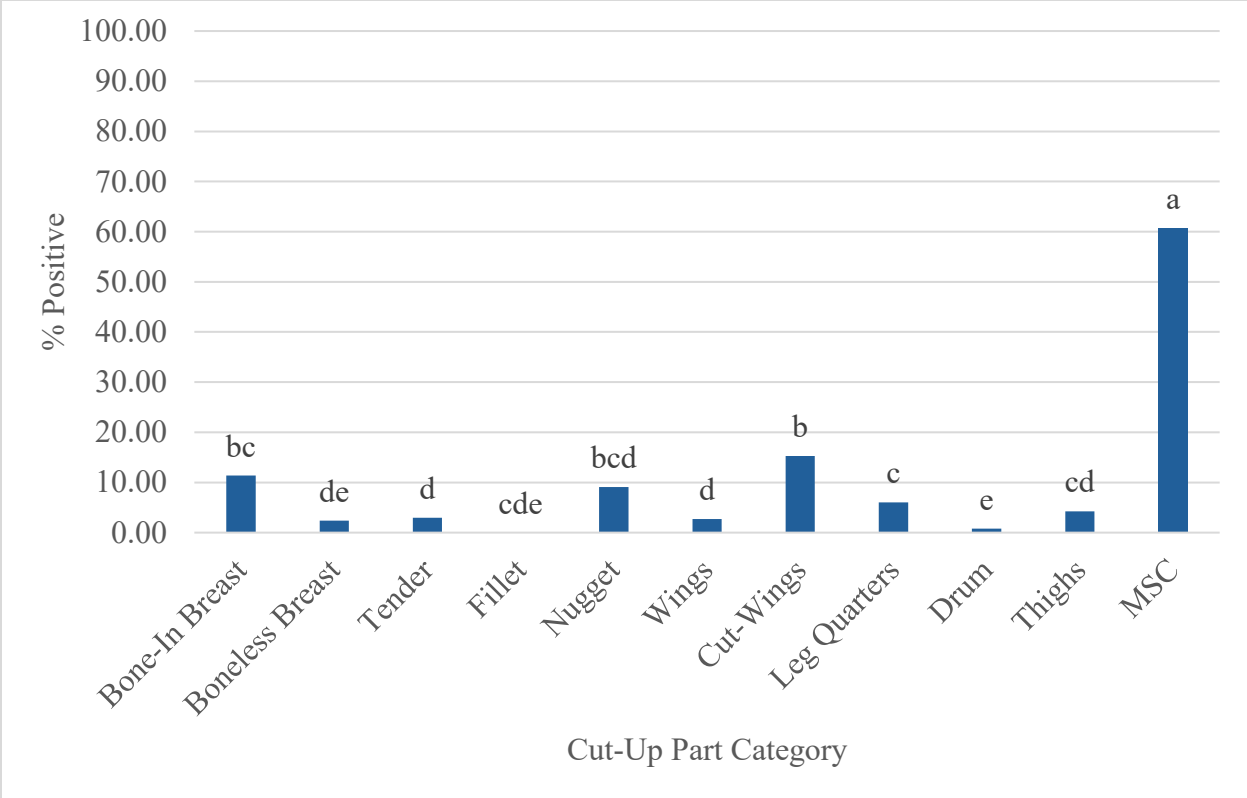
**Figure 3.10:** Baseline *Campylobacter* Bio-map. The chart represents a simulation model of *Campylobacter* prevalence per stage without interventions or chlorine.



**Figure 3.11:** Integrator *Campylobacter* Prevalence Bio-map. This figure represents the pooled prevalence per stage obtained from commercial integrators. The figure includes results from the receiving, scalding, rehang, whole birds after chill, cut-up parts, and MDM stages. Samples after the post-chill stage went through a PAA application. Distinct letters on top of the stage indicate statistically significant differences ( $P < 0.05$ ).



**Figure 3.12:** *Campylobacter* Simulation and Validation Bio-maps. This figure represents the baseline simulation with the validation data obtained from the integrator data.



**Figure 3.13:** Integrator *Campylobacter* Prevalence per Parts Categories. This figure represents the cut-up parts broken down by the categories obtained from the pooled results from commercial integrators. All cut-up parts categories were treated with PAA. Distinct letters on top of the stage indicate statistically significant differences ( $P < 0.05$ ).

## CHAPTER 4

### EXPOSURE ASSESSMENT MODEL OF *CAMPYLOBACTER* CONCENTRATION IN U.S. BROILER PROCESSING PLANTS<sup>3</sup>

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<sup>3</sup> Rivera, R.E.; Wang, J.; Mishra, A.; Thippareddi, H.; and Singh, M. To be submitted to Poultry Science

## Abstract

Poultry processing establishments are important in the control of *Campylobacter* in poultry products. *Campylobacter* is commonly associated with poultry products and is a bacterial pathogen that causes gastrointestinal infection in the United States. Recent evidence, performed by quantitative microbial risk assessments (QMRA) shows that concentrating efforts in lowering *Campylobacter* microbial load may help reduce illness risk in the public. QMRAs have not included *Campylobacter* load from parts and comminuted poultry products in their exposure assessments. A systematic review and meta-analysis were performed to develop *Campylobacter* estimates with and without the use of interventions on cut-up parts, and comminuted poultry. The initial concentration from literature data was  $4.81 \log_{10}$  CFU/mL. Log changes (LC) were calculated to indicate changes in *Campylobacter* concentration. The scalding stage resulted in an LC of  $-2.86 \log_{10}$  CFU/mL and the chilling stage an LC of  $-1.48 \log_{10}$  CFU/mL. Both stages provided significant reductions from the initial contamination ( $P < 0.05$ ). A baseline model representing *Campylobacter* concentrations at various processing stages without interventions was constructed. *Campylobacter* contamination in whole birds was reduced to  $1.38 \log_{10}$  CFU/mL, cut-up parts to  $0.79 \log_{10}$  CFU/mL, and comminuted product to  $0.45 \log_{10}$  CFU/mL. The model was validated using comparisons against U.S. commercial processing facilities. Whole bird concentrations from the processing plants had a concentration of  $0.72 \log_{10}$  CFU/mL and cut-up parts concentrations of up to  $1 \log_{10}$  CFU/mL. Chemical interventions improve the efficacy of *Campylobacter* reductions in cut-up parts and comminuted products. Single intervention scenarios demonstrated that post-chill immersion interventions after chill can improve reduction capabilities up to 99.99%. A multi-hurdle approach can reduce *Campylobacter* to undetectable levels. Chicken parts *Campylobacter* concentrations need to be included in QMRA.

Keywords: *Campylobacter*, QMRA, Poultry parts, Comminuted poultry, Interventions

## Introduction

Poultry processing plants serve as a principal control location for the presence of *Campylobacter* in poultry products. *Campylobacter* is a bacterial pathogen that causes gastrointestinal infection in the United States. *Campylobacter* is commonly associated with the consumption of poultry products (Berghaus et al., 2013; Ellis-Iversen et al., 2012; Overesch et al., 2020). The Foodborne Diseases Active Surveillance Network (FoodNet) indicates that about 20 cases are diagnosed each year for every 100,000 people. The Center for Disease Control and Prevention (CDC) estimates *Campylobacter* infections affect 1.5 million U.S. residents every year (CDC, 2022b). In 2019, the Interagency Food Safety Analytics Collaboration (IFSAC) reported that over 80% of non-dairy foodborne illnesses were attributed to chicken, other seafood (such as shellfish) and turkey, with *Campylobacter* illnesses most often linked to chicken meat (IFSAC, 2021). *Campylobacter* illnesses from poultry products alone costs an estimated \$6.9 billion a year in medical costs and loss of quality of life (Scharff, 2020).

The U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) is the agency responsible to develop and monitor food safety standards for poultry. To reduce pathogen contamination, USDA-FSIS introduced the Pathogen Reduction; Hazard Analysis and Critical Control Points rule (PR: HACCP rule) in 1996. FSIS has relied on controlling pathogens by developing performance standards aimed at reducing prevalence at the processing plant (USDA-FSIS, 1996). Since the introduction of the PR: HACCP rule, there have been several baseline surveys that determine the state of *Campylobacter* prevalence in poultry processing establishments and adjust the performance standards. The first baseline survey, conducted in 1995, found that 88.2% of chicken carcasses were *Campylobacter* positive. By the exploratory sampling survey in 2019, that number had decreased to 18.3% of carcasses (Williams et al., 2021). Since the publication of the latest baseline survey, the *Campylobacter* prevalence has stayed constant. USDA-FSIS raw chicken carcass sampling dataset shows that 20.94% of samples were positive for *Campylobacter* for the 2022 calendar year, up from 18.8% in 2021 (USDA-FSIS, 2023a). The FSIS raw chicken parts sampling dataset shows that 16.75% of samples were positive for *Campylobacter*

for the 2022 calendar year, up from 15.16% in 2021 (USDA-FSIS, 2023a). The FSIS raw comminuted chicken sampling dataset shows that 5.93% of samples were positive for *Campylobacter* for the 2022 calendar year, down from 6.28% in 2021 (USDA-FSIS, 2023a). Despite a reduction in overall *Campylobacter* prevalence since 1995, the reported *Campylobacter* illness rate have stayed steady for many years with no significant reduction since the introduction of the PR: HACCP rule (CDC, 2022b).

The regulatory approach has targeted pathogen prevalence reduction at the processing plant level. Recent evidence shows that concentrating efforts in lowering *Campylobacter* microbial load may help reduce illness risk in the public. This evidence is being obtained through the development of quantitative microbial risk assessments (QMRA). QMRAs are becoming widely used to analyze intervention strategies for microbial control along the food supply chain. It allows for comparisons between interventions for adequate selection of intervention strategies (Dogan et al., 2019). There are several QMRAs that characterize *Campylobacter* contamination throughout the farm-to-fork continuum. A microbial risk analysis between QMRAs demonstrated that there is not a defined criteria where model comparisons can be done effectively and there is still a need to incorporate assessments from the different production components to characterize risk (Chapman et al., 2016). QMRAs can be started using systematic reviews and meta-analysis (SR-MA) to obtain baseline data to construct processing plant models to evaluate process improvements.

Currently, *Campylobacter* reduction is heavily addressed through antimicrobial chemical interventions. Poultry processors are constantly adjusting interventions to meet performance standards (Wideman et al., 2016). The systematic reviews provide data on available and experimental interventions and can be used to develop models that identify where its use is most effective. Recent SR-MA and QMRAs are limited to using whole carcass *Campylobacter* population data and do not include comparisons on poultry cut-up parts and comminuted poultry (Chapman et al., 2016; Dogan et al., 2022; Dogan et al., 2019; Golden & Mishra, 2020; Keener et al., 2004; Sahin et al., 2015). Cut-up parts and comminuted poultry are the most consumed raw product in the U.S. and interventions must be evaluated and characterized to reduce

exposure risk (NCC, 2023). Comminuted product includes chicken that has been ground, chopped, shredded, or minced. Therefore, *Campylobacter* population analysis in chicken cut-up parts and comminuted product in the U.S. should be collected and incorporated into future QMRAs. This will help develop better exposure assessments, improve dose-response analysis and estimate accurate population illness risk.

The objective of this study is to estimate the *Campylobacter* concentration levels in chicken parts and comminuted product that can potentially reach consumers. Concentrations will be obtained by 1) developing baseline *Campylobacter* concentration in chicken cut-up parts and comminuted product in U.S. processing plants through a systematic review and meta-analysis and 2) estimating the efficacy of processing interventions in reducing *Campylobacter* concentration in cut-up chicken parts, and comminuted product through simulation modeling.

## Materials and Methods

### Systematic review and meta-analysis

*Campylobacter* population at each stage of poultry processing was estimated by developing a flow chart depicting the common processing steps from receiving to grinding in the U.S. (Fig. 4.1). *Campylobacter* concentration at the receiving stage of an establishment was determined as the initial concentration. The chicken processing model includes scalding, feather picking/ rehang, evisceration, carcass washing, immersion chilling, parts cut-up and grinding (comminuted) as standard operations of processing in the U.S. Final *Campylobacter* concentration data of cut-up parts, or comminuted chicken were collected to evaluate process and intervention efficacy. Additionally, the changes in *Campylobacter* population in each subsequent processing stage up to the grinding stage were estimated.

Each step was evaluated for its effect on *Campylobacter* population with and without interventions. *Campylobacter* concentration from data without interventions or chlorine was designated as the control or baseline group. *Campylobacter* concentration levels from published literature were extracted for each

processing stage to develop a baseline model using trials without reported use of interventions or reported chlorine use. Chlorine was used as part of the baseline since this intervention has historically been used for pathogenic bacterial control. It was assumed that commercial processing plant studies without reported use of interventions in control trials were using chlorine at the time of sampling.

*Campylobacter* concentration is the amount in logarithm base 10 of colony forming units per mL of sample ( $\log_{10}$  CFU/mL). Data for risk assessment inputs were obtained through a systematic review of literature and meta-analysis.

#### Literature search strategy

A systematic review was adapted from Golden and Mishra (2020), and Sargeant and O'Connor (2014) to address the following research question:

1. How does *Campylobacter* contamination on broiler carcasses change at each stage of processing from receiving to chicken parts and comminuted product in the U.S.?
2. What is the efficacy of chemical intervention and processing equipment on reducing *Campylobacter* contamination and their interactions in the U.S.?

To address this question, the Web of Science ([www.webofknowledge.com](http://www.webofknowledge.com)) and PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) online databases were searched using the following keywords aimed at addressing the research question: (“*Campylobacter*” OR “*Campylobacter jejuni*” OR “*C. jejuni*”), AND (“United States” OR “U.S.”) AND (“Poultry” OR “Broiler” OR “Chicken”) AND “Intervention” AND “Processing” AND (“Concentration” OR “Isolation”). Historical literature up to January 2023 that reported the data in the U.S. was retrieved. In the absence of geographic description of the study, the location was inferred by the first and corresponding address. Additional studies were identified by searching review articles or other reference lists by hand. Studies included peer-reviewed journal articles only. All references were managed by the EndNote citation manager (Endnote 20, Clarivate Analytics, Philadelphia, PA). Duplicates were removed from EndNote by using the “find duplicates” function or manually.

### Inclusion criteria

Abstracts were screened to determine eligibility with the following criteria included: 1) English language; 2) peer-reviewed journal articles; 3) primary research studies, excluding reviews; 4) interventions tested at a processing stage; 5) intervention tested on whole carcasses, cut-up parts, or ground chicken product. The concentration in before-after studies in U.S. commercial broiler processing environments, interventions tested in pilot plants, or challenge studies needs to be reported for including in the meta-analysis.

Commercial processing establishment before-after studies measure the effect of an intervention on naturally occurring bacterial levels, while challenge studies establish an initial bacterial level by artificial inoculation of bacteria to have a uniform initial concentration before the trial. Before-after studies can be performed in commercial or pilot processing plants that test intervention effect on naturally occurring bacterial levels. Challenge studies overstate microbial loads and may overestimate effectiveness of an intervention compared to what may be present at a commercial processing facility. Challenge studies were included as a data source to analyze an intervention where commercial processing data was insufficient or unavailable while considering the limits of these types of studies.

Commercial processing establishment before-after studies in different languages other than English or regions other than the U.S. were excluded from review. The data was used to develop a baseline for U.S. processing establishments. Following the initial screening, full-text articles were obtained for the remaining studies and analyzed for inclusion in model assessment. Studies with uncertain eligibility were reviewed and discussed by the authors until a consensus was reached.

Along with the previously mentioned screening criteria, details on the type of intervention used, application method, and data to perform a meta-analysis (i.e., samples size, mean, standard deviation, confidence intervals, standard error of the mean, number of positive samples for prevalence) were evaluated.

## Data extraction

Articles deemed eligible were screened and the extracted data were stored on Microsoft Excel Spreadsheets. Data relevant for analysis included the study type, processing step, type of intervention, intervention application method, initial and final *Campylobacter* prevalence, or concentration with or without an intervention, concentration unit of measurement, standard deviation, and sample size. The data were directly collected if the table is available, whereas the Plot Digitizer tool (Plot Digitizer, 3.1.5, 2024, <https://plotdigitizer.com>) was used to extract the mean and error values from the figures. All *Campylobacter* concentrations are reported as log<sub>10</sub> CFU/mL. Conversions to log<sub>10</sub> CFU/mL from log<sub>10</sub> CFU/g, log<sub>10</sub> CFU/cm<sup>2</sup>, or log<sub>10</sub> CFU/carcass were performed using the conversions recommended by Appendix A of the Joint Food and Agriculture Organization/ World Health Organization Risk Management Tool for the Control of *Campylobacter* and *Salmonella* in Chicken Meat (JEMRA, 2009). In the case of study presented a standard error (SE) or standard error of the mean, then the standard deviation (SD) was calculated as  $SD = SE \times \sqrt{n}$ , where n is the sample size. In the case of study that presented a 95% confidence interval (95% CI), then SD was calculated as  $SD = (Upper\ Confidence\ Interval - Lower\ Confidence\ Interval) \div (2 \times Critical\ T - value) \times \sqrt{n}$ .

The mean value of the concentration change of a specific stage in processing was calculated as the difference before and after interventions. The SD of the concentration change was calculated by formula reported in the Chapter 16.132 of Cochrane Handbook for Systematic Reviews of Interventions (Higgins & Green, 2008).

The concentration changes of each processing stage and included interventions were described by its Log Change (LC) by using the equation  $LC = C - C_i$  where  $C$  is the concentration after processing stage and  $C_i$  is the initial concentration before the processing stage. A  $LC < 0$  indicates a decrease in concentration, a  $LC = 0$  indicates no change, and a  $LC > 0$  indicates an increase in concentration. LC provide

a description of the level of contamination before and after a processing stage or intervention (Dogan et al., 2022).

#### Quality assessment of included studies

Quality assessment was often included in systematic reviews and meta-analysis to determine the quality of the evidence presented by each study. However, selection bias could happen since the types of quality scores can affect the interpretation of meta-analysis (Stone et al., 2019), therefore, quality scores for the included studies were not determined.

#### Data analysis

The collected data was subjected to a random effect model for meta-analysis to determine the *Campylobacter* concentrations, concentration changes and intervention efficacy (instruments and chemicals) at various stages of poultry processing. The results were presented as both tables and forest plots in accordance with Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA 2020) guidelines. The meta-analysis was conducted using the meta package (Schwarzer, 2007) in R software 4.0.2 (R core Team, 2021), and the summary statistics were presented as forest plots for visualization. Treatment effects were further separated by Tukey's HSD using `glht`

Function in `multcomp` package of R. The estimated concentrations or concentration change in the forest plot is displayed along with a 95% confidence interval (CI) along with the tau-squared ( $\tau^2$ ) variance that describes the variance between the studies (Higgins et al., 2019) and  $I^2$  as the measure of heterogeneity. The value of  $I^2$  up to 40% was considered low, 30–60% was considered moderate, 50–90% was considered substantial, and beyond 75% was considered high (Deek et al., 2019).

#### Test for publication bias due to small-study effects

The limitations of statistical tests to quantify asymmetry in funnel plots and recommended these tests only be conducted when there is enough studies ( $n > 10$ ) and low heterogeneity ( $I^2 < 50\%$ ).

Unfortunately, none of the meta-analyses examined in this study met these criteria, so publication bias could not be appropriately assessed.

## Exposure Assessment

### Processing plant module overview

The first objective of this module was to estimate *Campylobacter* concentration levels in whole birds, cut-up chicken parts, and comminuted poultry when chlorine or no reported interventions were recorded during the SR-MA. *Campylobacter* concentration ( $\log_{10}$  CFU/ mL) was estimated at each processing step from receiving until the cut-up or grinding stages. The second objective was to estimate the efficacy of interventions in improving the processing capabilities in reducing *Campylobacter* populations in whole birds, cut-up chicken parts, and comminuted chicken.

### *Campylobacter* concentration baseline estimate

The processing plant model starts with the initial *Campylobacter* concentration at the receiving stage before slaughter. Initial *Campylobacter* concentrations were obtained from SR-MA results. The processing model includes scalding, feather picking/ rehang, evisceration, carcass washing, carcass chilling, parts cut up and grinding as standard processing stages in the U.S. Baseline model inputs were obtained by pooling trials without reported interventions or chlorine use were recorded during the SR-MA. The LC derived from the SR-MA. LC values were fit as a normal distribution. Distributions were simulated using Monte Carlo simulation by Latin Hypercube Sampling with 10000 iterations using @Risk (version 8.4.1 (Build10), Palisade Company LLC, New York, USA). A baseline *Campylobacter* concentration level in  $\log_{10}$  CFU/mL for chicken cut-up parts, and comminuted chicken were obtained for intervention efficacy analysis.

### Baseline validation

*Campylobacter* concentrations recovered from pre-scalding (incoming concentration), post-scalding, post-pick, rehang, post-chill, and cut-up parts sampling were collected from routine testing of 31 commercial processing facilities in the U.S. over the period from 2018 to 2024. *Campylobacter* concentration was measured using various plating methods with a limit of detection (LOD) of 1 CFU/mL. *Campylobacter* concentration variation of cut-up parts including boneless skinless breast, drum, boneless breast fillet, leg quarter, nugget (breast trim), tender, bone-in thighs, and wing, were analyzed using one-way ANOVA and were further separated using Tukey HSD method in R (R Core Team, 2024). Significance values were set at 0.05.

### Intervention efficacy analysis

Single interventions obtained from the SR-MA were evaluated for its efficacy to improve a processing plant's ability to reduce *Campylobacter* concentration levels in whole birds, cut-up parts and comminuted chicken. A single intervention LC data was used to replace a stage (such as replacing immersion chilling with air chilling), or it was added to the baseline model as an additional step in the processing chain (such as adding a post-chill dip tank between chilling and cut-up) to obtain an adjusted *Campylobacter* concentration level estimate for whole bird, cut-up parts and comminuted chicken. Results from the interventions scenarios were expressed as 1. Mean *Campylobacter* concentration in CFU/mL with its 95% CI and 2. Intervention efficacy calculated using equation:

$$\text{Intervention efficacy} = \frac{\text{Concentration}_{\text{baseline}} - \text{Concentration}_{\text{intervention}}}{\text{Concentration}_{\text{baseline}}} \times 100, \quad \text{where}$$

$\text{Concentration}_{\text{baseline}}$  refers to the *Campylobacter* concentration for either whole birds, cut-up parts or comminuted chicken and  $\text{Concentration}_{\text{intervention}}$  refers to the *Campylobacter* concentration for the alternative intervention scenario. In addition, scenarios using multiple pre-chill and post-chill intervention scenarios were developed to observe *Campylobacter* concentrations in whole birds, cut-up parts and comminuted chicken utilizing multiple interventions.

## Results

### Search results

The initial search criteria produced 2,261 studies. After removing duplicates and screening the titles and abstracts, 181 records were retained for full text screening. After full text screening, 72 records were retained for analysis. 29 records were excluded due to missing sample number, variation data, and units of measurements that could not be converted to the log<sub>10</sub> CFU/mL. A total of 18 commercial plants before/after studies, 10 pilot plant before/after studies, 5 pilot plant challenge studies, and 11 lab scale challenge studies, totaling 44 studies were included for the risk assessment. The overview of the systematic review process is illustrated in Fig 4.2.

### Characteristics of included studies

The characteristics of the included studies in the meta-analysis and risk assessment model are presented in Table 4.1. 6 studies reporting *Campylobacter* concentrations at receiving or prior to the scalding step were used to determine an incoming load. 27 studies used chlorine or did not report an intervention in its control group. The control group consisted of trials without reported use of an intervention or chlorine use. Chlorine was used as part of the baseline since this intervention has historically been used for pathogenic bacterial control. It was assumed that commercial processing plant studies without reported use of interventions in control trials were using chlorine at the time of sampling. These were used to determine the baseline *Campylobacter* concentration and concentration change for each processing stage.

27 studies reported intervention trials other than the control group to control *Campylobacter* concentration. The studies reported the use of 18 different treatment types against *Campylobacter* concentration. The interventions that were included for meta-analysis were acidified sodium chlorite (ASC n=3), air chill (AC n=1), cloacal plug (CP n=3), cloacal wash (CW n=3), cetylpyridium chloride (CPC n=3), chlorine dioxide (ClO<sub>2</sub> n=1), high scalding pH (n=1), peroxyacetic acid (PAA n=10), lysozyme (n=1), trisodium phosphate

(TSP n=1), hydrogen peroxide (n=1), formic acid (FA n=1), sulfuric acid + sodium sulfate solution (SSS n=1), rescald (n=1), steam (n=1), tripotassium phosphate (TPP n=1), ultraviolet light (UV n=2), pulsed electric field (PEF n=1). Chemical applications were applied either through an immersion application (dip tank) (n=25) or a spray application (n=17).

#### Meta-analysis for *Campylobacter* concentration per processing stage for control group

The estimated *Campylobacter* concentration from the control group (without intervention or chlorine) at the various processing stages is presented in Figure 4.3. 9 stages were identified with varying *Campylobacter* concentrations with receiving (incoming load) being 4.81 log<sub>10</sub> CFU/mL (95% CI: 3.91 to 5.72), Scalding being 1.32 log<sub>10</sub> CFU/mL (95% CI: -0.33 to 2.97), Feather Picking being 3.05 log<sub>10</sub> CFU/mL (95% CI: 2.21 to 3.90), Rehang being 2.71 log<sub>10</sub> CFU/mL (95% CI: 2.30 to 3.13), Evisceration being 3.27 log<sub>10</sub> CFU/mL (95% CI: 2.40 to 4.14), Carcass Wash (IOBW) being 1.97 log<sub>10</sub> CFU/mL (95% CI 1.01 to 2.94), Carcass Chill (Immersion Chiller) being 0.99 log<sub>10</sub> CFU/mL (95% CI: 0.50 to 1.48), Cut-Up Parts being 0.57 log<sub>10</sub> CFU/mL (95% CI: -0.92 to 2.06). The test for differences between the initial concentration and the other processing stages were statistically significant ( $P < 0.05$ ). The *Campylobacter* concentration estimates in scalding, feather picking, rehang, evisceration, and carcass wash stages are significantly different from the initial concentration ( $P < 0.05$ ) but not between each other ( $P > 0.05$ ). The carcass chilling stage has significantly lower *Campylobacter* concentrations when compared to previous processing stages ( $P < 0.05$ ) and the reduction is maintained throughout the cut-up stage. *Campylobacter* concentration estimates for comminuted chicken could not be established because the included studies for this stage were lab challenge studies. *Campylobacter* concentrations were overestimated due to artificial inoculation levels and a proper assessment of *Campylobacter* concentration in this stage was not achieved. Low heterogeneity existed in the analysis between groups ( $I^2 = 0\%$ ,  $P > 0.05$ ).

### Meta-analysis for *Campylobacter* concentration changes per processing stage for control group

Log change was calculated for the comparison of the *Campylobacter* concentration change (no intervention or chlorine) for the various processing stages (Figure 4.4). 7 stages were identified with Scalding being  $-2.86 \log_{10}$  CFU/mL (95% CI:  $-4.15$  to  $-1.56$ ), Feather Pick being  $1.17 \log_{10}$  CFU/mL (95% CI:  $0.28$  to  $2.06$ ), Evisceration being  $0.13 \log_{10}$  CFU/mL (95% CI:  $-0.74$  to  $0.99$ ), Carcass Wash (IOBW) being  $-0.39 \log_{10}$  CFU/mL (95% CI:  $-1.04$  to  $0.25$ ), Carcass Chill (Immersion Chiller) being  $-1.48 \log_{10}$  CFU/mL (95% CI:  $-1.94$  to  $-1.02$ ), Cut-Up Parts being  $-0.58 \log_{10}$  CFU/mL (95% CI:  $-0.89$  to  $-0.26$ ), and Comminuted (Ground) being  $-0.35 \log_{10}$  CFU/mL (95% CI:  $-0.85$  to  $0.15$ ).

Scalding represents the highest concentration reduction due to a washing effect and some *Campylobacter* inactivation by temperature. Post-Pick represents a significant increase ( $P < 0.05$ ) in *Campylobacter* concentrations due to cross contamination from cloacal extrusion and the spread of *Campylobacter* from feather pickers to carcass. Significant reductions are represented in immersion chilling due to a washing off effect ( $P < 0.05$ ). In addition, reductions in meat temperatures may cause some inactivation of *Campylobacter*. Subsequent processes for cut-up or grinding also represent reductions in *Campylobacter* concentrations. Low heterogeneity existed in the analysis between groups ( $I^2 = 0\%$ ,  $P > 0.05$ ).

### Meta-analysis for interventions against *Campylobacter*

Several pre-chill and post-chill interventions were compared for their *Campylobacter* concentration change. Pre-chill interventions against *Campylobacter* included analysis for scalding and feather picking applications (Figure 4.5) and pre-chill chemical applications (Figure 4.6). A treatment to increase pH was used at the scalding stage. The concentration change being  $-2.84 \log_{10}$  CFU/mL (95% CI:  $-5.04$  to  $-0.64$ ). A  $\text{ClO}_2$  wash used at the feather picking stage, along with CW, and CP were the main interventions applied prior to the rehang stage. The concentration changes for  $\text{ClO}_2$  being  $0.98 \log_{10}$  CFU/mL (95% CI:  $-2.21$  to  $4.17$ ), CW being  $2.09 \log_{10}$  CFU/mL (95% CI:  $-0.47$  to  $4.67$ ), and CP being  $2.30 \log_{10}$  CFU/mL (95% CI:

1.19 to 3.41). A rescald application was also included after feather pick with concentration change being -0.25 log<sub>10</sub> CFU/mL (95% CI: -1.61 to 1.11). The test for subgroup differences resulted in the high pH treatment being significantly different in its reduction capability compared to rescald (P < 0.05). The test for subgroup differences among the feather picking interventions resulted in ClO<sub>2</sub> treatment being significantly different (P < 0.05) compared to CP and CW. Low heterogeneity existed in the analysis between groups (I<sup>2</sup> = 0%, P > 0.05).

Immersion and spray applications were evaluated prior to the chilling stage. Immersion application concentration change being -1.11 log<sub>10</sub> CFU/mL (95% CI: -1.99 to -0.23), spray applications being -0.77 log<sub>10</sub> CFU/mL (95% CI: -1.28 to -0.27). The effect of air chilling on *Campylobacter* concentration change was evaluated with the effect being -1.05 log<sub>10</sub> CFU/mL (95% CI: -2.11 to 0.00). The test for subgroup differences was not significant (P > 0.05), and there was low heterogeneity between studies, but not statistically significant (I<sup>2</sup> = 0%, P > 0.05).

The chemical interventions analyzed as immersion interventions at pre-chill were PAA and Lysozyme (Figure 4.7). PAA as an immersion treatment concentration change being -1.13 log<sub>10</sub> CFU/mL (95% CI: -2.04 to -0.21). Lysozyme concentration change being -0.90 log<sub>10</sub> CFU/mL (95% CI: -4.21 to 2.41). The test for subgroup differences was not significant (P > 0.05), and there was low heterogeneity between studies, but not statistical significance (I<sup>2</sup> = 0%, P > 0.05).

The chemical interventions analyzed as spray interventions at pre-chill were PAA, CPC and treatments with high temperature water and steam (Figure 4.8). PAA as a spray treatment concentration change being -0.50 log<sub>10</sub> CFU/mL (95% CI: -1.07 to 0.07). CPC concentration change being -1.56 log<sub>10</sub> CFU/mL (95% CI: -5.69 to 2.57). High temperature and steam spray treatment concentration changes being -1.81 log<sub>10</sub> CFU/mL (95% CI: -2.95 to -0.68). The test for subgroup differences was significant (P < 0.05), and there was low heterogeneity between studies, but not statistically significant (I<sup>2</sup> = 0%, P > 0.05).

Several post-chill interventions were categorized as immersion or spray applications (Figure 4.9). Interventions, such as UV and PEF, were also extracted from systematic review and included as additional physical interventions for further study. Post-chill immersion application concentration change being  $-1.87 \log_{10}$  CFU/mL (95% CI:  $-2.28$  to  $-1.45$ ), spray applications being  $-1.22 \log_{10}$  CFU/mL (95% CI:  $-1.76$  to  $-0.69$ ). PEF did not offer an effect on *Campylobacter* concentration in chicken meat with its concentration change being  $0.03 \log_{10}$  CFU/mL (95% CI:  $-1.04$  to  $1.09$ ) and UV being  $-1.46 \log_{10}$  CFU/mL (95% CI:  $-2.12$  to  $-0.79$ ). Immersion treatments have a higher concentration change effect at reducing *Campylobacter* concentrations in chicken products as a post chill application. The overall reduction is not statistically significant ( $P > 0.05$ ) from spray and UV applications. PEF was less effective as a post-chill intervention being statistically significant ( $P < 0.05$ ) from immersion, spray, and UV. Heterogeneity was high between immersion ( $I^2 = 58\%$ ,  $P < 0.05$ ) and spray groups ( $I^2 = 63\%$ ,  $P < 0.05$ ) indicating that the intervention application methods as overall groups should be interpreted carefully.

There were several chemical interventions analyzed for mean *Campylobacter* concentration change as post-chill immersion treatments (Figure 4.10). ASC being  $-0.68 \log_{10}$  CFU/mL (95% CI:  $-1.62$  to  $0.27$ ), CPC being  $-4.29 \log_{10}$  CFU/mL (95% CI:  $-5.75$  to  $-2.84$ ), FA being  $-1.80 \log_{10}$  CFU/mL (95% CI:  $-2.58$  to  $-1.02$ ), HP being  $-2.92 \log_{10}$  CFU/mL (95% CI:  $-4.05$  to  $-1.80$ ), PAA being  $-1.79 \log_{10}$  CFU/mL (95% CI:  $-2.29$  to  $-1.29$ ), SSS being  $-1.70 \log_{10}$  CFU/mL (95% CI:  $-2.32$  to  $-1.08$ ), and TSP being  $-1.69 \log_{10}$  CFU/mL (95% CI:  $-2.06$  to  $-1.33$ ). CPC was the most effective chemical intervention against *Campylobacter* as a dip treatment ( $P < 0.05$ ). Hydrogen Peroxide (HP) was also effective as a post-chill immersion intervention. Only one study was included in this analysis. FA, PAA, SSS, and TSP also represent effective interventions at reducing *Campylobacter* concentrations. Each are statistically less effective than CPC ( $P < 0.05$ ). Nevertheless, they are effective when compared to spray treatments. Several studies were included for PAA analysis representing moderate heterogeneity ( $I^2 = 42\%$ ,  $P > 0.05$ ). ASC is the least effective intervention of immersion treatments.

There were several chemical interventions analyzed for *Campylobacter* concentration change as post-chill spray treatments (Figure 4.11). CPC being  $-0.75 \log_{10}$  CFU/mL (95% CI:  $-1.56$  to  $0.06$ ), FA being  $-0.70 \log_{10}$  CFU/mL (95% CI:  $-1.48$  to  $0.08$ ), HP being  $-2.29 \log_{10}$  CFU/mL (95% CI:  $-3.53$  to  $-1.05$ ), PAA being  $-1.52 \log_{10}$  CFU/mL (95% CI:  $-2.59$  to  $-0.45$ ), SSS being  $-0.50 \log_{10}$  CFU/mL (95% CI:  $-1.28$  to  $0.28$ ), and TPP being  $-1.53 \log_{10}$  CFU/mL (95% CI:  $-3.10$  to  $0.05$ ). All treatments were less effective as a spray treatment when compared to immersion treatments. SSS was statistically the least effective treatment ( $P < 0.05$ ). PAA analysis representing high heterogeneity ( $I^2 = 79\%$ ,  $P > 0.05$ ) indicating that the intervention application results should be interpreted carefully. Some subgroups in this meta-analysis included only a single study due to limited available literature, which reduces statistical power and increases the potential for bias, limiting the reliability and interpretability of the results. However, these results were still presented in the forest plot as a reference for the reader's interest, highlighting potential research gaps in *Campylobacter* concentration changes during poultry processing.

## Exposure Assessment

### Baseline model and model validation

The baseline model is defined as a basic commercial chicken processing plant in the U.S. including scalding, feather picking/rehang, evisceration, carcass washing by inside-outside bird washers (IOBW), immersion chilling, parts cut-up and grinding without reported interventions or chlorine use from the SR-MA. Grinding is not available in most chicken processing plants, but ground product is available in several further processed products (NCC, 2023). Ground products, such as mechanically separated chicken (MSC), often go to further processes that include a lethality step. Therefore, ground products are seldom included in raw ready-to-cook pathogen analysis. The input parameters for the baseline processing model are described in (Table 4.2).

The simulation estimated *Campylobacter* concentration to be incoming at  $4.81 \log_{10}$  CFU/mL (95% CI:  $4.05$  to  $5.57$ ), at Scalding  $1.95 \log_{10}$  CFU/mL (95% CI:  $0.62$  to  $3.26$ ), at Feather Picking  $3.11 \log_{10}$

CFU/mL (95% CI: 1.62 to 4.63), at Evisceration 3.25 log<sub>10</sub> CFU/mL (95% CI: 1.57 to 4.91), at Carcass Wash 2.86 log<sub>10</sub> CFU/mL (95% CI: 1.09 to 4.62), and Whole Birds after Immersion Chill 1.38 log<sub>10</sub> CFU/mL (95% CI: -0.42 to 3.17). *Campylobacter* concentration after cut-up was estimated at 0.80 log<sub>10</sub> CFU/mL (95%CI: -1.02 to 2.61), and 0.45 log<sub>10</sub> CFU/mL (95% CI: -1.42 to 2.31) (Figure 4.12). The model output suggests that a processing plant can reduce *Campylobacter* concentration up to 3 logs and be effective food safety control with minimal interventions. The simulated data presents typical areas of decrease concentration, such as, scalding, carcass washing, and immersion chilling. Feather picking resulted in the step with the highest concentration. This stage typically increases concentration possibly through leakage of intestinal content and cross-contamination due to the pressure that is exerted on the carcass at this stage. A rehang estimate could not be included. LC for rehang could not be calculated from the SR-MA due to limitations in the number of studies. Post-feather pick carcasses pass through steps, such as feet and hock cutters, followed by hot rehang. The time between stages last a few seconds so there is the possibility that *Campylobacter* concentration differences are minimal between stages, unless interventions are placed between stages. Therefore, feather picking and rehang steps are represented as one stage. Subsequent processing steps represent additional *Campylobacter* concentration reduction and correction of previous increases.

Validation of the final *Campylobacter* concentration for cut-up parts and comminuted chicken was done by comparing the model with the *Campylobacter* concentration estimates obtained from data collected from commercial processing plants in the U.S. A bio-map comparing *Campylobacter* concentrations from the baseline simulation and the commercial processing plant results is represented in Figure 4.13. *Campylobacter* concentrations recovered from pre-scald (incoming concentration), post-scald, post-pick, hot rehang, post-chill, and cut-up parts sampling were collected from routine testing of 31 commercial processing facilities in the U.S. over the period from 2018 to 2024. *Campylobacter* concentration was measured using various plating methods with a limit of detection (LOD) of 1 CFU/mL. The processing plants reported using PAA for chill, post-chill and cut-up processes.

The *Campylobacter* concentration pattern from the commercial processing plants was comparable to the patterns obtained in the simulation (Figure 4.12). The incoming concentration at the receiving stage was 4.04 log<sub>10</sub> CFU/mL. The concentration after scalding fell to 1.46 log<sub>10</sub> CFU/mL followed by an increase to 4.20 log<sub>10</sub> CFU/mL after feather picking. The decrease after scalding was significantly different from receiving and the subsequent increase was significant when compared to the scalding stage (P < 0.05). The subsequent decrease to 1.90 log<sub>10</sub> CFU/mL at the rehang stage was significantly different from the feather picking stage (P < 0.05). Most of the processing plants represented in the data include a PAA or Chlorine spray treatment prior to the rehang stage, significantly reducing *Campylobacter* concentrations (P < 0.05). Significant reductions were represented after post-chill where whole bird samples averaged *Campylobacter* concentrations of 0.45 log<sub>10</sub> CFU/mL (P < 0.05). *Campylobacter* estimates for cut-up parts from the commercial processing data are 0.60 log<sub>10</sub> CFU/mL. Subsequent steps such as cut up and deboning do not significantly change *Campylobacter* concentrations (P > 0.05). Data from comminuted products were not obtained due to product not tested because the company produces comminuted product for further processing, thus not subject to testing under USDA-FSIS regulation. Validation of *Campylobacter* concentrations for the baseline simulation of this product type could not be achieved.

A comparison of *Campylobacter* concentrations between the baseline simulation and the validation data from commercial integrators is shown in Figure 4.14. The *Campylobacter* patterns between simulation and validation are similar. The concentration from the validation data is lower than the simulation. The validation data from commercial processing plants reported the use of PAA at various steps, resulting in lower *Campylobacter* concentrations. The single intervention and multiple intervention simulations from the intervention efficacy analysis (Tables 4.4, 4.5, and 4.6) were compared with the validation data. The *Campylobacter* concentrations from the single intervention and multiple intervention simulations resulted in similar *Campylobacter* concentrations as the validation results. This indicates that the input parameters to build the model are adequate and the baseline model is a representation of *Campylobacter* concentrations without interventions.

The data obtained from the commercial processing plants were organized in separate parts categories (Figure 4.15). Results were pooled per category to compare *Campylobacter* concentration between parts. The concentration between parts resulted in boneless skinless breast 0.99 log<sub>10</sub> CFU/mL, drumsticks 0.43 log<sub>10</sub> CFU/mL, boneless fillets 0 log<sub>10</sub> CFU/mL, legs 0.83 log<sub>10</sub> CFU/mL, breast trim used for nuggets 0.30 log<sub>10</sub> CFU/mL, tenders 0.67 log<sub>10</sub> CFU/mL, bone-in thighs 0.94 log<sub>10</sub> CFU/mL, and wings 0.70 log<sub>10</sub> CFU/mL were compared for differences between cut-up parts. *Campylobacter* concentration estimates for parts were at or below 1 log<sub>10</sub> CFU/mL. The concentration comparison between part types was not significant ( $P > 0.05$ ). All parts were sampled after all PAA applications.

### Scenario analysis

Based on the results of the SR-MA, 3 different single intervention application methods (air chill, spray, and immersion) and 5 of the most studied chemical interventions (ASC, CPC, PAA, TPP, and TSP) were selected to analyze its capacity of changing a processing plant's ability to control *Campylobacter* in cut-up parts or comminuted chicken for a total of 24 single intervention simulations. The input parameters for the processing model with interventions are described in Table 4.3. Table 4.4, 4.5, and 4.6 includes the *Campylobacter* concentrations estimates for all single intervention scenarios for whole birds, cut-up parts and comminuted product respectively. *Campylobacter* concentrations were converted to CFU/mL because final concentration estimates were very low to accurately determine intervention efficacy. The baseline *Campylobacter* estimates for whole birds was 23.44 CFU/mL (95% CI: 0.06 to 10715.19), cut-up parts was 6.31 CFU/mL (95% CI: 0.01 to 3801.89) and for comminuted chicken 2.82 CFU/mL (95% CI: 0 to 2290.87).

Pre-chill and Post-chill applications represent process improvements as single interventions. Pre-chill immersion applications provide higher *Campylobacter* concentration reductions in whole birds, cut-up parts and comminuted chicken when compared to pre-chill spray applications. PAA as pre-chill immersion represents a 92.41% reduction for whole birds, 92.59% for cut-up parts, and comminuted chicken. PAA was less effective as a pre-chill spray representing reductions of 67.64% for whole birds,

68.38% for cut-up parts, and comminuted chicken. CPC is a more effective spray treatment at the pre-chill stage representing reductions of 99.72% for whole birds, 97.25% in cut-up parts, and comminuted chicken.

Air chilling was included as an alternative chilling stage. Air chill is predominantly used in Europe, but it has been implemented in various U.S. processing plants. Air chilling was not effective at reducing *Campylobacter* concentration when compared to the baseline model utilizing immersion chilling.

Post-chill applications in general were more effective at reducing *Campylobacter* concentration. The largest improvements were achieved by utilizing post-chill immersion treatments in whole birds and cut-up parts. Applying post-chill dip treatment to whole bird carcasses reduced *Campylobacter* concentration by 98.52% in whole birds, 98.59% in cut-up parts, and 98.55% for comminuted chicken. Reductions of 94.50% for cut-up parts, and 94.38% for comminuted chicken respectively were achieved by applying an intervention post-cut-up. Post-chill and post-cut-up spray applications are also effective but less so than an immersion application. Post-chill spray application on whole bird carcasses reduces *Campylobacter* by 93.39% in whole birds, 93.83% on cut-up parts, and 93.69% on comminuted chicken. Improvements of 75.45% are achieved for cut-up parts and comminuted chicken if the spray interventions are applied at post-cut-up.

Each antimicrobial also presented differences in effectiveness. All included antimicrobial chemicals were more effective as a post-chill immersion treatment than a spray treatment. CPC is the most effective intervention chemical when applied as a post-chill immersion treatment. CPC reduces *Campylobacter* concentrations by 99.99% in whole birds, cut-up parts, and comminuted chicken when it's applied to whole bird carcasses post-chill. 99.98% reduction is achieved when applied post-cut-up. However, CPC was less effective when used in a spray application. Its effectiveness as a spray application reduces *Campylobacter* by 80.50% in whole birds, 81.80% in cut-up parts, and 81.38% in comminuted chicken when used as a whole carcass spray. Its effectiveness is reduced to 27.56% for cut-up parts and comminuted chicken when used as a post-cut-up spray.

PAA is effective as a post-chill intervention. PAA as an immersion application on whole birds represents reductions of 98.22% in whole birds, 98.30% in cut-up parts, and 98.26% in comminuted chicken. PAA as an immersion application as a post-cut-up intervention represents improvements of 93.39% in cut-up parts, and 93.24% in comminuted chicken. PAA as a post-chill spray application on whole birds represents reductions of 96.69% in whole birds, 96.91% for cut-up parts, and 96.84% for comminuted chicken. PAA spray applications as a post-cut-up interventions represent improvements of 87.70% for cut-up parts and comminuted chicken. PAA is most effective as a post-chill immersion when compared to a spray application.

TSP and TPP are also effective antimicrobials as immersion and spray treatment. ASC is the least effective overall as a post-chill immersion treatment.

Several intervention efficacy analyses were performed by comparing *Campylobacter* estimates using PAA spray and dip applications at multiple interventions at different processing stages. Results suggest that *Campylobacter* concentrations can be at or near undetectable levels when a combination of interventions are applied in the process (Data not shown).

## Discussion

### Meta-analysis for mean *Campylobacter* baseline concentration per processing stage

The SR-MA identified that the concentration varies per stage, and all stages impact *Campylobacter* concentrations. *Campylobacter* concentrations for whole bird carcasses and cut-up parts are significantly reduced after the carcass chilling stage when immersion chilling is used. The SR-MA demonstrates that the chilling and subsequent stages have the capability of reducing *Campylobacter* concentrations with just water or chlorine. The average LC for each stage was also consistent with the variation pattern observed with the *Campylobacter* concentration per stage. This pattern is similar to other bio-mapping studies from commercial processing plants performed in the U.S. and abroad (Betancourt-Barszcz et al., 2024; Chavez-Velado et al., 2024; DeVillena et al., 2022; Kingsbury et al., 2023; Vargas et al., 2023). A baseline

*Campylobacter* concentration for comminuted products from the SR-MA could not be established due to a lack of before-after studies from samples obtained from commercial processing facilities. LC levels used for the baseline simulation allowed to establish a *Campylobacter* concentration estimate for comminuted chicken. The result suggests that reductions achieved from immersion chill and the cut-up stage can be sustained through a grinding process and increases in *Campylobacter* concentrations during grinding do not occur, given that the conditions that prevent growth (e.g. time and temperature) are maintained. This estimate could not be compared with data from commercial processing or with published studies. In the U.S., most comminuted chicken product goes to further processing and will likely go through a thermal inactivation stage. Before-after studies using product from commercial processing facilities in the U.S. were not found because most ground poultry is not subject to regulatory sampling because these are not destined to be sold as a raw ground product.

The SR-MA to establish baseline values presented limitations. The heterogeneity and within study variation was low for both concentration and concentration change analysis. The study required dividing the studies into several subgroups and even though a mean value was established and was verified through additional bio-mapping studies, the subgroup analysis had very few studies with sufficient data points to perform within study and between study variation analysis.

#### Meta-analysis for interventions against *Campylobacter*

Most analyzed interventions could reduce *Campylobacter* concentration in whole bird carcasses and on cut-up parts. Most interventions are rarely examined, and, in many cases, only single data points were obtained. Heterogeneity and between study variability was difficult to assess due to the low number of studies per intervention group. For example, cloacal plug studies resulted in preventing some cross contamination during the feather picking stage, thus limiting *Campylobacter* concentration increases when compared to the control without cloacal plugs. The meta-analysis resulted in the concentration changes being higher than the baseline values for feather picking. There were only two studies observing this

intervention and perhaps more studies can provide more observations and test improvements to the intervention.

In other groups, such as, post-chill chemical interventions, heterogeneity was high because there were more studies to analyze and a mix of before-after studies with lab challenge studies. Studies had different sampling methods, sample numbers, and sample matrices that contribute to variability. PAA is the most studied chemical intervention and widely used in the U.S. PAA is applied either through immersion or spray application before and after the carcass chiller. PAA is also widely used in carcass chillers, but none of the studies obtained from the SR-MA observed dwell times like a typical immersion chiller in processing plants and it was decided to limit PAA application studies to pre-chill or post-chill. The results for PAA are consistent to past reviews (Cano et al., 2021; Oyarzabal, 2005). The decision to use PAA over other available chemical antimicrobials is due to cost and a processing plant's ability to implement and manage interventions.

Lesser-known interventions, such as cloacal plugs, cloacal washes, UV, etc. were included to provide a comparison of these against wider known interventions. Chemical interventions provide increased reduction levels overall, but additional research on these interventions can result in optimization of these technologies and develop them into alternatives or additional interventions complementing current ones.

### Exposure assessment

The baseline model bio-map simulation is similar to concentrations observed from the SR-MA and the validation data. The *Campylobacter* concentration data from whole birds and parts are higher in the baseline simulation when compared to the validation data. The commercial processing plants reported the use of PAA at various stages and this likely contributed to lower *Campylobacter* concentrations. The concentration patterns per stage were similar in both bio-maps. The results from the intervention scenarios were important to validate the model. When interventions were incorporated, *Campylobacter* concentrations were like those observed from the validation data. The baseline model is an adequate

representation of a U.S. processing plant without interventions. The input parameters used to construct the model were adequate and these can be used to develop more simulations using various intervention scenarios, where different settings can be studied and compared against the baseline model.

The baseline model suggests that low post-chill *Campylobacter* concentrations are maintained through the cut-up and grinding process. Further reductions for cut-up parts and comminuted chicken can be attributed to the reduction in meat temperature that may influence bacterial outgrowth, and operational sanitation practices that minimize cross contamination. The commercial data showed that interventions like PAA lowers whole carcass *Campylobacter* concentrations, but it does not have a significant effect on reducing concentrations further after the cut-up stage. Applying interventions at the cut-up stage helps maintain low numbers and may have a carryover effect to subsequent stages like grinding or packaging. The different cut-up parts did not show significant differences in *Campylobacter* concentration per part type. Processing interventions can even out *Campylobacter* throughout the carcass and reduce variability between parts. This causes parts to have similar *Campylobacter* concentrations as whole carcasses and potentially providing the same risk of exposure to the public, regardless of part type. The comminuted simulation results can serve as a benchmark for future studies since the SR-MA and the baseline simulation could not be compared to commercial processing plant data.

Simulating a chicken processing plant presented several limitations. The SR-MA to develop the baseline included before-after studies with samples obtained from commercial processing plants or pilot plants using samples with natural *Campylobacter* contamination. There are limitations in the availability of trials for cut-up parts and comminuted product in literature, from commercial processing plants, and USDA-FSIS. Access to perform controlled trials in U.S. commercial facilities is limited and only internal testing from integrators was available. Additional observations on the effect on *Campylobacter* concentration using reduced intervention levels or chlorine in cut-up parts, and comminuted product are not available because regulatory limitations do not allow for testing without interventions. Some uncertainties include the sampling locations and sampling matrices to determine initial concentration. Even though the initial

concentration was comparable to initial *Campylobacter* concentrations obtained from commercial processing plants, the SR-MA contained studies where incoming load was determined by sampling at different locations before the scald and sampled several matrices from carcass rinses to cecal content (Berghaus et al., 2013; Berrang, Buhr, et al., 2000; Kotula & Pandya, 1995; Mead et al., 1995; Potturi-Venkata et al., 2007; Stern & Robach, 2003). Another uncertainty is the impact of cross contamination on *Campylobacter* levels throughout the process. Cross contamination in feather picking is known to occur, but the rate of cross-contamination per bird and how much equipment contributes to cross contamination throughout subsequent steps was not factored in the simulations process perhaps underestimating *Campylobacter* concentrations in the model. The effect on the different sampling locations could not be included. A source of variability included the wide range of sample rinse volumes, detection methods, and sample matrices. Carcass rinse data extracted from the studies ranged from 200 mL to 400 mL. There are differences in the amount of *Campylobacter* concentrations obtained from different rinse volumes (Williams et al., 2010). The type of rinse and the type of enrichment and plating method have different sensitivities that may influence concentration data from studies (Gonsalves et al., 2016; Hiatt, 2017; Line et al., 2001). The SR-MA was sufficient in providing data for the simulation models.

### Intervention efficacy analysis

The intervention efficacy analysis demonstrated that single interventions could have a significant effect on improving a processing plant's ability of reducing *Campylobacter* concentrations. Air chilling was included in the scenario analysis as an alternative chilling stage. Air chilling was ineffective for *Campylobacter* control. Pre-chill and post-chill interventions may need to be validated as control measures with air-chillers. Post-chill immersion interventions, particularly PAA and CPC, were the most effective interventions. Pre-chill interventions are less effective than post-chill interventions. *Campylobacter* is present at higher levels and chicken carcasses may not be exposed to the intervention for long periods of time prior to chilling. Intestinal content, debris, or tissues that contain high levels of *Campylobacter* may still be present at pre-chill stages. Post-chill interventions are applied after all major *Campylobacter*

contamination sources like feathers, viscera, and intestinal content have been removed and the carcasses have gone through a washing process to remove all visible debris. This allows the chemical to act on the actual product and not compete with other organic material that can lower its effectiveness. There may be a carryover factor in these results that may overestimate the results. Chemical interventions, like PAA, are the last hurdle that is applied in a commercial processing setting before packaging or grinding.

The multiple intervention scenarios analyzing the application of PAA resulted in undetectable concentrations of *Campylobacter*. However, these levels are overestimated, and these concentration levels may not reflect a real-world scenario. It is important to note that single interventions are effective, but a multi-hurdle system, including pre-chill and post-chill interventions is most effective against *Campylobacter*.

The single intervention and multiple interventions model present *Campylobacter* concentrations after chemical interventions comparable to the commercial processing bio-map. These results validate that the baseline model without interventions presents a possible outcome of *Campylobacter* concentrations if interventions are not applied and it can be used as a starting point to model future exposure assessments modules. However, the model presents several limitations. Much of the data utilized for intervention efficacy analysis was extracted from a limited number of studies. PAA is the most used chemical antimicrobial in processing thus more studies are available, particularly for pre- and post-chill applications. Models studying the variations in immersion chilling will allow for better assessments. The limited number of studies limits the amount of data points used to reduce variation in the results. The general risk assessment model only analyzed overall intervention data. It did not consider different concentrations of the chemical, contact time and pH levels, which can influence the effectiveness of many of these interventions. Other lesser-known interventions from the SR-MA were not included because of the limited number of studies available, and commonly used chemicals were only considered for this analysis.

In conclusion, the SR-MA provided sufficient data to build a baseline model that presented a possible scenario of *Campylobacter* concentration levels in chicken parts and comminuted product from U.S. poultry

processing plants without interventions. Estimates for concentration and concentration changes can be calculated through the SR-MA without simulation. This simulation model is an option to observe and analyze data changes overtime and it can be modified by incorporating testing results from integrators and future SR-MA. The simulation model can be used to compare the efficacy of interventions used in different processing plants to assess its ability to reduce *Campylobacter* concentration. The model can be improved by obtaining more data of concentrations before and after the cut-up process and comminuted product. The model can also be modified to evaluate what contributes to differences between individual cut-up parts and ground product categories. Chicken cut-up parts and comminuted product undergoing a multi-hurdle approach to *Campylobacter* control has similar concentrations to whole birds.

This assessment shows that a typical U.S. processing plant has the ability to achieve reductions in *Campylobacter* concentration without the use of interventions. Additional reductions are achieved when incorporating interventions to whole birds after the chilling process, where concentrations below 1 log<sub>10</sub> CFU/mL can be obtained. Adding additional interventions after the cut-up process maintain similar concentrations that were achieved by the previous stages and minimize the effect of cross contamination. Interventions after the cut-up stage reduces the variation between cut-up parts categories, reducing the possibility of exposure to levels above 1 log<sub>10</sub> CFU/mL from parts and comminuted product. The likelihood of exposure to an infectious dose of *Campylobacter* from cut-up parts and comminuted product is similar to the likelihood of exposure from whole birds. Cut-up parts should be included in future public health risk assessments since this is the product type that is mostly consumed in the U.S., thus increasing the *Campylobacter* exposure risk. A processing plant that implements multiple interventions can lower concentrations but cannot provide products free of *Campylobacter*. Interventions in subsequent steps in the production chain must also be assessed to reduce exposure risk. Additional data is needed to assess comminuted chicken as a risk factor, and additional chemical and non-chemical interventions must be assessed to have alternatives available for use.

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**Table 4.1: Characteristics of Included Studies from Systematic Review**

<b>Reference</b>	<b>Processing Stage</b>	<b>Study type</b>	<b>Location</b>	<b>Equipment</b>	<b>Treatment</b>	<b>Sample Type</b>	<b>Unit of Enumeration</b>
Berghaus et al. (2013)	Receiving (Incoming Load)	Commercial plant before/after study	Receiving	Transport coops	None	Carcass Rinse	log MPN/carcass
Berrang, Buhr, et al. (2000)	Receiving (Incoming Load)	Commercial plant before/after study	Kill step	Blood tunnel	None	Carcass and Viscera Samples	log CFU/g
Kotula and Pandya (1995)	Receiving (Incoming Load)	Commercial plant before/after study	Scalding	Scalder	None	Skin/Feathers /Feet Samples	log CFU/g
Mead et al. (1995)	Receiving (Incoming Load)	Commercial plant before/after study	Kill step	Automatic killing machine	None	Skin/ Cecal Samples	log CFU/g
Potturi-Venkata et al. (2007)	Receiving (Incoming Load)	Commercial plant before/after study	Farm samples	None	None	Fecal Samples	log CFU/mL
Stern and Robach (2003)	Receiving (Incoming Load)	Commercial plant before/after study	Farm samples	None	None	Fecal Samples	log CFU/g
DeVillena et al. (2022)	Receiving (Incoming Load)	Commercial plant before/after study	Receiving	None	None	Carcass Rinse	log CFU/mL
Berrang et al. (2003)	Scalding	Commercial plant before/after study	Scalding	Scalding bath	None	Carcass Rinse	log CFU/mL
Berrang, Windham, et al. (2011)	Scalding	Commercial plant before/after study	Scalding	Scalding bath	None/ High pH	Carcass Rinse	log CFU/mL
Berrang and Dickens (2000)	Scalding	Commercial plant before/after study	Scalding	Spray Cabinet	Chlorine	Carcass Rinse	log CFU/mL

**Table 4.1 (Continued): Characteristics of Included Studies from Systematic Review**

<b>Reference</b>	<b>Processing Stage</b>	<b>Study type</b>	<b>Location</b>	<b>Equipment</b>	<b>Treatment</b>	<b>Sample Type</b>	<b>Unit of Enumeration</b>
Berrang et al. (2001)	Feather Picking	Pilot plant before/after study	Post-Pick	Feather Picker	None/Chlorine/ Cloacal Plug	Sponge	log CFU/mL
Berrang and Dickens (2000)	Feather Picking	Commercial plant before/after	Post-Pick	Feather Picker	Chlorine	Carcass Rinse	log CFU/mL
Berrang, Dickens, et al. (2000)	Feather Picking	Pilot plant before/after study	Post-Pick	Scalding Tanks and Spray	Post Immersion rescald/ Post spray rescald	Carcass Rinse	log CFU/mL
Berrang et al. (2006a)	Feather Picking	Pilot plant before/after study	Post-Pick	Feather Picker	Cloacal Wash with Vinegar	Sponge	log CFU/mL
Berrang et al. (2006b)	Feather Picking	Pilot plant before/after study	Post-Pick	Feather Picker	Cloacal Wash with Organic Acids	Sponge	log CFU/mL
Berrang, Meinersmann, et al. (2011)	Feather Picking	Commercial plant before/after	Post pick	Feather Picker	None/ ClO <sub>2</sub>	Carcass Rinse	log CFU/mL
Berrang, Windham, et al. (2011)	Feather Picking	Commercial plant before/after	Post-Pick	Post pick dip Post-Pick tank	Chlorine	Carcass Rinse	log CFU/mL
Berrang et al. (2018)	Feather Picking	Pilot plant before/after study	Post-Pick	Feather Picker	None/Cloacal Plug	Sponge	log CFU/mL
Musgrove et al. (1997)	Feather Picking	Commercial plant before/after	Post pick	Scald and pickers	Cloacal plug	Carcass Rinse	log CFU/mL
Berghaus et al. (2013)	Rehang	Commercial plant before/after	Rehang	Rehanger	None	Carcass Rinse	log MPN/ Carcass

Table 4.1 (Continued): Characteristics of Included Studies from Systematic Review

Reference	Processing Stage	Study type	Location	Equipment	Treatment	Sample Type	Unit of Enumeration
Berrang et al. (2007)	Rehang	Commercial plant before/after study	Rehang	Rehang	Chlorine	Carcass Rinse	log CFU/mL
DeVillena et al. (2022)	Rehang	Commercial plant before/after study	Rehang	Rehang	Chlorine/ PAA	Carcass Rinse	log CFU/mL
Northcutt, Berrang, et al. (2003)	Evisceration	Pilot plant before/after study	Post-Evisceration	In-line evis equipment	None	Carcass Rinse	log CFU/mL
Meredith et al. (2013)	Evisceration	Commercial plant before/after study	Post-Evisceration	In-line evis equipment	None/Cloacal Wash with Organic	Carcass Swabs	log CFU/cm <sup>2</sup>
Berrang and Dickens (2000)	Evisceration	Commercial plant before/after study	Post-Evisceration	In-line evis equipment	Chlorine	Carcass Rinse	log CFU/mL
DeVillena et al. (2022)	Evisceration	Commercial plant before/after study	Post-Evisceration	In-line evis equipment	Chlorine	Carcass Rinse	log CFU/mL
Oyarzabal et al. (2004)	Carcass Wash	Commercial plant before/after study	Carcass washing	IOBW	None	Carcass Rinse	log CFU/ml
Berrang and Dickens (2000)	Carcass Wash	Commercial plant before/after study	Carcass washing	IOBW	Chlorine	Carcass Rinse	log CFU/ml
Berghaus et al. (2013)	Carcass Wash	Commercial plant before/after study	Carcass washing	IOBW	None	Carcass Rinse	log MPN/carcass
James et al. (2007)	Carcass Wash	Pilot plant challenge study	Carcass washing	Pre-Chill Spray	Steam	Skin Rinse	log CFU/cm <sup>2</sup>

**Table 4.1 (Continued): Characteristics of Included Studies from Systematic Review**

Reference	Processing Stage	Study type	Location	Equipment	Treatment	Sample Type	Unit of Enumeration
Li et al. (2002)	Carcass Wash	Pilot plant challenge study	Carcass washing	IOBW	Chlorine/High Temperature Wash	Carcass Rinse	log MPN/carcass
Zhang et al. (2011)	Carcass Wash	Commercial plant before/after study	Carcass washing	Pre-Chill Spray	CPC	Carcass Rinse	log CFU/mL
DeVillena et al. (2022)	Carcass Wash	Commercial plant before/after study	Carcass washing	IOBW/Pre-Chill Spray	Chlorine/PAA	Carcass Rinse	log CFU/mL
Zhang et al. (2011)	Carcass Chill	Commercial plant before/after study	Chiller	Immersion chiller	Chlorine/Air Chill	Carcass Rinse	log CFU/mL
Stern and Robach (2003)	Carcass Chill	Commercial plant before/after study	Chiller	Immersion chiller	Chlorine	Carcass Rinse	log CFU/carcass
Potturi-Venkata et al. (2007)	Carcass Chill	Commercial plant before/after study	Chiller	Immersion chiller	None	Carcass Rinse	log CFU/mL
Oyarzabal et al. (2004)	Carcass Chill	Commercial plant before/after study	Chiller/Post-Chill	Immersion chiller/Post-Chill Tank	ASC	Carcass Rinse	log CFU/mL
Northcutt et al. (2006)	Carcass Chill	Pilot plant before/after study	Chiller	Immersion chiller	None	Carcass Rinse	log CFU/mL
Northcutt, Smith, et al. (2008)	Carcass Chill	Commercial plant before/after study	Chiller	Immersion chiller	Chlorine	Carcass Rinse	log CFU/mL
Northcutt, Cason, et al. (2008)	Carcass Chill	Pilot plant before/after study	Chiller	Immersion chiller	None	Carcass Rinse	log CFU/mL

**Table 4.1 (Continued): Characteristics of Included Studies from Systematic Review**

Reference	Processing Stage	Study type	Location	Equipment	Treatment	Sample Type	Unit of Enumeration
Huezo et al. (2007)	Carcass Chill	Pilot plant before/after study	Chiller	Immersion chiller/Air Chiller	None	Carcass Rinse	log CFU/mL
Cason et al. (1997)	Carcass Chill	Commercial plant before/after	Chiller	Immersion chiller	None	Carcass Rinse	log CFU/ carcass
Berrang and Dickens (2000)	Carcass Chill	Commercial plant before/after	Chiller	Immersion chiller	Chlorine	Carcass Rinse	log CFU/mL
Berrang et al. (2008)	Carcass Chill	Pilot plant before/after study	Chiller	Immersion chiller/Air Chiller	None	Carcass Rinse	log CFU/mL
Berrang et al. (2007)	Carcass Chill	Commercial plant before/after	Chiller/ Post-Chill	Immersion chiller	Chlorine	Carcass Rinse	log CFU/mL
Berghaus et al. (2013)	Carcass Chill	Commercial plant before/after	Pre-Chill/ Chiller	IOBW/ Immersion Chiller	None	Carcass Rinse	log MPN/carcass
Smith et al. (2015)	Carcass Chill	Lab challenge study	Post-Chill	Dip Tank/Spray Cabinet	None/ Chlorine/ PAA	Carcass Rinse	log CFU/mL
Nagel et al. (2013)	Carcass Chill	Pilot plant challenge study	Post-Chill	Dip Tank	None/Chlorine/ PAA	Carcass Rinse	log CFU/mL
DeVillena et al. (2022)	Carcass Chill	Commercial plant before/after	Post-Chill	Dip Tank	None/Chlorine/ PAA/ Lysozyme	Carcass Rinse	log CFU/mL
Bourassa et al. (2021)	Parts	Lab challenge study	Post-Chill processing	Dip Tank/Spray Cabinet	None/HP/ PAA	Wing Rinse	log CFU/mL

**Table 4.1 (Continued): Characteristics of Included Studies from Systematic Review**

<b>Reference</b>	<b>Processing Stage</b>	<b>Study type</b>	<b>Location</b>	<b>Equipment</b>	<b>Treatment</b>	<b>Sample Type</b>	<b>Unit of Enumeration</b>
Gonzalez et al. (2021)	Parts	Lab challenge study	NA	Dip Tank/Spray Cabinet	None/SSS/FA/PAA	Wing Rinse	log CFU/mL
Gunther et al. (2016)	Parts	Lab challenge study	NA	UV	UV	Skin Homogenate	log CFU/mL
Haughton, Lyng, et al. (2012)	Parts	Lab challenge study	NA	UV/PEF	UV/PEF	Skin/Boneless Breast	log CFU/g
Hinton and Ingram (2005)	Parts	Lab challenge study	NA	Spray Cabinet	TPP	Skin Rinse	log CFU/mL
Kataria et al. (2020)	Parts	Lab challenge study	NA	Dip Tank	PAA	Wing Rinse	log CFU/g
Kumar et al. (2020)	Parts	Lab challenge study	NA	Dip Tank/Spray Cabinet	PAA	Boneless Breast Rinse	log CFU/mL
Sarjit and Dykes (2015)	Parts	Lab challenge study	NA	Spin Chiller	None/Chlorine/TSP	Breast Meat with Skin	log CFU/ cm2
Vaddu et al. (2021)	Parts	Lab challenge study	NA	Dip Tank	PAA	Wing Rinse	log CFU/mL
Zang et al. (2018)	Parts	Pilot plant challenge study	NA	Dip Tank	None/Chlorine/ASC/ PAA/ CPC	Various Parts	log CFU/mL
DeVillena et al. (2022)	Parts	Commercial plant before/after	Post-Cut-Up	Dip Tank	Chlorine/PAA	Wing Rinse	log CFU/mL

**Table 4.1 (Continued): Characteristics of Included Studies from Systematic Review**

Reference	Processing Stage	Study type	Location	Equipment	Treatment	Sample Type	Unit of Enumeration
Chen et al. (2014)	Comminuted	Pilot plant challenge study	Cut up /Grinding	Dip Tank/ Spray	None/Chlorine/ PAA/ CPC	Ground Chicken Breast/Thighs	log CFU/g
Park et al. (2017)	Comminuted	Lab challenge study	NA	Dip tanks	None/Chlorine/ PAA	Ground Chicken Breast	log CFU/g

**Table 4.2: Input Parameters for Baseline Model Simulation from the SR-MA**

Processing Stage	Concentration Change Distribution	Unit
Receiving (Initial Concentration)	Normal(4.81,0.46)	log <sub>10</sub> CFU/mL
Scalding	Normal(-2.86,0.66)	log <sub>10</sub> CFU/mL
Feather Picking	Normal(1.17,0.45)	log <sub>10</sub> CFU/mL
Evisceration	Normal(0.13,0.44)	log <sub>10</sub> CFU/mL
Carcass Wash	Normal(-0.39,0.33)	log <sub>10</sub> CFU/mL
Carcass Chill	Normal(-1.48,0.23)	log <sub>10</sub> CFU/mL
Cut Up Parts	Normal(-0.58,0.16)	log <sub>10</sub> CFU/mL
Comminuted Chicken	Normal(-0.35,0.26)	log <sub>10</sub> CFU/mL

**Table 4.3: Input Distributions of Processing Interventions from the SR-MA Used for Intervention Efficacy Analysis**

Intervention Type	Concentration Change Distribution	Unit
1. Pre-Chill Immersion Treatment	Normal(-1.11,0.45)	log <sub>10</sub> CFU/mL
a. PAA	Normal(-1.13,0.47)	log <sub>10</sub> CFU/mL
2. Pre-Chill Spray Treatment	Normal(-0.77,0.27)	log <sub>10</sub> CFU/mL
a. PAA	Normal(-0.50,0.29)	log <sub>10</sub> CFU/mL
b. CPC	Normal(-1.56,2.11)	log <sub>10</sub> CFU/mL
3. Air Chiller	Normal(-1.05,0.54)	log <sub>10</sub> CFU/mL
4. Post-Chill Immersion Treatment - Whole Carcass	Normal(-1.87,0.21)	log <sub>10</sub> CFU/mL
a. PAA	Normal(-1.79,0.25)	log <sub>10</sub> CFU/mL
b. ASC	Normal(-0.68,0.57)	log <sub>10</sub> CFU/mL
c. CPC	Normal(-4.29,0.85)	log <sub>10</sub> CFU/mL
d. TSP	Normal(-1.69,0.46)	log <sub>10</sub> CFU/mL
5. Post-Chill Spray Treatment - Whole Carcass	Normal(-1.22,0.27)	log <sub>10</sub> CFU/mL
a. PAA	Normal(-1.52,0.55)	log <sub>10</sub> CFU/mL
b. CPC	Normal(-0.75,0.95)	log <sub>10</sub> CFU/mL
c. TPP	Normal(-1.53,1.18)	log <sub>10</sub> CFU/mL
4. Post-Cut-Up Immersion Treatment - Cut-Up Parts	Normal(-1.87,0.21)	log <sub>10</sub> CFU/mL
a. PAA	Normal(-1.79,0.25)	log <sub>10</sub> CFU/mL
b. ASC	Normal(-0.68,0.57)	log <sub>10</sub> CFU/mL
c. CPC	Normal(-4.29,0.85)	log <sub>10</sub> CFU/mL
d. TSP	Normal(-1.69,0.46)	log <sub>10</sub> CFU/mL
5. Post-Cut-Up Spray Treatment - Cut-Up Parts	Normal(-1.22,0.27)	log <sub>10</sub> CFU/mL
a. PAA	Normal(-1.52,0.55)	log <sub>10</sub> CFU/mL
b. CPC	Normal(-0.75,0.95)	log <sub>10</sub> CFU/mL
c. TPP	Normal(-1.53,1.18)	log <sub>10</sub> CFU/mL

**Table 4.4: Intervention Efficacy Analysis for Single Intervention Scenarios for Whole Birds**

Scenario	Concentration (CFU/mL)	Lower 95% CI	Upper 95% CI	Intervention Efficacy (%)
Baseline	23.44	0.06	10715.19	-
Pre-Chill Immersion	1.95	0.00	1071.52	91.68
a. Whole Bird Dip Tank - PAA	1.78	0.02	173.78	92.41
Pre-Chill Spray	4.07	0.06	275.42	82.62
a. Whole Bird Spray - PAA	7.59	0.11	549.54	67.64
b. Whole Bird Spray - CPC	0.07	0.00	5623.41	99.72
Air Chiller	64.57	0.65	6025.60	Not effective
Post Chill Dip Tank Whole Birds	0.35	0.00	174.98	98.52
a. Whole Bird Dip Tank - PAA	0.42	0.00	212.81	98.22
b. Whole Bird Dip Tank - ASC	5.25	0.01	3539.97	77.61
c. Whole Bird Dip Tank - CPC	0.00	0.00	1.41	99.99
d. Whole Bird Dip Tank - TSP	0.51	0.00	306.20	97.81
Post Chill Spray Whole Birds	1.55	0.00	837.53	93.39
a. Whole Bird Spray - PAA	0.78	0.00	498.88	96.69
b. Whole Bird Spray - CPC	4.57	0.00	7620.79	80.50

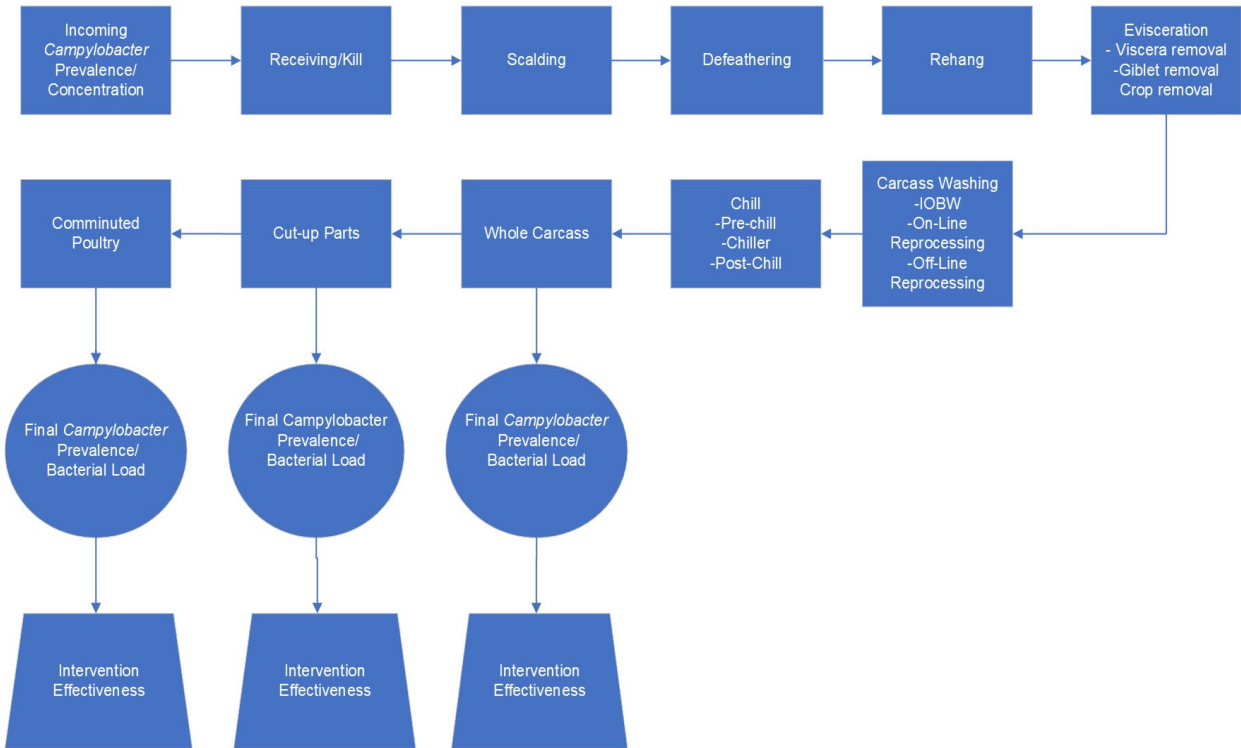
**Table 4.5: Intervention Efficacy Analysis for Single Intervention Scenarios for Cut-up Parts**

Scenario	Concentration (CFU/mL)	Lower 95% CI	Upper 95% CI	Intervention Efficacy (%)
Baseline	6.31	0.01	3801.89	-
1. Pre-Chill Immersion Treatment	0.50	0.00	363.08	92.06
a. PAA	0.47	0.00	46.77	92.59
2. Pre-Chill Spray Treatment	1.07	0.01	64.57	83.02
a. PAA	2.00	0.03	151.36	68.38
b. CPC	0.17	0.00	1380.38	97.25
3. Air Chiller	16.98	0.16	1659.59	Not Effective
4. Post-Chill Immersion Treatment - Whole Carcass	0.09	0.01	3749.73	98.59
a. PAA	0.11	0.00	65.01	98.30
b. ASC	1.32	0.00	1177.61	79.11
c. CPC	0.00	0.00	0.46	99.99
d. TSP	0.13	0.00	100.93	97.91
5. Post-Chill Spray Treatment - Whole Carcass	0.39	0.00	263.03	93.83
a. PAA	0.19	0.00	161.06	96.91
b. CPC	1.15	0.00	2443.43	81.80
c. TPP	0.19	0.00	539.51	96.91
6. Post Cut-Up Immersion Treatment - Cut-Up Parts	0.35	0.00	170.22	94.50
a. PAA	0.42	0.00	199.99	93.39
b. ASC	5.25	0.01	3515.60	16.82
c. CPC	0.00	0.00	1.41	99.98
d. TSP	0.51	0.00	356.45	91.87
7. Post Cut-Up Spray Treatment - Cut- Up Parts	1.55	0.00	746.45	75.45
a. PAA	0.78	0.00	572.80	87.70
b. CPC	4.57	0.00	7095.78	27.56
c. TPP	0.76	0.00	2123.24	87.98

**Table 4.6: Intervention Efficacy Analysis for Single Intervention Scenarios For Comminuted**

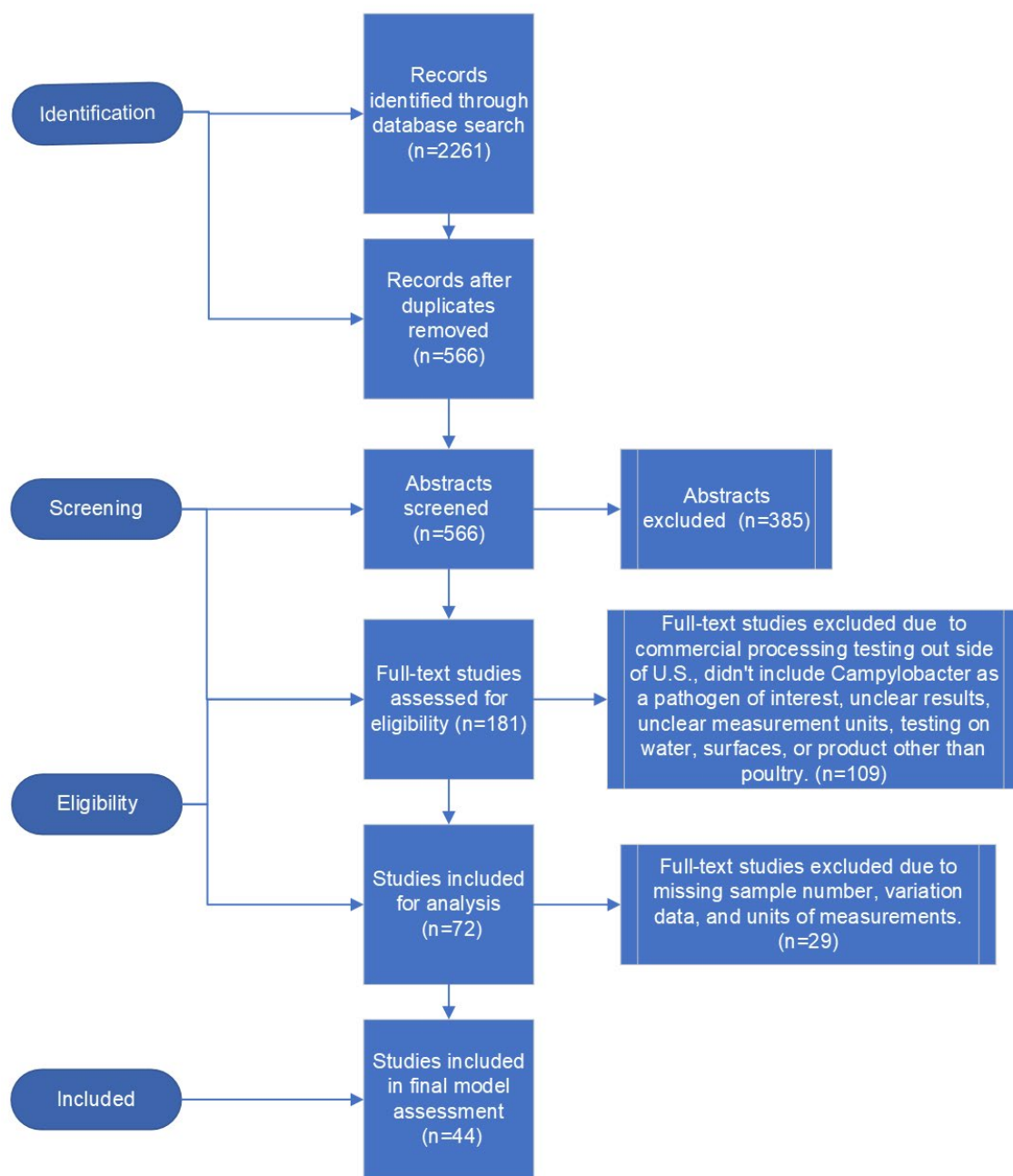
**Product**

Scenario	Concentration (CFU/mL)	Lower 95% CI	Upper 95% CI	Intervention Efficacy (%)
Baseline	2.82	0.00	2290.87	-
1. Pre-Chill Immersion Treatment	0.23	0.00	199.53	91.87
a. PAA	0.21	0.00	21.88	92.59
2. Pre-Chill Spray Treatment	0.48	0.01	39.81	83.02
a. PAA	0.89	0.01	70.79	68.38
b. CPC	0.08	0.00	660.69	97.25
3. Air Chiller	7.59	0.07	794.33	Not Effective
4. Post-Chill Immersion Treatment - Whole Carcass	0.04	0.00	2249.05	98.55
a. PAA	0.05	0.00	40.36	98.26
b. ASC	0.60	0.00	732.82	78.62
c. CPC	0.00	0.00	0.26	99.99
d. TSP	0.06	0.00	58.88	97.86
5. Post-Chill Spray Treatment - Whole Carcass	0.18	0.00	148.25	93.69
a. PAA	0.09	0.00	92.47	96.84
b. CPC	0.52	0.00	1393.16	81.38
c. TPP	0.09	0.00	292.42	96.91
6. Post Cut-Up Immersion Treatment - Cut-Up Parts	0.16	0.00	100.69	94.38
a. PAA	0.19	0.00	118.03	93.24
b. ASC	2.34	0.00	1901.08	16.82
c. CPC	0.00	0.00	0.81	99.98
d. TSP	0.23	0.00	192.31	91.68
7. Post Cut-Up Spray Treatment - Cut- Up Parts	0.69	0.00	504.66	75.45
a. PAA	0.35	0.00	309.03	87.70
b. CPC	2.04	0.00	4236.43	27.56
c. TPP	0.35	0.00	1132.40	87.70

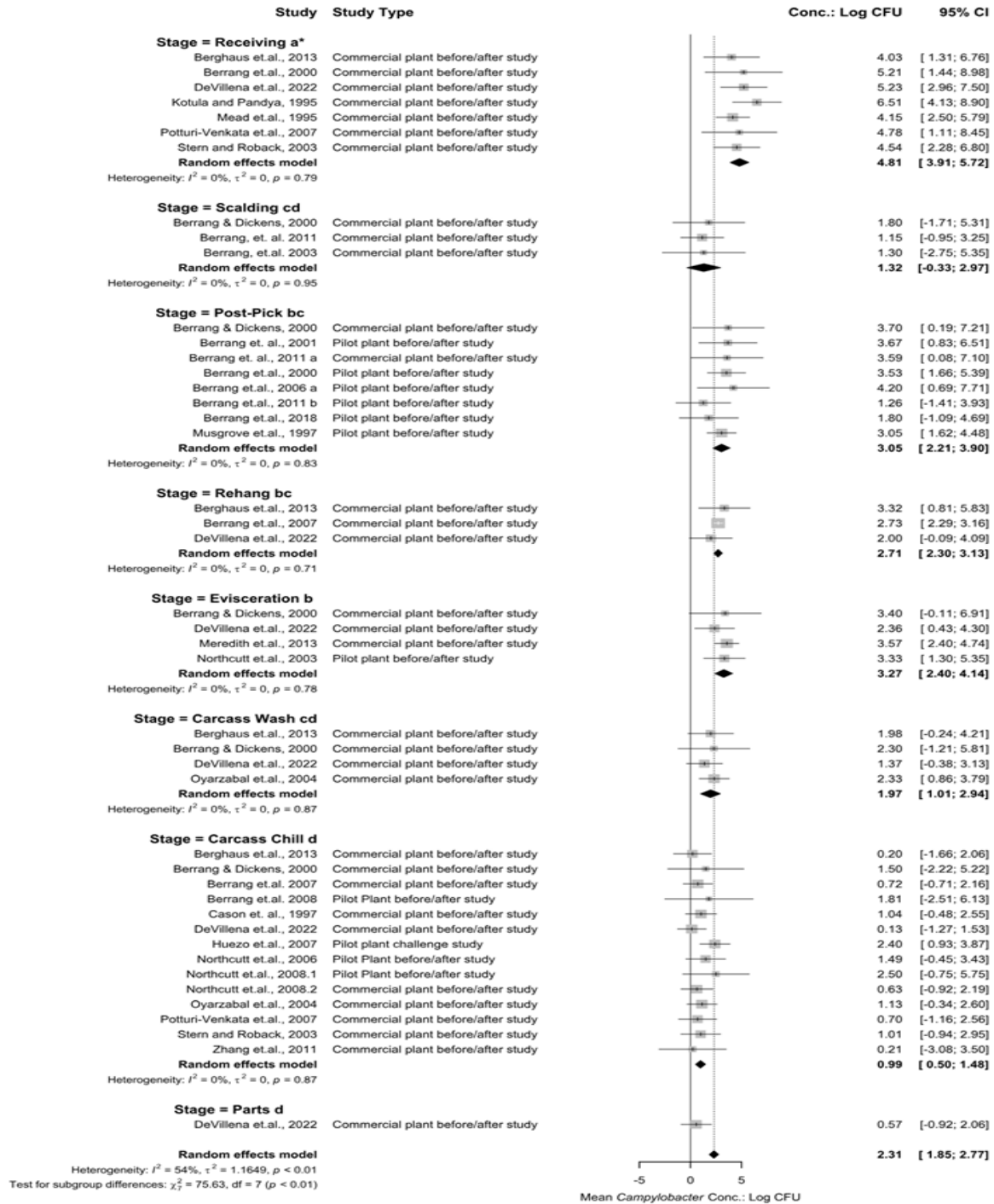


**Figure 4.1:** Flow Diagram of Poultry Processing Stages Used for Exposure Assessment

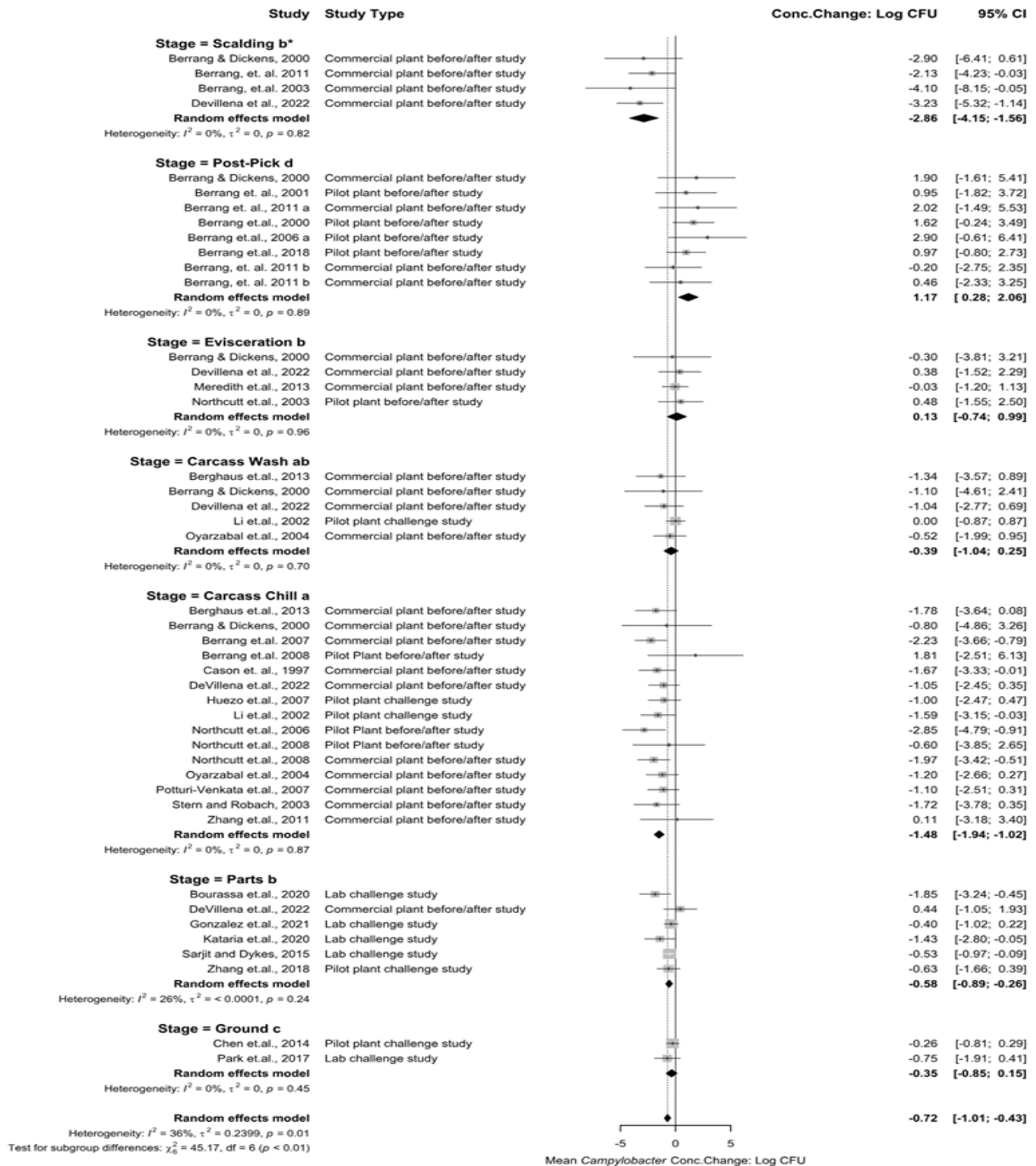
\*IOBW = Inside/Outside Bird Washer



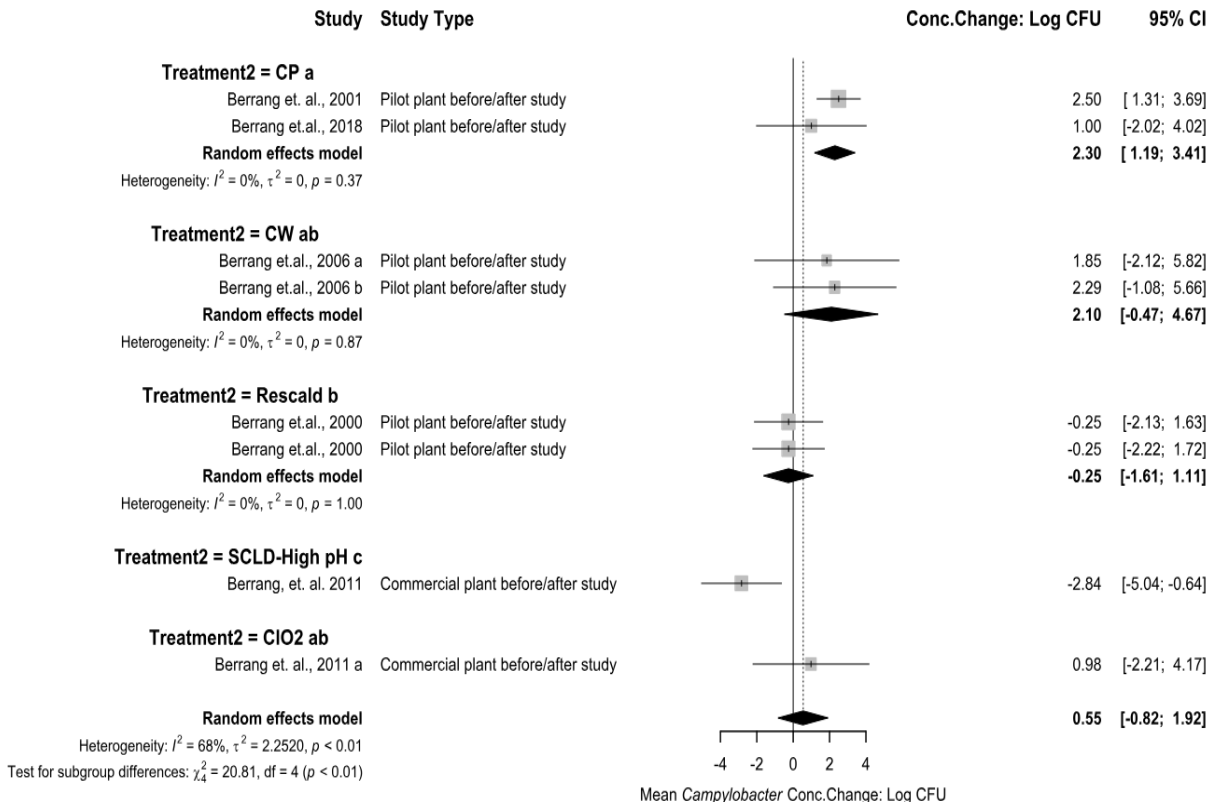
**Figure 4.2:** Flow Chart of the Systematic Review Process



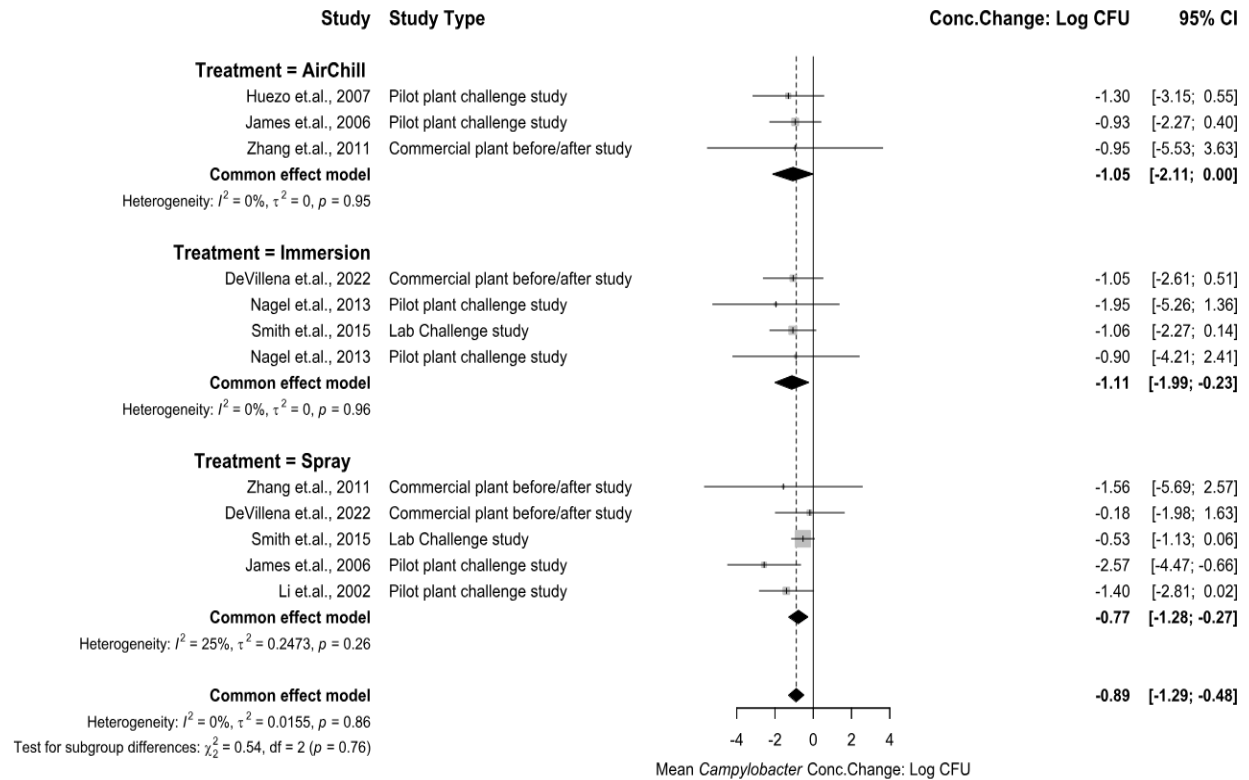
**Figure 4.3:** *Campylobacter* Concentration per Stage Without Reported Interventions or Chlorine. The random effects model results represent the mean concentration per stage for the control group that includes studies without reported interventions or chlorine. Distinct letters next to the stage description indicate statistically significant differences ( $P < 0.05$ ).



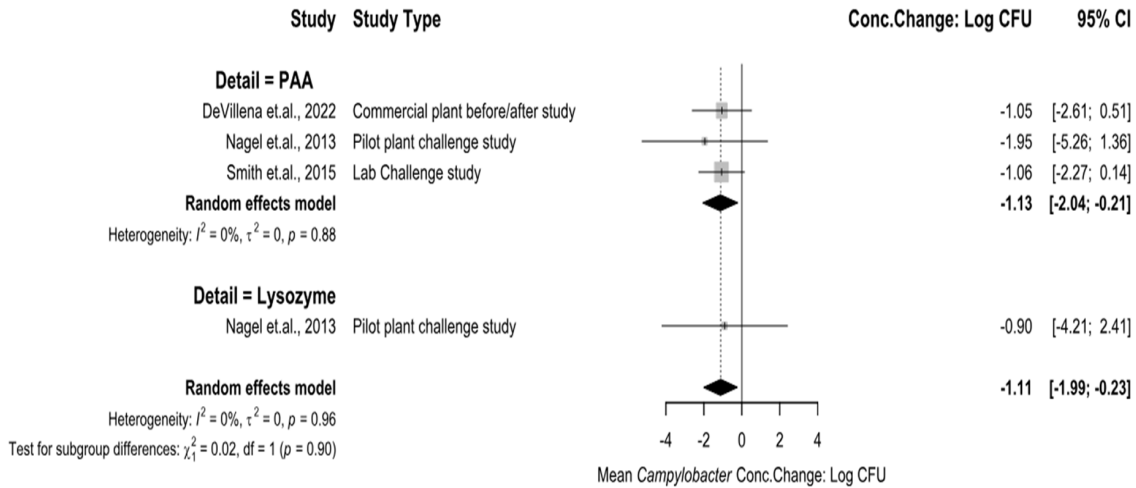
**Figure 4.4:** *Campylobacter* Concentration Change per Stage Without Reported Interventions or Chlorine. The random effects model results represent the mean concentration change per stage for the control group that includes studies without reported interventions or chlorine. Results  $> 0$  indicate an increase in concentration. Results  $< 0$  indicate a decrease. Results  $= 0$  indicate no change. Distinct letters next to the stage description indicate statistically significant differences ( $P < 0.05$ ).



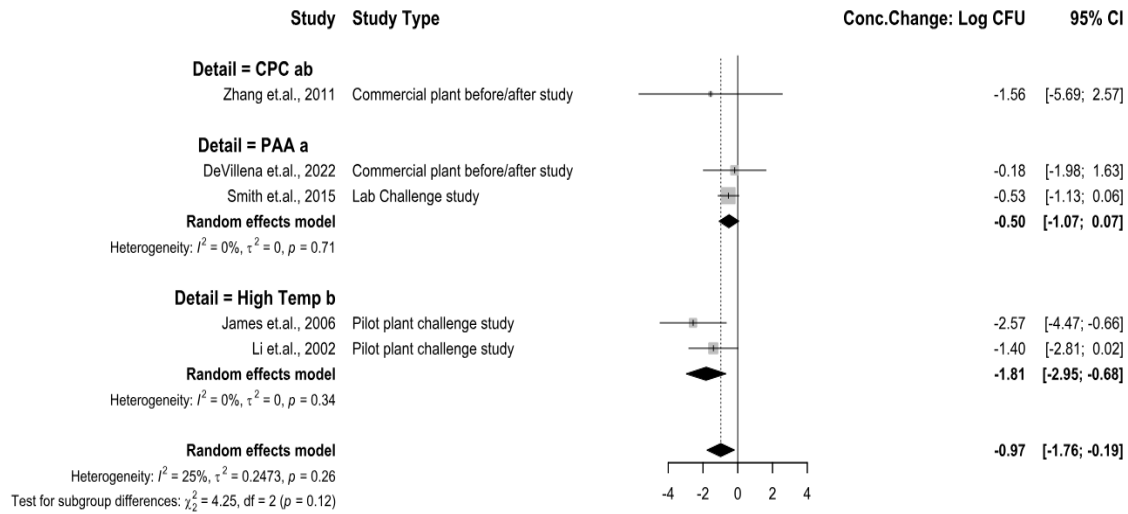
**Figure 4.5:** *Campylobacter* Concentration Change of Interventions at Scalding and Feather Picking. The random effects model results represent the mean concentration change of included interventions at the scald and feather picking stages. Results  $> 0$  indicate an increase in concentration. Results  $< 0$  indicate a decrease. Results  $= 0$  indicate no change. Distinct letters next to the stage description indicate statistically significant differences ( $P < 0.05$ ).



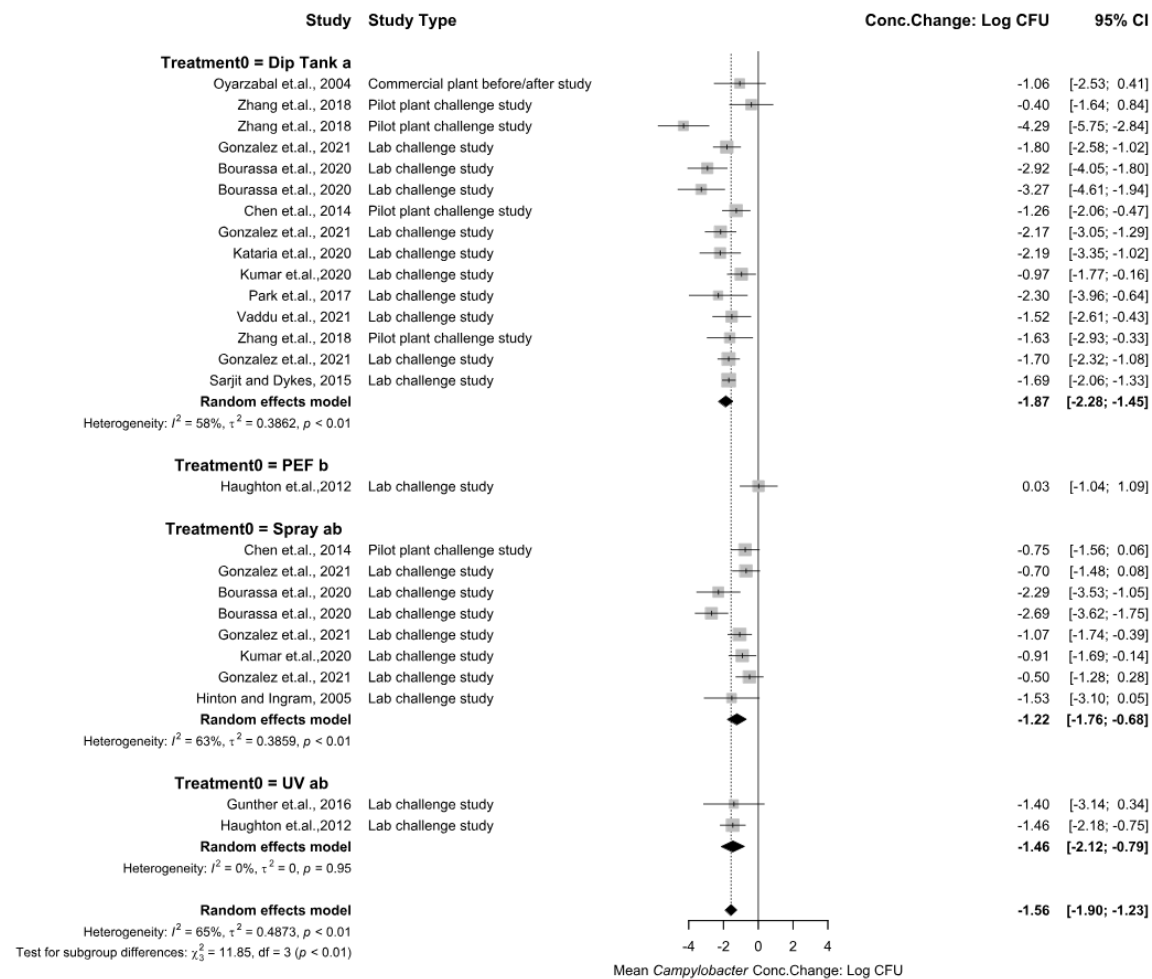
**Figure 4.6:** *Campylobacter* Concentration Change of Intervention Application at Pre-chill and Air Chilling Stages. The random effects model results represent the mean concentration change of included interventions at the pre-chill and air chill picking stages. Results > 0 indicate an increase in concentration. Results < 0 indicate a decrease. Results = 0 indicate no change. Distinct letters next to the treatment description indicate statistically significant differences ( $P < 0.05$ ).



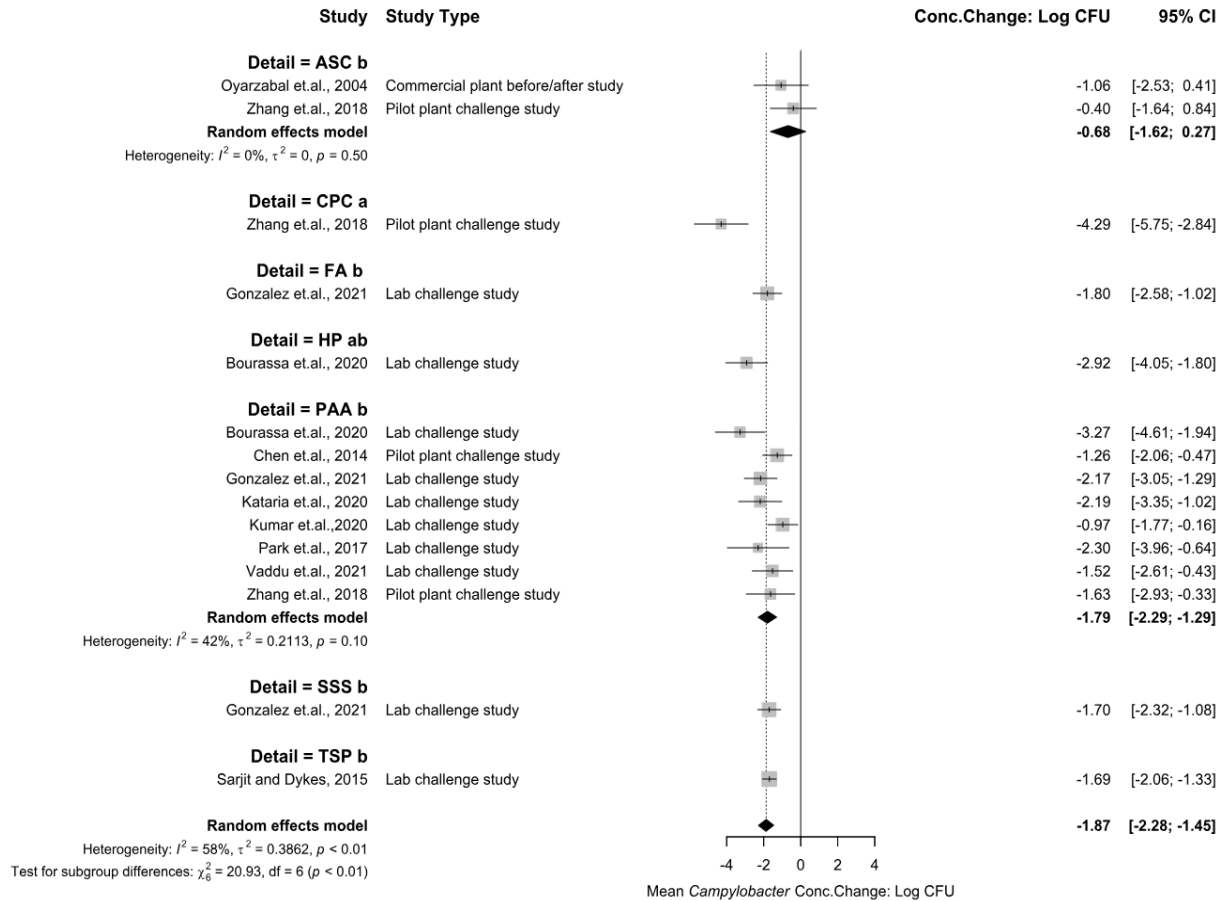
**Figure 4.7:** *Campylobacter* Concentration Change of Immersion Interventions at Pre-chill Stage. The random effects model results represent the mean concentration change of included immersion interventions at the pre-chill stage. Results > 0 indicate an increase in concentration. Results < 0 indicate a decrease. Results = 0 indicate no change. Distinct letters next to the treatment. description indicate statistically significant differences ( $P < 0.05$ ).



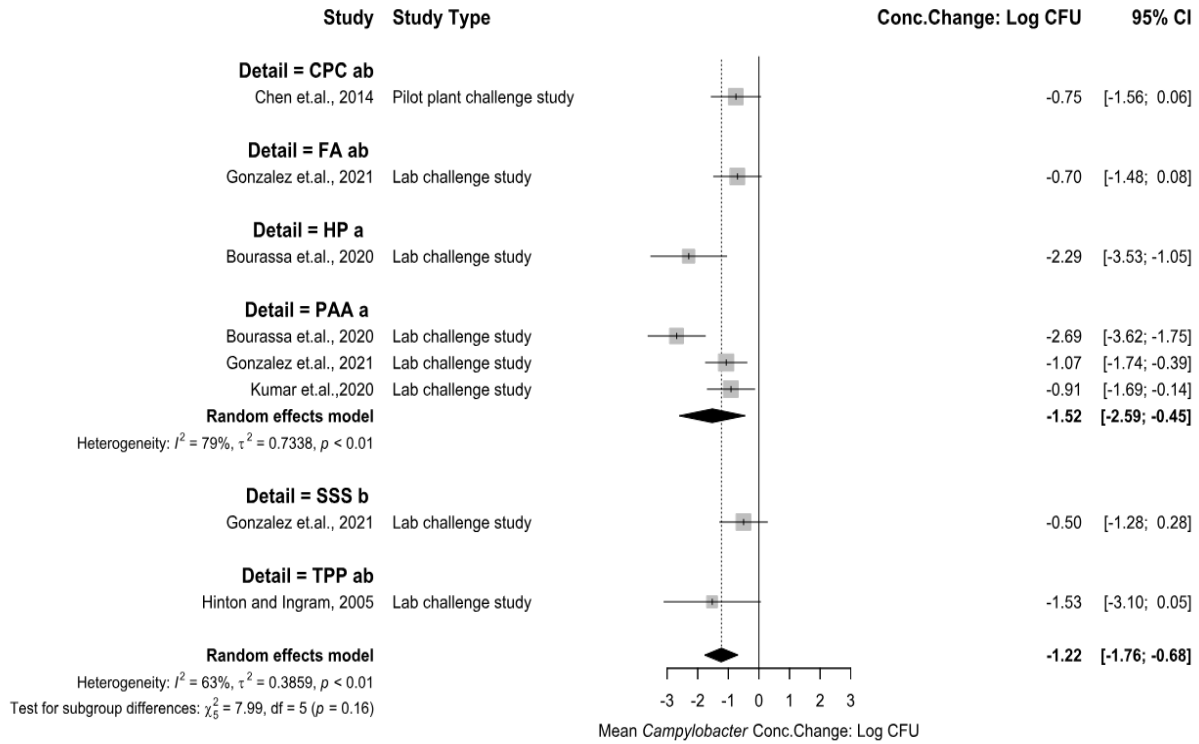
**Figure 4.8:** *Campylobacter* Concentration Change for Spray Interventions at Pre-chill Stage. The random effects model results represent the mean concentration change of included spray interventions at the pre-chill stage. Results  $> 0$  indicate an increase in concentration. Results  $< 0$  indicate a decrease. Results  $= 0$  indicate no change. Distinct letters next to the treatment description indicate statistically significant differences ( $P < 0.05$ ).



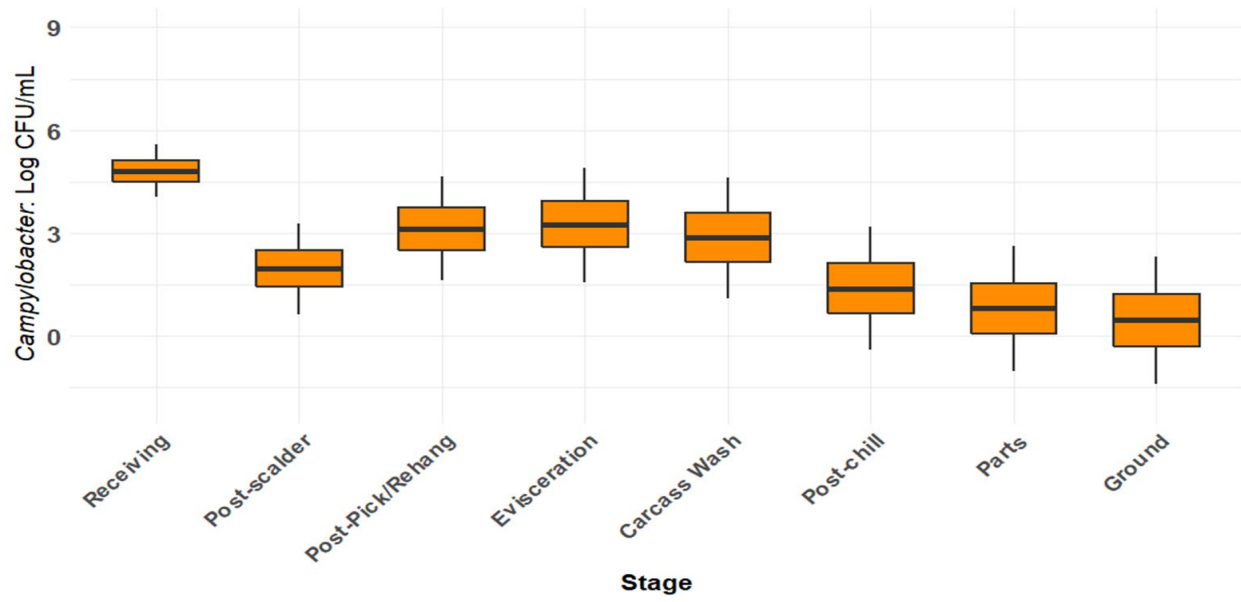
**Figure 4.9:** *Campylobacter* Concentration Change of Intervention Applications at Post-chill Stage. The random effects model results represent the mean concentration change of included spray interventions at the pre-chill stage. Results  $> 0$  indicate an increase in concentration. Results  $< 0$  indicate a decrease. Results  $= 0$  indicate no change. Distinct letters next to the stage description indicate statistically significant differences ( $P < 0.05$ ).



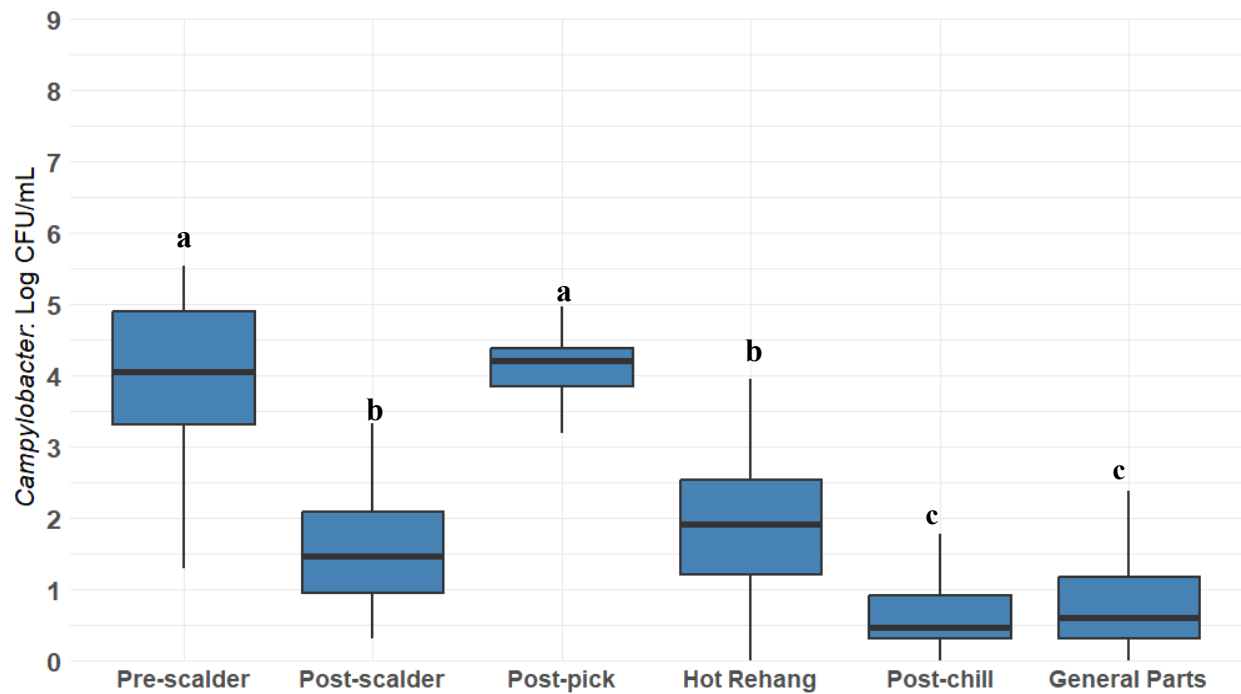
**Figure 4.10:** *Campylobacter* Concentration Change for Immersion Interventions at Post-chill Stage. The random effects model results represent the mean concentration change of included immersion interventions at the post-chill stage. Results  $> 0$  indicate an increase in concentration. Results  $< 0$  indicate a decrease. Results  $= 0$  indicate no change. Distinct letters next to the treatment description indicate statistically significant differences ( $P < 0.05$ ).



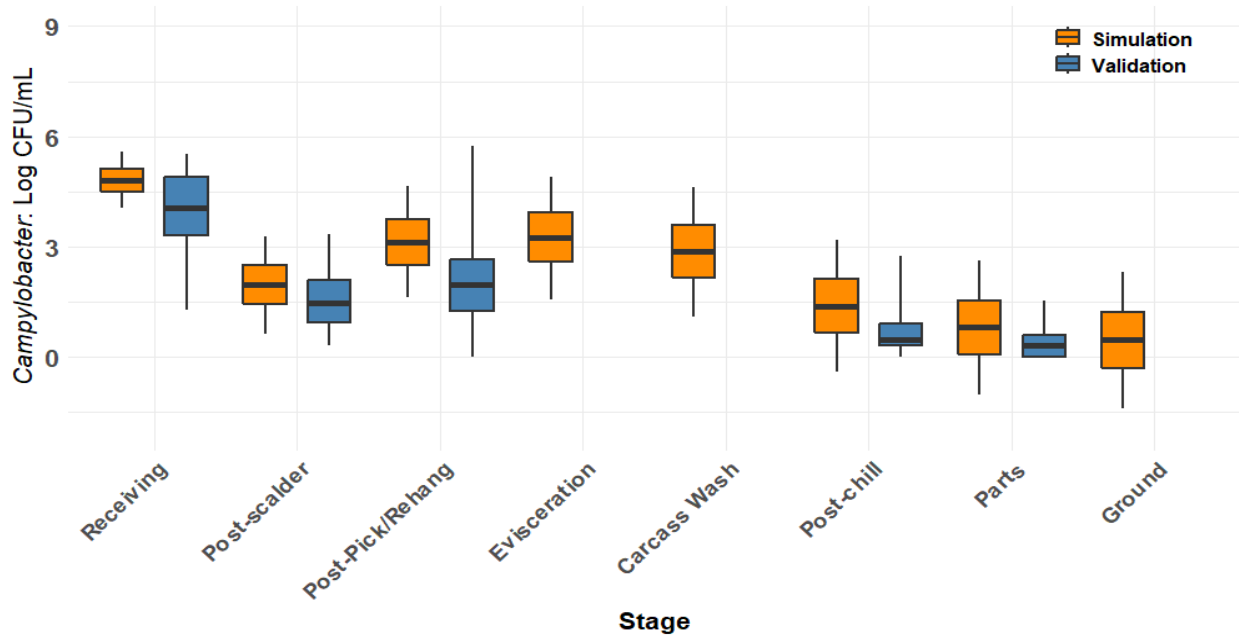
**Figure 4.11:** *Campylobacter* Concentration Change for Spray Interventions at Post-chill Stage. The random effects model results represent the mean concentration change of included spray interventions at the post-chill stage. Results  $> 0$  indicate an increase in concentration. Results  $< 0$  indicate a decrease. Results  $= 0$  indicate no change. Distinct letters next to the treatment description indicate statistically significant differences ( $P < 0.05$ ).



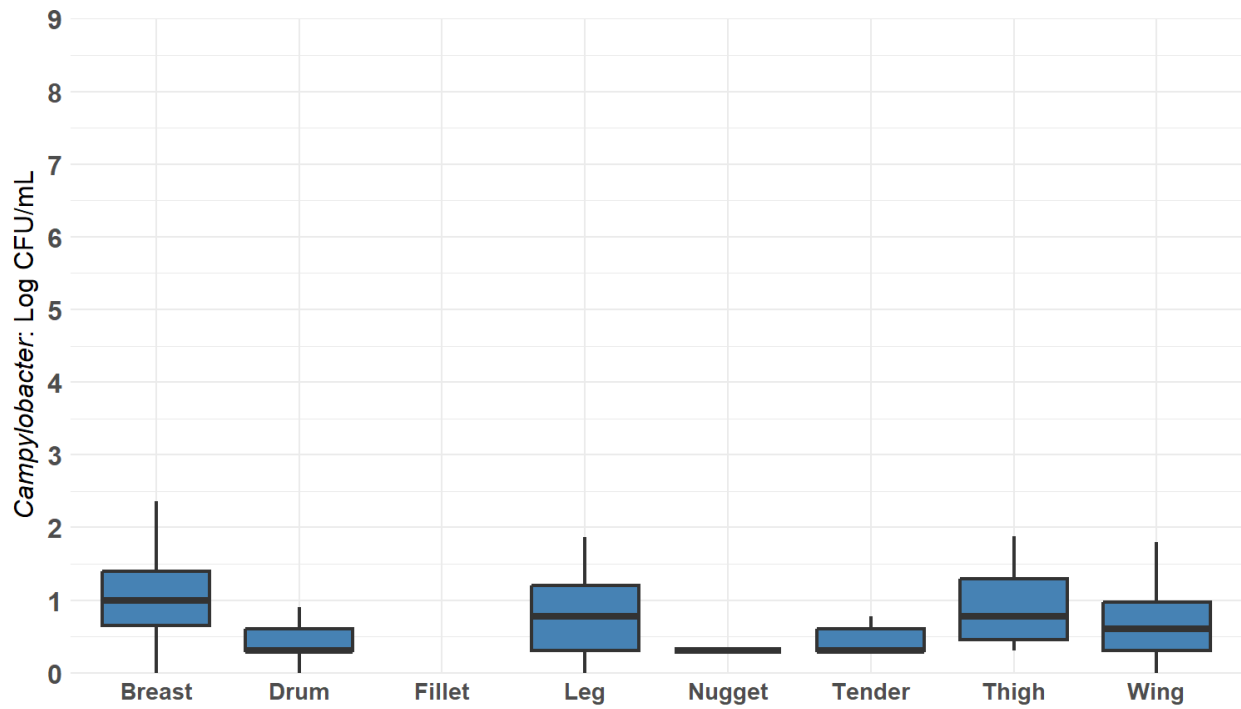
**Figure 4.12:** Baseline *Campylobacter* Bio-map. The chart represents *Campylobacter* concentrations per stage without interventions or chlorine



**Figure 4.13:** Commercial Processing Plant *Campylobacter* Bio-map. This figure represents the concentrations obtained from commercial processing plants. The difference between concentrations between feather picking and rehang is because the processing plants include an intervention between stages. The type of intervention is undisclosed. The data provided by the commercial integrator was used for model validation. Distinct letters on top of the stage indicate statistically significant differences ( $P < 0.05$ ).



**Figure 4.14:** Baseline *Campylobacter* Bio-map with Commercial Processing Plant Bio-map. The chart represents *Campylobacter* concentrations per stage for the baseline model (orange) and the validation model obtained from integrator data (blue). The commercial integrator only provided testing results for the Receiving, Scalding, Feather Picking, Rehang, Post-chill, and Parts Stages.



**Figure 4.15:** Commercial Integrator *Campylobacter* Concentrations per Parts. This figure represents the general cut-up parts broken down by the categories obtained from the testing results obtained from commercial processing plants. All cut-up parts categories were treated with PAA.

## CHAPTER 5

### CONCLUSIONS

The U.S. broiler has invested in *Campylobacter* reduction interventions since the implementation of the 2015 *Campylobacter* performance standards for chicken parts. *Campylobacter* reduction has been achieved through the implementation of antimicrobial interventions and monitoring using various microbial verification tests, with 49% of establishments meeting the performance standard for parts. The industry would have fallen below the USDA-FSIS 2015 estimate of processing establishments meeting the *Campylobacter* performance standard for chicken parts that was set at 52% (USDA-FSIS, 2015a). The *Campylobacter* illnesses attributed to chicken have remained unchanged since the implementation of the performance standard (IFSAC, 2021).

Many of the antimicrobials that are used for meeting the *Campylobacter* performance standards are similar to those used in *Salmonella* control programs and processing establishments (Zhang et al., 2018). All survey responses indicated that there is a *Salmonella* control program in conjunction with the *Campylobacter* control program. Current interventions allow processing establishments to meet the *Campylobacter* performance standards for chicken parts, but *Campylobacter* specific interventions need to be evaluated separately from *Salmonella* control interventions. There is a need for additional microbial verification testing so that USDA-FSIS and poultry processing establishments can precisely assess *Campylobacter* presence and improve process efficacy.

The exposure assessment indicated that chicken cut-up parts can have a higher *Campylobacter* prevalence than whole birds. A higher prevalence increases the likelihood of exposure to the consumer. *Campylobacter* concentrations in cut-up parts are similar to the concentration in whole birds. Even though a higher prevalence exists in cut-up parts compared to whole birds, the risk of exposure is lowered by

reducing the concentration in cut-up parts. Post-chill chemical interventions are capable of reducing concentration variations in parts categories, reducing the risk to consumers of being exposed to an infectious dose.

The simulation model is an option to observe and analyze data changes overtime without the need for additional sampling events. The simulation model provides scenarios that can be used to compare the efficacy of interventions. The comparisons can be made to observe differences between processing plants and between different processing stage settings. The model can be refined and strengthened when testing results from commercial processing plants is used in substitution to an SR-MA.

The model can be improved by obtaining more *Campylobacter* population data before and after the cut-up process. The model can also be modified to evaluate what factors contribute to differences between individual cut-up parts and ground product categories. Chicken cut-up parts and comminuted product undergoing a multi-hurdle approach to *Campylobacter* control has similar populations to whole birds. *Campylobacter* exposure from cut-up parts can be more widespread if controls are not applied. Processing plants are capable of reducing the likelihood of widespread exposure to *Campylobacter* when interventions are used but are insufficient to completely eliminate *Campylobacter*. Interventions at subsequent steps may help to reduce exposure.

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