

Equivalency of peroxyacetic acid to chlorine as a shell egg sanitizing rinse

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ABSTRACT In the United States, all shell eggs processed under the USDA Agricultural Marketing Service voluntary grading standards must receive a shell sanitizing rinse of 100–200 ppm chlorine or its equivalent after leaving the washing process. A study was conducted to determine the concentration of peroxyacetic acid (PAA) which would be equivalent to 100–200 ppm chlorine (Cl) in reducing target organisms under the required washing conditions for shell eggs. Three isolates of *Salmonella* spp. (Enteritidis, Braenderup, and Typhimurium), as well as *Enterobacter cloacae* were used as inocula. Sanitizing treatments were negative control; deionized water; 100 and 200 ppm Cl; and 50–500 ppm PAA (7 concentrations). Considering all isolates tested, 100 and 200 ppm chlorine had 2.6 and 2.3 log cfu/mL cultural organisms remaining on shell surface; 50 and 100 ppm peracetic acid had 1.9 and 1.0 log cfu/mL cultural organisms remaining, respectively, compared with untreated

control average of 3.8 log cfu/mL ($P < 0.001$). *Salmonella* Typhimurium was least resistant to shell sanitizer treatments. Peroxyacetic acid concentrations >250 ppm did not produce significant reductions in microbial populations as PAA concentration increased. Culturing for the prevalence of viable and injured organisms, 400–500 ppm PAA resulted in fewer eggs ($P < 0.0001$) being positive for *Salmonella* spp. *E. cloacae* was culturable via enrichment from 99.4% of inoculated eggs, regardless of sanitizer treatment. The results of this study indicate that 50–100 ppm PAA is equivalent to 100–200 ppm chlorine in reducing egg surface microorganisms. The use of 400–500 ppm PAA resulted in a lower incidence of viable, but not culturable, *Salmonella* spp. on the shell surface. *E. cloacae* resulted in almost 100% viable, but not culturable, organism recovery for all sanitizing treatments and should be considered as an indicator organism when studying processing facility sanitation procedures.

Key words: shell egg, sanitizing rinse, peroxyacetic acid, chlorine, egg safety

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INTRODUCTION

In the United States, eggs processed under USDA voluntary grade certification must receive a sanitizing rinse of 100–200 ppm chlorine or its equivalent as the eggs exit the washer (USDA, 2004). United States egg producers participating in the voluntary National Organic Program certification (NOP) must remove all chlorine compounds on the surface of organic products with a potable water rinse (USDA, 2019b). Therefore, NOP-certified organic egg producers need to use an additional potable water rinse to meet voluntary grade

certification and NOP standards. This additional rinse can increase wastewater and costs.

Peracetic acid and/or peroxyacetic acid (PAA) have been shown to be reduce levels of *Staphylococcus* spp., *Listeria* spp., *Escherichia coli*, *Enterobacter cloacae*, *Salmonella* spp., and *Enterococcus* spp., as well as other organisms (Pao and Davis, 1999; Beuchat et al., 2004; Brinez et al., 2006; Rossi et al., 2007; Walter et al., 2009; Jones, 2010; Ding and Yang, 2013; Neo et al., 2013; Rosado de Castro et al., 2017; Mohammad et al., 2018). Peracetic acid compounds have been shown to be effective in the presence of organic matter (Kitis, 2004; Rossi et al., 2007; Flores et al., 2014). Recent research has focused on PAA compounds in produce washing (Pao and Davis, 1999; Beuchat et al., 2004; Walter et al., 2009; Fraise et al., 2011; Neo et al., 2013; Singh et al., 2018) with greater microbial reduction found on smooth surface produce (Pao and Davis, 1999; Singh et al., 2018).

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The present study was conducted to determine the equivalency of PAA to 100 and 200 ppm chlorine as a shell egg surface sanitizing rinse. The study was conducted with NOP-certified organic eggs challenged with 4 different organisms associated with shell eggs.

MATERIALS AND METHODS

Egg Sourcing and Handling

On four occasions, 720 organic (USDA, 2019a) brown shell, unwashed eggs were obtained from a local commercial egg producer. Across the egg collection times, eggs ranged from 2 to 6 d after lay when obtained. All eggs were held in refrigeration within 36 h of lay in accordance with US requirements (FDA, 2009). On arrival at the laboratory, eggs were placed in 4°C storage. Approximately 72 h later, eggs were washed in accordance with the methods and conditions described by Jones et al. (2014) with the exception that the shell sanitizing rinse was not conducted. Washed, intact eggs were placed on clean pulp flats and divided among 4 half-case boxes (maximum volume $n = 180$ eggs). Packaged eggs were returned to 4°C storage until use later in the week.

Preparation of Inoculum

Four isolates, all trained to be resistant to 200 ppm nalidixic acid (NAL), collected from egg production and processing environments were used for the study: *Salmonella* Braenderup (SB), Enteritidis (SE), and Typhimurium (ST), as well as *E. cloacae* (EC). *E. cloacae* was selected as an indicator organism as it has been frequently identified in the shell egg processing environment (Jones and Musgrove, 2008). The day before inoculation, a single isolate was placed in 10 mL buffered peptone water (BPW, Acumedia, Lansing, MI) at 37°C for 18–24 h. The following morning, fresh inoculum was introduced into 7 L BPW to achieve a target concentration of approximately 6 log cfu/mL. Inoculum concentration was monitored before inoculations began, mid-point, and end of inoculations each day using the enumeration methods described in sample collection and analysis section of this article. Average inoculum concentrations are presented in Table 1. Isolate and replicate concentrations of inoculums were not statistically different.

Treatments

Shell sanitizing rinse treatments were prepared with either sodium hypochlorite (Cl; Clorox Germicidal

Table 1. Inoculum concentrations for all isolates and replicates (± 0.41 log cfu/mL).

Isolate	Replicate 1	Replicate 2	Replicate 3
<i>Enterobacter cloacae</i>	6.30	6.10	6.45
<i>Salmonella</i> Braenderup	6.84	6.66	6.39
<i>Salmonella</i> Enteritidis	6.70	6.34	6.50
<i>Salmonella</i> Typhimurium	5.73	6.46	6.34

Bleach, Clorox Company, Oakland, CA) or PAA (Pera-san A, Enviro Tech Chemical Services, Modesto, CA). A summary of treatments and actual concentrations for each isolate replication is presented in Table 2. The night before treatment, 500 mL of deionized water was added to clean plastic spray bottles (16 oz plastic spray bottle, DilaBee, www.dilabee.com) for each treatment and placed in a 52°C water bath. Each morning, fresh shell sanitizing rinse treatments were prepared and concentration confirmed (Cl-K-2513; PAA-K-7913; V-2000 Multi analyte photometer; CHEMetrics, Midland, VA). The fresh deionized water treatment was confirmed each day to be free of Cl and PAA. After preparation, sanitizing rinse treatments were maintained at 52°C to mimic the temperatures generally experienced in US commercial shell egg processing. Facilities participating in the voluntary USDA egg grading program must use a shell sanitizing rinse at or greater than wash water temperature (USDA, 2004).

Egg Inoculation and Treatment Application

A single isolate was inoculated on any given day with each isolate inoculation replicated on 3 occasions. The night before inoculation, up to 180 washed eggs were placed at room temperature. Eggs were inoculated as described by Jones and Musgrove (2005) with 12 intact eggs placed in the sterile stainless steel basket for each inoculation group. After draining, eggs were placed on a sterilized polymer egg flat under a biological safety cabinet to dry for 10 min. One egg was then aseptically placed in a sterile sample bag as an untreated control. One egg was placed on egg rolling device under the biological safety cabinet and sprayed for 10 s with deionized water as the water treatment. The remaining 10 eggs were then placed on the roller device and sprayed for 10 s with the designated treatment. The water and sanitizing rinse treated eggs were placed on separate sterilized plastic egg flats and allowed to dry under the biological safety cabinet for 10 min. The process was repeated with the egg rolling device rinsed with deionized water between treatments until all treatments were used for the isolate replication.

Sample Collection and Analysis

After treated eggs were dried, each egg was aseptically placed into a sterile sample bag. Shell surface rinses of each egg were collected as per the procedures of Jones et al. (2002) using 10 mL BPW. If an egg became broken during sampling, it was excluded from analysis. Rinsates were appropriately diluted in sterile phosphate-buffered saline and duplicate plated on appropriate media (brilliant green sulfa agar with 200 ppm NAL (BGS-NAL; BGS—Acumedia; NAL—Sigma—Aldrich, St. Louis, MO) for SB, SE, and ST; standard methods agar with 200 ppm NAL (SMA-NAL; SMA—Acumedia) for EC) and incubated 18–24 h at 37°C before enumeration. The remaining rinsate was enriched for 18–24 h at 37°C before being plated onto the previously described

Table 2. Treatment concentrations for all replicates (ppm).

Shell sanitizing rinse (ppm)	<i>Enterobacter cloacae</i>			<i>Salmonella</i> Braenderup			<i>Salmonella</i> Enteritidis			<i>Salmonella</i> Typhimurium		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Cl 100	106.0	102.8	94.8	101.6	96.8	98.8	96.0	105.2	102.4	99.2	106.4	99.2
Cl 200	204.0	212.8	207.6	194.0	201.6	193.6	202.4	197.6	204.8	209.6	201.6	208.8
PAA 50	50.0	52.2	50.6	53.0	49.4	47.6	52.6	47.8	50.4	52.0	53.6	51.8
PAA 100	103.0	96.8	98.0	92.0	98.8	95.2	98.8	103.2	102.6	101.2	101.0	102.8
PAA 175	179.0	171.5	172.2	168.7	177.1	168.0	175.7	176.4	178.5	182.7	175.7	180.6
PAA 250	252.0	255.0	257.5	247.0	255.0	256.0	244.0	261.0	258.5	248.0	259.0	249.0
PAA 325	321.0	323.7	318.5	325.0	326.3	322.4	332.8	314.6	327.6	339.3	339.0	318.5
PAA 400	406.0	393.6	408.8	389.0	427.0	387.2	404.8	385.6	391.5	419.2	404.8	404.0
PAA 500	524.0	482.0	523.0	494.0	488.0	489.0	496.0	496.0	496.0	506.0	514.0	488.0

Abbreviations: Cl, chlorine; PAA, peroxyacetic acid; Rep, replicate.

media to determine prevalence after incubation for 18–24 h at 37°C.

Statistical Analysis

Enumerated values for each isolate were subjected to an analysis of variance with the general linear model (SAS Institute, 2002). Data were pooled across isolates for average impact of treatment with treatment and replicate as the main effects. Data were also sorted by isolate and analyzed with treatment and replicate as the main effects to determine impact of shell sanitizing rinse treatment on each isolate. Similar models were used for the chi-square analysis of the microbial prevalence data.

Within an isolate, the maximum number of samples for each shell sanitizing rinse treatment is $n = 30$, with deionized water ($n = 27$) and untreated controls ($n = 27$) representing each inoculation dip set, allowing for a maximum number of eggs sampled for an isolate being $n = 324$. Any eggs cracked during sample collection were removed. Data presented in this article represent total eggs sampled as EC, $n = 322$; SB, $n = 322$; SE, $n = 321$; ST, $n = 324$.

RESULTS

The overall microbial levels detected in shell rinsates, across all isolates, were impacted by a treatment*replicate interaction ($P < 0.0001$; Figure 1). Untreated controls had 3.5–4 log cfu/mL shell rinsate after inoculation and drying. Deionized water, Cl 100, and Cl 200 treatments resulted in 1.8–2.8 log cfu/mL rinsate cultural organisms remaining on the shell surface. Peroxyacetic acid 100 (0.6–1.5 log cfu/mL) reduced bacterial loads more than PAA 50 (1.6–2.2 log cfu/mL). Greater than 175 ppm PAA as a shell surface sanitizer produced biologically similar microbial loads, generally less than 0.5 log cfu/mL across all isolates combined.

The effect of shell sanitizing rinse treatments on each isolate is shown in Table 3. Differences in microbial levels found in shell rinsates were seen for all isolates used. The response to the various shell sanitizing rinse treatments varied among the isolates. Differences in microbial counts obtained by traditional culturing that are less than 1 log cfu/mL can be statistically significant but may not be biologically significant due to the nature of cultural microbiology. *Salmonella* Enteritidis levels were decreased in a similar manner for deionized water, Cl 100, Cl 200, and PAA 50 (0.9–1.6 log cfu/mL;

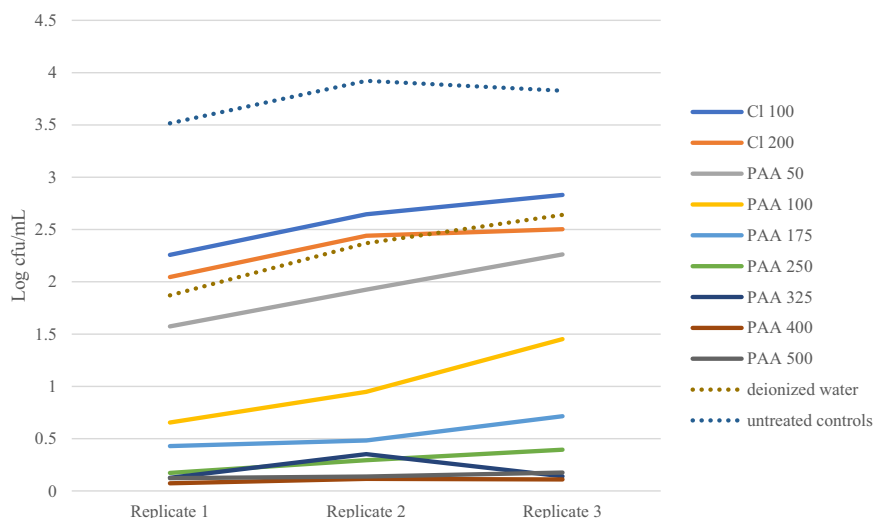


Figure 1. Interaction of shell sanitizing rinse treatment (ppm) and replicate on overall levels of cultural organisms present in shell surface rinsates with all isolates combined ($P < 0.0001$). Abbreviations: Cl, chlorine; PAA, peroxyacetic acid.

$P < 0.0001$). Peroxyacetic acid 100 and PAA 175 produced similar reductions in SE (2.5–2.75 log cfu/mL). Peroxyacetic acid greater than 250 ppm resulted in consistently low SE levels, regardless of PAA concentration. A similar trend was observed for SB.

Both EC and ST produced treatment*replicate interactions ($P < 0.001$). Across the replicates, deionized water and Cl 100 had almost identical levels of EC cultural organisms remaining after treatment (2.0–2.75 log cfu/mL; Figure 2). Peroxyacetic acid 50 and PAA 100 had distinctly separate reductions in shell rinsate EC levels across the replicates. The reductions observed for PAA 50 and PAA 100 did not cross with levels of detected cultural organisms for other treatments. Peroxyacetic acid concentrations of 175 ppm and greater produced similar levels of organism detected in shell rinsates. Unlike other isolates tested, PAA 50 had varied outcomes across replicates on the reduction of ST (Figure 3). Furthermore, PAA 100 had a similar level of ST reduction as greater than or equal to 175 ppm PAA treatments for first 2 replicates. Compared with the other isolates tested, ST appears to be more susceptible to the shell sanitizing rinse treatments used in the present study.

While microbial levels were found to be decreased by shell sanitizing rinse treatments, the prevalence of the isolates in shell rinsates indicate viable, but not culturable, cells remained (Table 4). Overall, greater reductions in microbial prevalence were seen at 400 and 500 ppm PAA when all isolates were combined. This was pronounced for SE ($P < 0.0001$) with 80% detection for PAA 325 and 33.33 and 44.83% positive samples at 400 and 500 ppm PAA, respectively. *Salmonella* Braenderup experienced a drop in detection between PAA 250 (75.86%) and PAA 325 (43.33%; $P < 0.0001$). *Salmonella* Typhimurium was the most susceptible to sanitizing shell rinse treatment with only 13.33 and 6.67% prevalence in rinsates from the PAA 400 and PAA 500 treatments ($P < 0.0001$). There was no difference in EC prevalence with 96.55% detection in PAA 500. When enumerated levels within isolates (Table 3) are compared with prevalence data (Table 4), ST had no detectable counts for PAA 175 yet a corresponding

93.33% prevalence in the same rinsates. For SB, PAA 500 produced no detectable counts in shell rinsates and only a 10.34% prevalence when the samples were enriched.

Of the total 1,289 enumerated samples in the study, 41% ($n = 528$) resulted in no detectable counts in appropriate dilutions. After enrichment and subsequent selective plating on appropriate media containing 200 ppm NAL, 319 samples (60% of no detectable count samples; 25% of total enumerated samples) were found to be positive for the inoculated organism indicating the presence of viable, but not culturable or injured organisms. The viable, but not culturable or injured organisms were primarily detected in PAA treatments (Table 5). The PAA concentrations producing the situation was dependent on the organism with EC having 80 and 57% of PAA 400 and PAA 500 samples, respectively, being viable, but not culturable. *Salmonella* Braenderup and SE experienced a high level of viable, but not culturable or injured organisms in PAA 325, PAA 400, and PAA 500 treatments. Both enumeration (Table 3) and prevalence (Table 4) data show that ST was most susceptible to the shell sanitizing rinse treatments, yet the PAA treatments greater than or equal to 175 ppm resulted in a high degree of viable, but not culturable or injured cells contributing to ST prevalence detection.

DISCUSSION

The effectiveness of Cl and PAA as food surface sanitizers has been explored with a wide array of outcomes. Much of this work has been conducted in produce. As was seen in the present study, often statistical differences are found with either Cl or PAA treatments being superior but counts are within a log cfu/mL raising question of the biological difference of the treatments (Beauchat et al., 2004; Neo et al., 2013). Pao and Davis (1999) and Singh et al. (2018) found that smooth surface or unscarred fruits benefited the most from Cl and PAA surface sanitizing treatments. While the egg has pores on the surface, overall the surface of a grade AA or A (USDA, 2000) should be smooth.

Table 3. Average counts (log cfu/mL) in shell rinsates for each isolate exposed to shell sanitizing rinse treatments.¹

Shell sanitizing rinse (ppm)	<i>Enterobacter cloacae</i>	<i>Salmonella</i> Braenderup	<i>Salmonella</i> Enteritidis	<i>Salmonella</i> Typhimurium
Cl 100	2.45	2.77 ^b	2.92 ^b	2.17
Cl 200	2.25	2.54 ^{b,c}	2.69 ^c	1.85
PAA 50	2.02	2.12 ^c	2.26 ^{b,c}	1.29
PAA 100	1.25	0.94 ^d	1.32 ^d	0.57
PAA 175	0.70	0.42 ^{d,e}	1.09 ^d	ND
PAA 250	0.58	0.18 ^e	0.34 ^e	0.04
PAA 325	0.53	0.03 ^e	0.18 ^e	0.09
PAA 400	0.20	0.08 ^e	0.03 ^e	0.10
PAA 500	0.43	ND ^e	0.03 ^e	0.11
Deionized water	2.44	2.39 ^{b,c}	2.34 ^c	2.00
Untreated controls	3.58	4.02 ^a	3.85 ^a	3.57
SE	±0.10	±0.10	±0.09	±0.09
<i>P</i> value	**	0.0001	0.0001	**

^{a,b}Means within a column with different superscript are significantly different; $P < 0.05$.

**Treatment and replicate interaction; $P < 0.001$.

Abbreviation: Cl, chlorine; ND, none detected; PAA, peroxyacetic acid.

¹Individual isolates, $n = 30$ for shell sanitizing rinse treatments; $n = 27$ for deionized water and untreated controls.

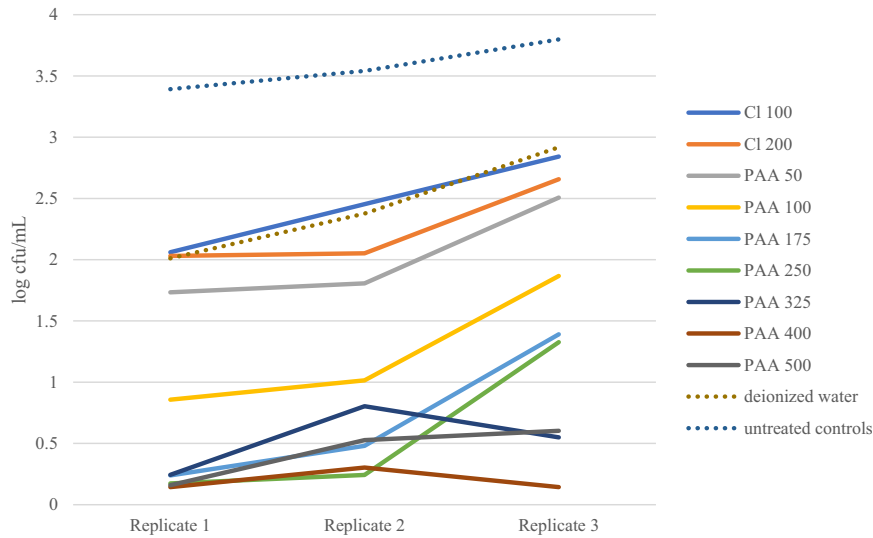


Figure 2. Interaction of shell sanitizing rinse treatment (ppm) and replicate on *Enterobacter cloacae* levels in shell surface rinsates ($P < 0.01$). Abbreviations: Cl, chlorine; PAA, peroxyacetic acid.

Vinayananda et al. (2017) found 100 ppm PAA and 200 ppm Cl reduced shell surface *E. coli* in a similar manner. In the present study, 100 ppm PAA reduced shell microbial levels both significantly and biologically compared to 200 ppm Cl across all four isolates tested. There were differences in how eggs were handled between the 2 studies, with the present study mimicking USDA guidance (USDA, 2004), including egg washing, while using subsequent inoculation techniques. Vinayananda et al (2017) did not include commercial egg washing practices, as methods were based on the accepted egg handling techniques where the research was conducted.

In the present study, the sanitizing treatments were applied to the shell surface after the washed eggs had dried, stored at 4°C, and then brought to room temperature. Commercial processing of shell eggs under USDA

voluntary grading (USDA, 2004) involves a shell sanitizing rinse applied immediately after washing, before the eggs are blown dry. In the present study, this was not possible because the eggs were inoculated just before the shell sanitizing treatments application. Eggs were not inoculated before washing because the washing conditions (pH 11, 48°C–50°C) would greatly reduce remaining inoculum. The intent of the present study was to determine the effectiveness of various levels of PAA as a shell surface sanitizing rinse as well as determine the levels of PAA which were equivalent to the sanitizing effectiveness of 100 and 200 ppm Cl. The egg and sanitizing treatment temperatures used in the present study should also allow for a more worldwide usage of the results since eggs are handled in a wide variety of manners around the globe. The present study examined the impact of shell sanitizing rinse treatments on

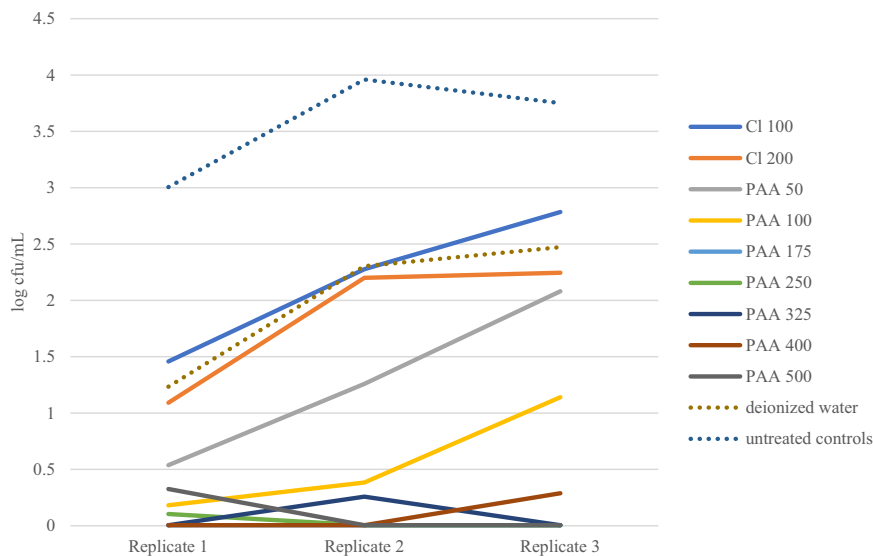


Figure 3. Interaction of shell sanitizing rinse treatment (ppm) and replicate on *Salmonella Typhimurium* levels in shell surface rinsates ($P < 0.01$). Abbreviations: Cl, chlorine; PAA, peroxyacetic acid.

Table 4. Prevalence of organism detection (percent) in shell rinsates after exposure to shell sanitizing rinse treatments.

Shell sanitizing rinse (ppm)	Overall ¹	<i>Enterobacter cloacae</i> ²	<i>Salmonella</i> Braenderup	<i>Salmonella</i> Enteritidis	<i>Salmonella</i> Typhimurium
Cl 100	100	100	100	100	100
Cl 200	100	100	100	100	100
PAA 50	100	100	100	100	100
PAA 100	100	100	100	100	100
PAA 175	92.31	100	76.67	100	93.33
PAA 250	78.99	96.67	75.86	83.33	60
PAA 325	68.33	100	43.33	80	50
PAA 400	45	100	33.33	33.33	13.33
PAA 500	39.32	96.55	10.34	44.83	6.67
Deionized water	100	100	100	100	100
Untreated controls	100	100	100	100	100
<i>P</i> value	<0.0001	0.535	<0.0001	<0.0001	<0.0001

Abbreviations: Cl, chlorine; PAA, peroxyacetic acid.

¹Combined results of all four isolates; n = 120 for shell sanitizer treatments; n = 108 for deionized water and untreated controls.

²Individual isolates, n = 30 for shell sanitizing rinse treatments; n = 27 for deionized water and untreated controls.

reducing 4 organisms on the shell surface using shell rinsates as the sampling medium. Additional research is needed to determine if the shell sanitizing rinse treatments impacted organisms within the shell matrix and membranes.

While the current USDA guidance requires a shell sanitizing rinse concentration of 100–200 ppm chlorine or its equivalence (USDA, 2004), the results of the present study show that deionized water was as effective as 100 and 200 ppm Cl in reducing all four isolates. For equivalency purposes, 50 ppm PAA was most similar to 100–200 ppm Cl in microbial enumerations and prevalence (100% prevalence for all organisms at these sanitizer concentrations). The allowable limits for PAA on produce and poultry carcasses and parts are 80 and 220 ppm, respectively (FDA, 2016). Based on the data from this study, it is recommended that the PAA equivalency to the shell sanitizing rinse capacity of 100–200 ppm Cl would be 50–100 ppm PAA. According to the data, this level of PAA greatly reduces microbial populations compared with 100–200 Cl.

In addition, this study finds that when assessing sanitizer effectiveness, it is important to monitor both enumerated levels of residual contamination, as well as the prevalence of the target organism. Enumerated levels of the isolates dropped dramatically as PAA

concentration increased, but the when the same rinsates were enriched, a high percentage of samples with no detectable counts from direct plating were positive for the target organism. This was particularly the case for *E. cloacae* which had almost 100% prevalence at even the highest concentrations of PAA. The resistance of EC to these very high sanitizer concentrations identifies it as a good indicator organism when assessing sanitation intervention strategies.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Table 5. Viable but not culturable shell rinsates detection after exposure to shell sanitizing rinse treatments.

Shell sanitizing rinse (ppm)	Overall ¹	<i>Enterobacter cloacae</i>	<i>Salmonella</i> Braenderup	<i>Salmonella</i> Enteritidis	<i>Salmonella</i> Typhimurium
Cl 100	0/120	0/30	0/30	0/30	0/30
Cl 200	1/120	0/30	0/30	0/30	1/30
PAA 50	8/120	0/30	1/30	0/30	7/30
PAA 100	39/120	5/30	10/30	5/30	19/30
PAA 175	63/108	14/29	13/23	8/28	28/28
PAA 250	66/94	14/30	18/22	17/25	17/18
PAA 325	63/82	18/30	12/13	20/24	13/15
PAA 400	44/54	24/30	8/10	9/10	3/4
PAA 500	33/46	17/28	3/3	12/13	1/2
Deionized water	1/108	0/27	0/27	0/27	1/27
Untreated controls	0/108	0/27	0/27	0/27	0/27

Abbreviations: Cl, chlorine; PAA, peroxyacetic acid.

¹Values represent number of samples without viable counts, yet positive for target organism after enrichment/total number of enriched samples positive for target organism.

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