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Comparison of Buffered Peptone Water to Neutralizing Buffered Peptone Water for *Salmonella* Detection from Commercially Slaughtered Whole Chicken Carcasses and Cut Chicken Parts

ABSTRACT

Poultry processors apply sanitizing interventions to reduce foodborne pathogen prevalence on fresh poultry. Nevertheless, chemical sanitizer application may subsequently result in carryover of sanitizer residues into rinse fluid collected during routine verification sampling. This may result in failure to detect viable pathogens, including *Salmonella enterica*. This study compared *Salmonella* detection from commercially slaughtered whole chicken carcasses and cut chicken parts (wings, thighs, split breasts, drumsticks) following sanitization and rinsing with either buffered peptone water (BPW) or neutralizing buffered peptone water (nBPW). *Salmonella* detection from commercial carcasses was observed only for nBPW-rinsed carcasses following sanitization; detection frequencies differed between two commercial establishments (Establishment A: 15.0%; Establishment B: 43.0%) (*P* < 0.0001). For cut chicken parts, statistical differences in *Salmonella* detection frequencies were detected among the parts types (wings: 16.0%; thighs: 4.0%; split breasts: 21.0%; drumsticks: 0.0%) (*P* < 0.0001). Neither the establishment nor the rinsing fluid composition influenced *Salmonella* detection in cut parts (*P* > 0.05). Data indicate that *Salmonella* detection may be influenced by the sanitizer selected, application methods, and rinsing medium formulation.

INTRODUCTION

Salmonella enterica may cross-contaminate fresh poultry products and be transmitted to consumers, potentially resulting in human disease *(8, 9, 40)*. Fresh poultry has been repeatedly implicated and identified in the occurrence of human foodborne disease outbreaks of salmonellosis in the United States *(3, 9, 21)*. Nonetheless, chicken meat consumption per individual in the U.S. has increased in recent years, with the U.S. per capita consumption of boneless, trimmed chicken increasing by 8.1 lbs from 2000 to 2015 *(18, 25)*. Between March 2013 and July 2014, whole chickens, cut parts, and marinated products produced by a U.S. poultry processor were identified as transmitting

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S. Heidelberg to consumers, resulting in a multistate disease outbreak *(6, 7)*. In 2014, poultry was linked to 14% of food-borne disease outbreaks and outbreak-associated illnesses in the U.S., second only to finfish (21%) *(9)*.

In 1996, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) introduced a *Salmonella* performance standard for poultry products *(26)*. As a part of this program, the *Salmonella* Verification Program was initiated, wherein the USDA-FSIS assesses industry performance and the efficacy of antimicrobial interventions for reducing *Salmonella* prevalence in fresh poultry products *(15, 26)*. In 2014, the Modernization of Poultry Slaughter Inspection program was started by the USDA-FSIS to aid poultry harvest and fabrication establishments in taking measures to prevent *Salmonella* contamination and transmission to consumers *(32)*. Poultry processors implement antimicrobial interventions to reduce microbial contamination from chicken carcass surfaces *(3, 22, 23)* and parts *(15, 28, 33)*. Sanitizers, such as peracetic acid (PAA) and cetylpyridinium chloride (CPC), are approved as antimicrobial interventions to treat surfaces of poultry carcasses and parts (skin-on, skinless) *(1, 2, 3, 20, 33, 37, 39)*. CPC is a quaternary ammonium compound (QAC) used to a maximum allowed concentration of 0.8% with a requirement for rinse-off/removal *(17)*. PAA is a quaternary equilibrium mix of acetic acid and hydrogen peroxide, ultimately breaking down to acetic acid, water, and oxygen *(16)*. When applied as a dip or spray, PAA concentration may not exceed 2,000 ppm *(37)*.

The USDA-FSIS previously recommended that poultry carcasses or cut parts samples collected for pathogen detection testing should be allowed to drip excess sanitizer prior to rinse sampling to reduce the possibility of sanitizer carryover into the sampling bag *(31, 34)*. Gamble et al. *(15)* reported that buffered peptone water (BPW) as a carcass rinse fluid was unable to fully overcome sanitizer carryover while simulating 1 min carcass dripping when supplemented with sanitizers (CPC or PAA). Subsequently, USDA-FSIS issued Notice #41-16 instructing in-plant personnel to substitute Neutralizing Buffered Peptone Water (nBPW) for BPW during routine verification sampling of poultry carcasses and fresh cut parts *(35)*. This study was designed and performed to determine the potential impacts of sanitizer application and rinsing fluid composition on *Salmonella* detection from fresh chicken carcasses and cut parts. A preliminary study was conducted with *Salmonella*-inoculated young chicken carcasses undergoing sanitizer application prior to rinsing to determine whether sanitizer and/or rinse fluid composition impacted *Salmonella* detection under laboratory-controlled conditions. Subsequently, fresh broiler carcasses and cut pieces from two USDA-FSIS-inspected poultry slaughter and fabrication establishments were collected and rinsed to determine the impact of rinsing fluid composition under commercial processing conditions. The

null hypothesis for each trial was that rinse fluids with differing formulations would not differ in allowing for *Salmonella* detection from sanitizer-treated carcasses or cut parts.

MATERIALS AND METHODS

Preparation of *Salmonella* cocktail for evaluation of differences in *Salmonella* detection from inoculated whole chicken carcasses

Isolates (one per serovar) of *Salmonella* Typhimurium, Heidelberg, and Enteritidis (all recovered from a federally inspected poultry slaughter establishment) were revived from -80°C storage from the Food Microbiology Laboratory culture collection (Department of Animal Science, Texas A&M AgriLife Research, College Station, TX, USA). To revive and activate cultures for inoculation, each isolate was individually inoculated into 10 ml sterile tryptic soy broth (TSB; Becton, Dickinson and Co., Franklin Lakes, NJ, USA), followed by aerobic static incubation at 35 ± 2°C for 24 h. One loopful of each culture was then passed into 10 ml sterile TSB and incubated in identical fashion. Immediately prior to use, *Salmonella* cultures were decimally diluted in phosphate-buffered saline (PBS; EMD Millipore, Temecula, CA, USA) to a targeted inoculum of 10^6 CFU/ml. Tubes containing diluted cells were centrifuged ($2,191 \times g$; Jouan B4i centrifuge; Thermo-Fisher Scientific, Waltham, MA, USA) for 15 min at 25 ± 2°C, after which supernatants were poured off and pellets suspended in 10 ml sterile PBS. Suspensions of bacterial isolates then underwent two additional centrifugation and washing cycles similar to the first.

Cocktails of *Salmonella* isolates were prepared by mixing 5.0 ml of each of the three cultures into a 15 ml sterile conical tube and vigorously vortexing for at least 5 sec. The resulting mixture of cells was used as a cocktail in preliminary experiments determining *Salmonella* detection from inoculated whole chicken carcasses. To quantify the total number of *Salmonella* in the cocktail prepared for inoculation, 5.0 ml was decimally diluted in 0.1% peptone water (PW; Becton, Dickinson, and Co.), and dilutions were spread on surfaces of xylose lysine tergitol-4 (XLT4; Becton, Dickinson and Co.) agar, with Niaproof-4 (Sigma-Aldrich Co., St. Louis, MO, USA) substituted for tergitol-4. Inoculated Petri plates were incubated aerobically for 24 h at 35 ± 2°C; black or red colonies, with or without black centers, were counted. Counts were transformed to $\log_{10}$ CFU/ml following counting. The remaining cocktail was transferred to a sterile spray bottle for chicken carcass inoculation.

Inoculation of chicken carcasses with *Salmonella* cocktail

Five boxes, each containing 20 young chicken carcasses (*Gallus domesticus*) were purchased one day prior to study initiation. Three carcasses each were used for negative controls (non-inoculated, no sanitizer application: used to determine *Salmonella* counts on purchased carcasses) and

positive controls (inoculated: used to quantify efficiency of *Salmonella* inoculation). Five carcasses per replicate were used for each combination of sanitizer and rinsing medium; three identical replicates were completed (n = 15). Boxes containing carcasses were transported to the Texas A&M Poultry Science Research Center (College Station, TX, USA) and placed in a walk-in cooler (4 ± 2°C) for storage prior to testing. For *Salmonella* inoculation, a spray bottle containing prepared cocktail was used to spray-inoculate chicken carcasses. To prime the spray bottle, a researcher wearing a shoulder-length glove placed the inoculum-containing spray bottle into an empty polyethylene bag and sprayed three pumps (1.0 ± 0.1 ml/pump). After priming, the bag containing the carcass was opened and one spray of inoculum was applied 8 to 10 in. from the carcass surface. The bottle was then removed and the bag closed to prevent aerosol release; this process was completed for all inoculated carcasses. Two minutes after inoculation, carcasses were removed from bags and placed on a metal rack, anterior side facing upwards, without touching one another. Carcasses were held for 30 min at 25 ± 2°C for inoculum attachment. Following attachment, carcasses were aseptically transferred to polyethylene bags (15 × 20 in.; 12 liter capacity; VWR Int., Radnor, PA, USA) prior to sanitizer treatment.

Sanitizer preparation and application

A CPC working solution (8,000 ppm CPC) was prepared immediately prior to application by combining 14.7 liters sterile distilled water with 0.3 liters commercial CPC-containing solution (Cecure™, Safe Foods Corp., North Little Rock, AR, USA; 40 ± 2% active agent content, according to manufacturer guidance) and stirring. Ingoing CPC concentration was verified by use of a CPC titration kit (Safe Foods Cecure Titration Kit, Safe Foods Corp.). Two CPC application methods were applied: a carcass drench (CPC_a) and a carcass drench followed immediately by an 80 min chill in ice-cold water (CPC_b). Two liters CPC working solution were drenched per carcass for each treatment. The drench was accomplished by a researcher wearing shoulder-length gloves holding the carcass by the drumsticks while another poured 1.0 liter of CPC working solution over the carcass and into the interior cavity. Afterwards, the carcass-holding researcher removed one hand and poured an additional 500 ml over the drumstick area previously covered by the glove. The carcass-holding researcher then placed the gloved hand back onto the leg and removed the other hand; the process was then repeated for the opposite side. After the drench was applied, CPC_a-treated carcasses were allowed to drip for 1 min to remove excess sanitizer (30, 35). CPC_a-treated carcasses were then sprayed with 50 ± 5 ml sterile distilled water to simulate CPC rinse-off during poultry slaughter and sanitization. After water spray, carcasses were allowed to drip for an additional 1 min before being placed into a polyethylene bag. CPC_b-treated birds were allowed to drip

for 1 min after the CPC drench and then placed into an ice water bath for 80 min. Carcasses were afterwards removed from the bath and allowed to drip for 1 min before being sprayed with 50 ml sterile distilled water. Carcasses were then allowed to drip for 1 min and placed into a polyethylene bag for subsequent rinse-testing.

An aqueous PAA solution was prepared immediately prior to application, at an ingoing concentration of 2,000 ppm PAA (0.2%), by combining 14.8 liters sterile distilled water and 0.2 liters PAA (Promoat, Safe Foods Corp.; 14–17% PAA content) and stirring. Ingoing PAA concentration was tested with a hydrogen peroxide and peracetic acid test kit (LaMotte, Chestertown, MD, USA) prior to PAA application. Approximately 2.0 liters of working PAA sanitizer solution were applied onto carcasses, using the same drench method described for CPC-treated carcasses. After the drench was applied, PAA-treated carcasses were allowed to drip for 1 min to remove excess PAA solution, and a sterile distilled water spray application (50 ml) was then applied. The additional rinse was completed to simulate post-sanitization rinsing occurring in commercial establishment B for PAA-treated parts *(Fig. 1)* and to maintain the same process for testing the impact of CPC and PAA. The carcasses dripped for 1 min and were then placed into a polyethylene bag for subsequent rinse-testing.

Preparation of rinsing fluids

The poultry rinsing fluids PBS (control), BPW, and nBPW were prepared according to manufacturer instructions or USDA-FSIS guidance *(24)*. Dehydrated PBS and BPW media were mixed in sterile distilled water according to manufacturer instructions (Hardy Diagnostics, Santa Maria, CA, USA). After being dispensed into bottles, media were sterilized by autoclaving (121°C, 15 min) and thereafter refrigerated (5°C) until used. For preparation of nBPW, 20.0 g dehydrated BPW medium (Hardy Diagnostics), 7.0 g powdered refined soy lecithin (Alfa Aesar, Haverhill, MA, USA), and 1.0 g sodium thiosulfate (EMD Millipore) were added to 833 ml distilled water and stirred for 5 min. The resulting solution was autoclaved (121°C, 15 min). In a separate flask containing 167 ml distilled water, 12.5 g sodium bicarbonate (EMD Millipore) was dissolved by stirring, with gentle heating. This bicarbonate solution was then filter-sterilized (polyether-sulfone, 0.45 μm, VWR Int.) and added to the sterilized basal broth after it had been tempered to 55°C (final medium pH 7.5 ± 0.3 by calibrated pH probe). The nBPW was then stirred for 1 min after addition of bicarbonate solution and the combination was stirred frequently while being aliquoted into sterile screw-cap bottles to maintain the suspension of precipitate in the broth medium. Afterwards, bottles were refrigerated (5°C) until required for use. All rinse solutions were aliquoted into 100 ml volumes per previous reports indicating no difference in *Salmonella* recovery versus when larger volumes of poultry rinse medium were used *(10)*.
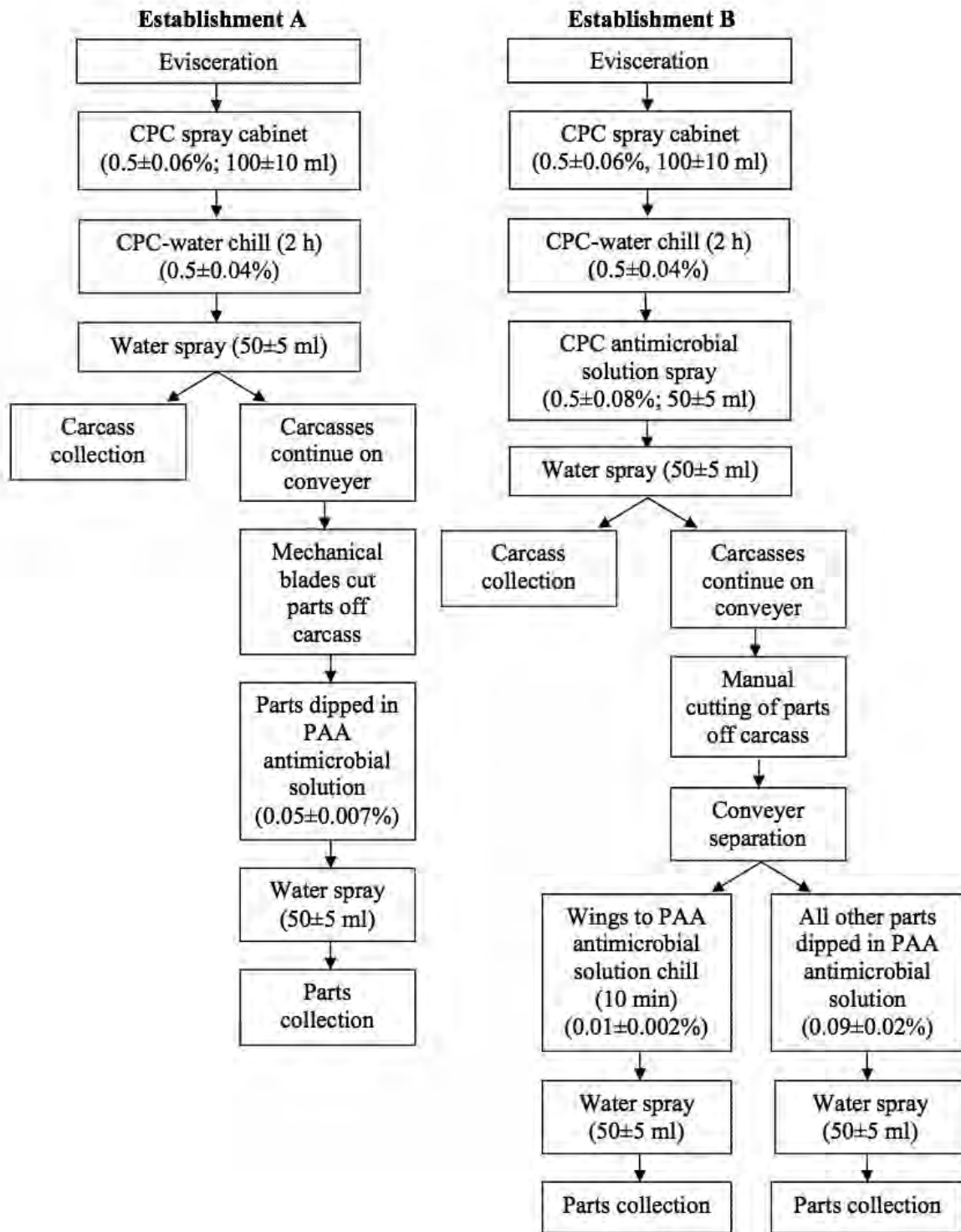
Establishment A

Evisceration
↓
CPC spray cabinet
(0.5±0.06%; 100±10 ml)
↓
CPC-water chill (2 h)
(0.5±0.04%)
↓
Water spray (50±5 ml)
↓ ↘
Carcass collection | Carcasses continue on conveyer
↓
Mechanical blades cut parts off carcass
↓
Parts dipped in PAA antimicrobial solution (0.05±0.007%)
↓
Water spray (50±5 ml)
↓
Parts collection

Establishment B

Evisceration
↓
CPC spray cabinet
(0.5±0.06%, 100±10 ml)
↓
CPC-water chill (2 h)
(0.5±0.04%)
↓
CPC antimicrobial solution spray
(0.5±0.08%; 50±5 ml)
↓
Water spray (50±5 ml)
↙ ↘
Carcass collection | Carcasses continue on conveyer
↓
Manual cutting of parts off carcass
↓
Conveyer separation
↙ ↘
Wings to PAA antimicrobial solution chill (10 min) (0.01±0.002%) | All other parts dipped in PAA antimicrobial solution (0.09±0.02%)
↓ ↓
Water spray (50±5 ml) | Water spray (50±5 ml)
↓ ↓
Parts collection | Parts collection

Figure 1. Flow process of establishments A and B for carcass and parts processing

Carcass rinsing and rinse fluid collection procedure

For *Salmonella*-inoculated chicken carcass samples, volumes of 100 and 30 ml of rinsing medium were utilized to rinse carcasses following sanitizer application and for pre-enrichment with 30 ml of collected rinse fluid, respectively. *Salmonella*-inoculated positive control carcass samples were placed into sterile polyethylene bags and rinsed with PBS. Inoculated and sanitizer-treated carcasses were placed into

polyethylene bags and 100 ml of BPW or nBPW was poured into the bag. The top of the bag was twisted several times to seal, and the carcass rinsed by moving the bag back and forth in an arc-like motion repeatedly for 1.0 min, rinsing both interior cavity and exterior surfaces of the carcass (30). The corner of the bag was then cut open with flame-sterilized scissors, and the rinsing fluid was drained into the container it had been poured from. Rinse fluid containers were sealed, placed into an insulated container with sanitized chilling pouches, and transported to the Food Microbiology Laboratory for analysis.

Testing of rinsing media for *Salmonella* detection on commercially slaughtered whole bird carcasses

To determine the sample count (n) necessary to achieve at least 80% statistical power, the calculation n = log(*P*)/log (*P'*) was utilized, where *P* is the chosen power (1-β) and *P'* is the proportion of carcasses or parts that are not likely contaminated (11). The values for *P'* for both carcasses and edible parts were determined based on USDA-FSIS-collected microbiological baseline data for *Salmonella* prevalence in raw young chicken carcasses and parts as well as data provided under non-disclosure agreement by cooperating commercial establishments (27, 29). Broiler carcasses (n = 20 each per rinse fluid type at each establishment for each replicate) were rinsed with PBS, BPW, or nBPW, yielding N = 60 broiler carcasses for each rinsing fluid over three identically completed replicates. Per rinse fluid type, three samples of 16 wings each, three samples of 9 thighs each, four samples of 4 breasts each and four samples of 11 drumsticks each were collected to ensure variety between light and dark meat pieces. This produced an n = 14 per replicate per establishment (N = 42 per establishment over three identically completed replicates). The numbers of pieces per sample correlated to the cooperating establishments' collection standards for numbers of pieces yielding an average of 4 lb equivalent to USDA-FSIS weight requirement for all chicken part samples for *Salmonella* detection sampling (36).

All parts collected from both facilities were taken from carcasses undergoing CPC treatment prior to fabrication. Carcasses were cut into parts in the sequence of wings, split breasts, thighs, and then drumsticks at both plants. Establishment A parts were mechanically fabricated, while plant B parts were manually fabricated by employees. Establishment A dipped cut parts into a PAA solution (0.05 ± 0.007%). Establishment B wings were submerged in a PAA-water chill (0.01 ± 0.002%) for 10 min while all other parts underwent a PAA dip (0.09 ± 0.02%). Ingoing PAA concentrations were verified with a hydrogen peroxide and peracetic acid test kit (LaMotte Co.). Sanitizer solution was collected from the PAA dip tank following sanitizer solution preparation by establishment personnel. All chicken parts samples were subjected to a water spray (50 ± 5 ml) as they exited the dip tank immediately prior to sample collection. Samples were collected from the conveyor line, allowed to drip for 1 min, and then placed into polyethylene bags for rinsing.

Rinse fluid (PBS, BPW, nBPW; 400 ml), prepared as already described, was poured into a bag containing either a whole chicken carcass or a set of parts that had undergone sanitizer treatment. The top of the bag was twisted several times, and the sample was rinsed by moving the bag back and forth in an arc motion (21 ± 3 in) for 1 min (30). After 1 min, the corner of the bag was cut with flame-sterilized scissors and sample rinse fluid was collected back into the container it had been poured from. After all rinse fluid samples were collected, samples were packed into a cooler, covered with ice packs, and transported to the Food Microbiology Laboratory for sample processing. Upon arrival at the laboratory, coolers were opened and samples checked for rinsate freezing; no evidence of sample liquid freezing was detected during experimental trials (data not shown).

Salmonella testing and confirmation

Salmonella testing/detection methods described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG; section 4.08) were used to recover inoculated *Salmonella* spp. from rinsed carcasses (inoculated, commercial) and parts (commercial) (31), with the modification that 30 ml sample rinse fluid was added to a bottle containing 30 ml sterile matching rinse fluid for pre-enrichment. Pre-enrichments were incubated at 35 ± 2°C for 24 h. Following incubation, 0.5 and 0.1 ml pre-enrichment solution was added to 10 ml tetrathionate (TT) (Hajna) broth (Becton, Dickinson and Co.) and 10 ml modified Rappaport-Vassiliadis broth (mRV; Sigma-Aldrich Co.), respectively, for selective enrichment. Selective enrichment broths were incubated at 42 ± 2°C for 24 h. Two 10 μl volumes from each selective enrichment post-incubation were streaked for isolation onto surfaces of brilliant green sulfa (BGS; Becton, Dickinson and Co.) agar and XLT4 (Becton, Dickinson and Co.) agar, with Niaproof-4 (Sigma-Aldrich Co.) substituted for tergitol-4. BGS and XLT4 Petri plates were aerobically incubated at 35 ± 2°C for 24 h prior to inspection. From each sample set, three colonies demonstrating typical *Salmonella* morphologies were randomly selected from BGS and XLT4 plates and picked for biochemical identification. Colonies were stabbed individually into triple sugar iron agar (TSI; Hardy Diagnostics) and lysine iron agar (LIA; Becton, Dickinson and Co.) slants into the butt of a slant, withdrawing and then streaking the needle across the surface of the slant. Slants were aerobically incubated at 35 ± 2°C for 24 h before being assessed for *Salmonella* (13, 31).

In the event an atypical *Salmonella* result occurred, the Rule-Out Reactions guidelines were followed to identify questionable TSI or LIA slants as *Salmonella*-positive or -negative (13). In instances where no selective agar plates (XLT4, BGS) bore presumptive *Salmonella* colonies following the initial incubation, plates were subsequently incubated at 35 ±

2°C for an additional 24 h. After the second incubation period, plates displaying no colony growth were recorded as negative and corresponding samples were coded *Salmonella*-negative. Presumptive-positive *Salmonella* colonies were then inoculated onto tryptic soy agar (TSA; Becton, Dickinson and Co.) slants and stored at 4 ± 2°C until polymerase chain reaction (PCR) confirmation of *Salmonella* was completed according to previously reported methods.

From all samples (carcasses, cut parts) collected at commercial facilities that showed *Salmonella* presumptive positive results, a TSA slant containing an isolated presumptive *Salmonella* was selected for PCR analysis and *Salmonella* confirmation. Slants were transported to the Quantitative and Functional Genomics Laboratory at Texas A&M University (Department of Poultry Science, College Station, TX) for confirmation of isolates as *Salmonella*. DNA was isolated and purified from each colony using an UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) according to manufacturer instructions. PCR was conducted targeting the *invA* gene, using methods and primers described previously *(5)*. PCR amplicons were visualized on a 1% agarose gel stained with BioRed (Phenix Research, Candler, NC, USA) and visualized under UV light, using a 100 bp ladder to determine appropriate amplicon size.

Statistical analysis

Carcass-derived rinsate samples were scored as 1 (*Salmonella* detected) or 0 (*Salmonella* non-detected) and grouped by rinse fluid and sanitizer treatment for each trial (inoculated trial, commercial trial). Chicken parts-derived rinsate samples from commercial parts samples were scored and data grouped in like fashion. Contingencies analysis (Chi-square; χ^2) was first completed for pathogen detection/non-detection data to test the null hypothesis that sanitizer and neutralizer pairings and *Salmonella* detection results were independent of one another (that rinsing medium composition did not influence *Salmonella* detection). For all samples, the total number of *Salmonella*-positive samples was divided by the total number of like samples collected, to determine the frequency of *Salmonella*-positive samples for each sanitizer and rinsing medium combination. For inoculated carcass trial data, analysis of variance (ANOVA) was used to detect statistically significant differences in the recovery of *Salmonella* as a function of experimental fixed effects (rinse fluid composition, sanitizer treatment) and the interaction of main fixed effects (significance set at $P = 0.05$). Similarly, for commercial carcasses and parts-derived data, analysis of variance was utilized to detect differences in *Salmonella* detection as a function of commercial processing scheme *(Fig. 1)*, rinsing medium composition, and the interaction of these effects. For all data, significant differences between means were identified by use of Student's *t*-test ($P < 0.05$). All analyses were performed using JMP Pro v13.0.0 (SAS Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Recovery of *Salmonella* from inoculated chicken carcasses as a function of sanitizer application and rinsing fluid composition

A *Salmonella*-inoculated carcass trial was completed prior to evaluation of rinsing fluids in a commercial establishment environment to test the effect of carcass sanitizer and neutralizer pairings on carcasses under conditions where pathogen presence/absence status was controlled. *Salmonella* mean inoculum was $6.0 ± 0.4 \log_{10}$CFU/ml; positive control carcasses were found to bear a mean *Salmonella* count of $2.5 ± 0.2 \log_{10}$CFU/ml following inoculation, attachment, and rinsing in PBS. Testing of negative control carcasses indicated a mean *Salmonella* load of $1.4 + 0.6 \log_{10}$ CFU/ml PBS, indicating inoculated carcasses bore at least 0.5 to $1.0 \log_{10}$CFU/ml higher *Salmonella* loads than non-inoculated carcasses, facilitating identification of presumptive *Salmonella*-contaminated carcasses as most likely those with contamination coming from inoculation. Contingency analysis of *Salmonella* detection or non-detection following sanitizing and neutralizer application was determined through Pearson's χ^2 analysis; *Salmonella* detection results for inoculated chicken carcasses were, as was expected, highly dependent upon sanitizer and neutralizer combination (Pearson's $\chi^2 = 37.95$; $P < 0.0001$). This subsequently led researchers to reject the null hypothesis that sanitizer + neutralizer pairing and *Salmonella* detection or non-detection were independent of one another.

Frequencies of *Salmonella* detection from inoculated chicken carcasses following sanitization with CPC or PAA and neutralization by BPW or nBPW are depicted in *Table 1*. Only the 0.2% PAA-treated, BPW-rinsed samples (PAA-BPW) yielded a detection frequency of < 100% of *Salmonella*-typical colonies. Overall, 53.3% of PAA-BPW-treated carcasses (8/15) were presumptive-positive for inoculated *Salmonella*; all other treatments yielded 100% presumptive-positive *Salmonella* detection for carcasses tested per treatment. Analysis of *Salmonella* detection frequencies from inoculated carcasses indicated the interaction of experimental main effects (rinse fluid formulation, carcass sanitizer treatment) was statistically highly significant with respect to *Salmonella* detection ($P < 0.0001$). Nevertheless, while the detection of *Salmonella* from BPW-rinsed carcasses was reduced compared with nBPW-rinsed carcasses following PAA application, rinsing medium formulation did not impact *Salmonella* detection for CPC$_a$ and CPC$_b$-sanitized carcasses ($P ≥ 0.05$) *(Table 1)*.

Researchers have previously reported reduced *Salmonella* recovery from inoculated BPW in which the application of 2,000 ppm PAA and a 1 min drip of the whole chicken carcass was simulated *(14, 15)*. Others have reported reductions of 2.0-2.1 $\log_{10}$CFU/ml rinse fluid in *Salmonella* counts from inoculated chicken carcasses when immersion-chilled in 0.1% PAA-infused waters and rinsed with BPW, reducing the likelihood

TABLE 1. Frequencies of *Salmonella enterica* detection (%) from inoculated whole chicken carcasses as a function of sanitizer treatment x rinsing medium interaction (*P* < 0.0001)[a]

Sanitizer Treatment[b]	Carcass Rinsing Fluid[c]	*Salmonella* Detection[d]
0.8% CPC[a]	BPW	100.0A
	nBPW	100.0A
0.8% CPC[b]	BPW	100.0A
	nBPW	100.0A
0.2% PAA	BPW	53.0B
	nBPW	100.0A

Pooled Standard Error = 5.0

[a]Mean *Salmonella* count from inoculated carcasses in PBS was 2.5 ± 0.2 $\log_{10}$CFU/ml.

[b]CPC: cetylpyridinium chloride; PAA: peroxyacetic acid. CPC[a] and CPC[b] were applied as 2.0 liter drench treatments followed by 1 min carcass drip + 50 ml sterile distilled water (CPC[a]) or 1 min carcass drip + 80 min ice cold sterile water immersion chilling (CPC[b]). PAA was drenched onto carcasses, followed by 1 min carcass drip without additional water spray.

[c]BPW: buffered peptone water; nBPW, neutralizing buffered peptone water. Prepared rinsing fluids were steam-sterilized (121°C, 15 min) and refrigerated prior to use.

[d]Samples were coded as *Salmonella*-positive (1) or negative (0). Values depict mean frequencies of *Salmonella*-positive samples from like-treated samples (n = 5 per replicate; 3 replicates). Values not sharing common letters (A, B) differ from one another at *P* = 0.05 as determined by analysis of variance and Student's *t*-test.

of pathogen detection seen with other sanitizing treatments *(22)*. Comparing varying levels of PAA and 0.003% chlorine solutions on inoculated chicken carcasses, Bauermeister et al. *(2)* found PAA levels as low as 0.0025% were more effective than chlorine in decreasing *Salmonella* counts. Gamble et al. *(15)* likewise reported failure to detect *Salmonella* from inoculated BPW following a simulated whole carcass treatment with 0.8% CPC and 1-min carcass dripping. In the current study, detection of *Salmonella* was consistently achieved on inoculated carcasses following 0.8% CPC application and 1-min carcass dripping prior to rinsing, regardless of rinsing fluid *(Table 1)*. Likewise, Gamble et al. *(14)* also reported that 7.0 g/liter lecithin was capable of neutralizing 0.8% CPC in rinse fluid, allowing *Salmonella* enumeration similar to numbers obtained from controls. In the current study, application of a second 1-min drip period and 50 ml sterile water spray or the 80 min post-drench water chilling potentially facilitated increased *Salmonella* detection for both CPC treatment processes by reducing the amount of sanitizer on carcasses going into rinsing *(Table 1)*. These findings suggest that: (i) the combination of rinsing fluid supplemented with sanitizer neutralizers and dripping periods >1 min is most effective for pathogen recovery and detection, and (ii) extended dripping may be a particularly important contributor to *Salmonella* detection during routine verification sampling on whole bird carcasses.

***Salmonella* recovery from commercially harvested chicken carcasses and chicken parts samples**

Figure 1 depicts the process flow of whole chicken carcasses and parts at cooperating inspecting poultry slaughter and fabrication establishments A and B. After evisceration, carcasses at both facilities underwent a sprayed CPC sanitizing treatment (0.50 ± 0.06%; 100 ± 10 ml) in a spray cabinet in which sanitizer was applied to exterior surfaces of carcasses and the interior cavity. Ingoing CPC concentration was verified using a CPC titration kit (Safe Foods Cecure Titration Kit, Safe Foods Corp.) following sanitizer solution preparation by establishment personnel. Carcasses collected from establishment A were subsequently chilled in a CPC-infused immersion chiller (0.50 ± 0.04%) for approximately 2 h, after which they received a water rinse upon exiting the chiller. Establishment B had an additional CPC-spray (50 ± 5 ml, 0.50 ± 0.09%) after the 2 h chill, after which carcasses received a water rinse (50 ± 5 ml) 1 min after CPC-spray application. Carcasses were selected at random, collected following the post-chill water spray, and allowed to drip for 1 min before being placed into polyethylene bags for rinsing.

Findings from experiments to determine detection of *Salmonella* from commercially harvested chicken carcasses as a function of rinsing fluid are presented in *Table 2*. For whole chicken carcasses, the method of CPC sanitization (1 method per establishment) of carcasses as well as the

TABLE 2. *Salmonella enterica* detection frequencies (%) from commercially slaughtered whole chicken carcasses as a function of commercial establishment x rinsing fluid interaction (*P* < 0.0001)

Establishment[a]	Rinsing Fluid[b]	*Salmonella* Detection[c]
A	PBS	0.0C
	BPW	0.0C
	nBPW	15.0A
B	PBS	0.0C
	BPW	0.0C
	nBPW	43.0B

Pooled Standard Error = 3.0

[a]Post-evisceration carcasses at both establishments underwent cetylpyridinium chloride (CPC) spray treatment (0.50 ± 0.06%; 100 ± 10 ml) in a cabinet applying sanitizer to exterior and interior surfaces. Carcasses collected from establishment A were immersed in CPC-infused chiller waters (0.50 ± 0.04%) for 2 h and then were sprayed with 50 ± 5 ml sterile water post-chilling. Establishment B applied a second CPC sanitizer treatment (100 ± 10 ml; 0.50 ± 0.09%) post 2-h chill, after which carcasses received a water rinse (50 ± 5 ml) 1 min after CPC application.

[b]PBS: phosphate buffered saline; BPW: buffered peptone water; nBPW, neutralizing buffered peptone water. Prepared rinsing fluids were steam sterilized (121°C, 15 min) and refrigerated prior to use.

[c]Values depicted are means of the frequencies of *Salmonella*-positive samples from like-treated samples (N = 60). Values not sharing common letters (A, B, C) differ from one another at *P* = 0.05 as determined by analysis of variance and Student's *t*-test.

TABLE 3. *Salmonella enterica* detection (%) from commercially prepared chicken parts (*P* < 0.0001)

Sample Type	Parts Type-Specific n/N[a]	*Salmonella* Detection[b]
Drumsticks	0/72	0.0 ± 0.0A
Split Breasts	14/70	21.0 ± 3.0B
Thighs	2/53	4.0 ± 4.0A
Wings	8/51	16.0 ± 4.0B

[a]Indicates sample count for each chicken parts type for which pathogen detection was confirmed divided by total sample count tested for *Salmonella*.

[b]Samples were coded as *Salmonella*-positive (1) or negative (0) following pathogen detection confirmation. Values depict means of the frequencies of *Salmonella*-positive samples from like-treated samples plus/minus the standard error of the mean. Values not sharing common letters (A, B) differ from one another at *P* = 0.05 as determined by analysis of variance and Student's *t*-test.

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formulation of rinsing fluid interacted to significantly influence the recovery of *Salmonella* from carcass surfaces (*P* < 0.0001). Whereas PBS- and BPW-rinsed carcasses did not bear detectable *Salmonella*, carcasses from both establishments rinsed in nBPW did bear detectable salmonellae. Fifteen percent of tested carcasses rinsed in nBPW from establishment A bore detectable *Salmonella*, while establishment B had a significantly higher rate of *Salmonella* recovery from nBPW-rinsed carcasses (43.0%) (*P* < 0.0001). Both establishments treated carcasses with a CPC spray (0.5%, 100 ml), while Establishment A followed up the initial spray with a 2 h chill in 0.5% CPC-infused cold water, after which carcasses were sprayed with water to remove residual CPC prior to collection for sampling. In

contrast, Establishment B immersion-chilled carcasses in CPC-containing water following the initial sanitizer spray, followed by a second CPC (0.5%) spray prior to the water spray to remove CPC residue before carcass collection for testing *(Fig. 1)*. Contingency analysis confirmed ANOVA results, indicating that *Salmonella* detection on commercial carcasses was highly dependent upon sanitization scheme by establishment and sample rinsing fluid (Pearson's χ^2=104.98; $P < 0.0001$).

At the outset of revival and preparation of presumptive *Salmonella* isolates for PCR confirmation, isolate slants from six differing parts samples (1 split breast sample, establishment A; 3 wings samples, establishment B; 1 thigh sample, establishment B; 1 split breast sample, establishment B) could not be successfully revived for subsequent sample processing, thereby preventing these samples from being confirmed for *Salmonella*. These samples were removed from statistical analysis, resulting in submission of 20 and 38 samples from Establishments A and B, respectively, for PCR *Salmonella* confirmation *(Table 3)*. Contingency analysis of commercial chicken parts samples and *Salmonella* detection dependency on rinsing medium formulation and establishment indicated that *Salmonella* detection results were dependent on parts type, establishment, and rinsing medium selection (Pearson's χ^2 = 38.30; $P = 0.024$). While drumsticks did not yield detectable *Salmonella enterica*, the pathogen was recovered from all other sample types (split breasts, thighs and wings). Analysis of variance indicated significantly higher *Salmonella* detection frequencies on split breasts (21.0%) and wings (16.0%), though these detection rates did not differ from one another at $P = 0.05$ *(Table 3)*. The recovery of *Salmonella* from commercial breast and wings samples were statistically higher than those from thighs and drumsticks *(Table 3)*. Of note, *Salmonella* recoveries were highest from samples containing parts cut from the whole carcass first and second (wings, split breasts), while pathogen recovery from parts cut later (thighs, drumsticks) did not bear detectable *Salmonella* or bore *Salmonella* only at a low prevalence.

Currently, there is concern regarding whether poultry sampling methods provide optimal opportunity for *Salmonella* detection from raw poultry. Bourassa et al. *(4)* reported, after performing neck skin, whole carcass rinsing and whole carcass enrichment comparisons on broilers following either air or immersion chilling, that the only way to determine carcasses as *Salmonella*-free would be to sample by whole carcass enrichment. Gamble et al. *(15)* reported that at 0 and 1 min of post-sanitization-simulated dripping of carcasses treated with PAA, CPC and acidified sodium chlorite, collected drip fluids displayed significant ($P < 0.0001$) sanitizer residual/carryover activity, with no samples yielding detectable *Salmonella*. These researchers concluded that a risk of false-negative results may arise during routine verification sampling in the commercial establishment if sanitizer carryover is

not effectively neutralized during rinsing. Additionally, *Salmonella* cells may not be detected when a 30-ml aliquot of sample rinsate is prepared for pre-enrichment from a 100 or 400 ml total rinsing fluid volume *(12, 31)*. The ability to detect *Salmonella* presence is in large part dependent upon the numbers of *Salmonella* present on the food at the point of sampling *(4, 38)*. Raw poultry carcasses have been reported to bear low *Salmonella* counts, with studies indicating chicken carcasses that are recorded as *Salmonella* positive typically have no more than 100 cells of *Salmonella*, though *Salmonella* counts are known to vary widely *(4, 19)*. Berghaus et al. *(3)* reported that *Salmonella* prevalence and populations decreased from the time live chickens arrived for slaughter (45.9% prevalence; $3.4 \pm 0.7 \log_{10}$ MPN per carcass) to the time they exited chlorinated immersion chilling (2.4% prevalence; $2.3 \pm 0.9 \log_{10}$ MPN per carcass). This demonstrates that antimicrobial interventions reduced the numbers of *Salmonella* on carcasses and may also explain the low numbers of *Salmonella*-positive commercial rinse samples.

Study findings suggest the use of nBPW provided for a higher rate of *Salmonella* recovery post-sanitization for commercially harvested whole carcasses than did BPW and PBS, although researchers were unable to determine *Salmonella* presence/absence status of commercially harvested carcasses prior to sample collection. Although rinsing medium did not exhibit a statistically significant interaction with chicken parts sample type with respect to *Salmonella* detection, parts type did significantly impact *Salmonella* detection, presumably due to cross-contamination from further carcass handling and/or incoming pathogen loads from whole birds entering the fabrication environment. On *Salmonella*-inoculated carcasses, rinsing PAA-treated birds in BPW resulted in lower recovery of *Salmonella* versus CPC-treated birds and PAA-treated birds rinsed in nBPW. These data indicate that in samples that bear *Salmonella*, nBPW exhibits enhanced neutralizing activity against sanitizer residues remaining on carcass surfaces post-1 min dripping, compared with other rinsing media.

To the authors' knowledge, this represents the first study comparing BPW and nBPW poultry rinse fluids in a commercial poultry abattoir on consequent *Salmonella* detection. Enhanced *Salmonella* recovery was seen for nBPW-rinsed commercial whole chicken carcasses compared with other rinsing media, but statistically higher detection of *Salmonella* from chicken parts treated with nBPW versus BPW was not detected. This may result from differences in *Salmonella* presence on carcasses entering fabrication/cutting, as well as pathogen-reducing effects of interventions on carcasses entering fabrication. Fresh poultry products safety is best protected through application of validated antimicrobial and sanitization interventions, utilizing routine sampling to verify intervention performance, in combination with proper food preparation in the domestic or foodservice kitchen. Nonetheless, the current study assists poultry

industry members in quantifying the utility of approved sanitizers for food safety protection via enhanced capacity to detect *Salmonella* during routine sampling.

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