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Research Paper

Exploring Washing Procedures for Produce Brush Washer

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ABSTRACT

Previous environmental monitoring projects in food production facilities have revealed inconsistencies in how produce brush washer machines are cleaned after use; thus, the study of effective sanitation procedures for these machines is needed. Four chlorine solution treatments (ranging from 25 to 200 ppm), as well as a water-only treatment, were tested for efficacy in reducing bacterial loads for a selected small brush washer machine. Results indicate that rinsing with the machine's power and water alone, a frequent practice among some produce processors, yielded a reduction of 0.91–1.96 log CFU per brush roller in bacterial counts, which was not statistically significant ($p > 0.05$). However, the chlorine treatments were found to be effective in reducing bacterial loads significantly, with higher concentrations being the most effective. The 200 ppm and 100 ppm chlorine treatments yielded bacterial reductions of 4.08 and 3.95 log CFU per brush roller, respectively, leaving bacterial levels statistically similar to the levels at postprocess decontamination, meaning these are the most effective at killing bacteria of all the chlorine concentrations tested. These data suggest the use of at least 100 ppm chlorine sanitizer solution is a good method to sanitize hard-to-clean produce washing machines, yielding an approximate 4 log CFU reduction of the inoculated bacteria.

Produce safety is a growing twenty-first century challenge, with one study finding that approximately 46% of all foodborne illness outbreaks in the United States from 1998 to 2008, as well as 38% of foodborne illness-related hospitalizations, could be attributed to produce (Painter et al., 2013). Fruits and vegetable row crops were found to be the leading causes of multistate foodborne disease outbreaks from 2010 to 2014 (Crowe et al., 2015). Additionally, more recent studies have shown that the number of produce-associated foodborne disease outbreaks in the US increased further from 2010 to 2017 (Carstens et al., 2019). Prevention of further outbreaks requires enhanced food safety practices at all stages of food production.

One critical stage is during postharvest processing of produce. Studies have shown that microbial cross-contamination can occur during washing and conveying of produce during processing (Castillo et al., 2004; Smolinski et al., 2018). A key measure for food processors to prevent microbial cross-contamination is to sanitize the equipment used during processing between production lots. However, many different types of machines are used in postharvest processing, and each type presents unique sanitation challenges. This is problematic as most widely published sanitation and food safety research fails to target the equipment used by small farmers, does not account for the time, resource, and manpower constraints of smaller-scale farmers, and does not illustrate the insufficiency of the strategies currently in use.

To better quantify these risks, an environmental monitoring program of a small farm in South Deerfield, Massachusetts was carried out over the 2014 and 2015 harvesting seasons (unpublished). In this program, researchers collected and analyzed samples from various sites around the facility using Aerobic Plate Counts (APCs) and Coliform counts, including an OESCO brush washer unit. Microbial loads of samples recovered from the brush washer ranged from <10 to 10^7 CFU/mL of coliforms (data not shown). This wide variability over the course of the growing season in both years showed no apparent trend or "baseline", indicating the variation was due to inconsistent implementation of sanitation protocols rather than seasonal variation. Produce brush washers, depending on size and type, have numerous harborage points for pathogens and can cause cross-contamination of produce throughout processing if proper sanitation procedures are not followed (Wang et al., 2021). Given that similar pieces of equipment have been implicated in cross-contaminating produce (Centers for Disease Control and Prevention, 2011; U.S. Food and Drug

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Administration, 2012), this machine may have a potentially high food safety risk that current practices do not effectively manage. There are currently few established sanitation standard operating procedures (SOPs) for such machines, but observations during the above-mentioned environmental monitoring program and similar programs found that most farmers simply rinsed the machines with water between produce lots, with one deep cleaning at the end of the production season. Such practices have not been experimentally validated and may be inadequate to prevent microbial cross-contamination. Therefore, there is a need to investigate the best practice for this specific piece of equipment, which would also inform the management of similar equipment.

The OESCO brush washer (Figs. 1 and 2) is used to rinse and brush debris off fruiting crops such as bell peppers, squash, eggplants, and cucumbers. Nine rollers inside the machine spin via an electric motor drive shaft and gear system at different rates to dislodge dirt and field debris from produce while allowing the produce to travel through the machine; nozzles above the rollers spray a fine mist of tap water over the produce as it travels through. This piece of equipment cannot be disassembled easily for cleaning and sanitizing, presenting a pressing food safety challenge. As there is currently no designated sanitation SOP for this equipment, the aim of this study was to determine the effectiveness of a range of sanitation procedures that could potentially increase microbial safety without placing undue burdens on small- and medium-scale food producers. These procedures included water-only rinsing, a method currently used by many smaller-scale farmers, versus 25–200 ppm chlorine treatments. This work illustrates the implications of current widely used sanitation practices on food safety while highlighting the need for more rigorous sanitation SOPs on farms, and could potentially serve as a valuable tool for food safety extension educators to help coach small- and medium-scale food producers in the implementation of more rigorous and effective sanitation procedures.

Materials and methods

Site Preparation. The washing unit was tagged out of operation and trials were conducted in the off-season to ensure that the unit was quarantined during this research. Prior (16 h) to the designated trial time, physical debris was removed, and the unit was washed with mechanical force using water and food-grade brushes and then disinfected with 10% chlorine bleach (Clorox Germicidal bleach solution (EPA Reg. No. 5813-102), The Clorox Company). The chlorine concentration was confirmed using a LaMotte-free chlorine test strip (Code 4250-BJ, La Motte).

All food contact surfaces of the unit (the interior, the input chute, and the output chute) were doused with the 10% Clorox Germicidal bleach solution. During application, the rollers were turned multiple times to ensure all sides were exposed to the solution. The machine was run for 30 s and then allowed to sit for 2 min, the recommended contact time for bleach on a food contact surface (McGlynn, 2016; U.S. Food and Drug Administration, 2015). The machine was then allowed to sit overnight to allow the chlorine to evaporate. The following day, the machine was confirmed to be free from residual chlorine by running a free chlorine test strip through the machine and observing for any color change. Overnight, after cleaning, and in between runs, the machine was covered with a plastic drop cloth to minimize any particulates settling on the machine.

Organism and Inoculation. An overnight culture of a non-pathogenic streptomycin-resistant *Escherichia coli* strain, Castellani and Chalmers (ATCC 35695, MC4100) was prepared by transferring a loopful of late-exponential phase culture into an Erlenmeyer flask containing 150 mL of BHI broth with 20 μg/mL streptomycin, similar to a protocol described by Annous et al., who evaluated the efficacy of cleaning procedures on a commercial flatbed brush washer (Annous et al., 2001). The flask was incubated at 37°C with 200 rpm shaking.

Figure 1. OESCO Brush Washer Unit, Exterior. (Source: Author)

Figure 2. OESCO Brush Washer Unit, Interior. (Source: Author)

Cell concentration was confirmed to be about 10^8 CFU/mL by spread plating.

As part of a random sampling plan, the rollers were divided into three sections, labeled A, B, and C. To account for the reduction in loads due to swabbing, only one-third of the roller, a 96 cm^2 area, was sampled for each time point. A random letter sequence generated using Excel prior to the start of the test determined in which order the thirds would be sampled. Five 0.1 mL aliquots of the culture were deposited at the base of the bristles of the designated rollers in an "x" pattern, totaling 0.5 mL of culture per one-third section of the roller (Fig. 3). This gave a mean total application of about 8 log CFU of *E. coli* per section.

Sampling. Sponge swab sticks with 10 mL buffered peptone water (3M) were used to collect all samples. While testing the water-only cleaning procedure, sampling took place at the following locations, in the following order, at each of the designated time points: Input Chute, Output Chute, Drain, Roller 1, Roller 3, Roller 9, and Interior Wall. For the Input Chute, Output Chute, and Interior Wall sites, an area of 200 cm^2 was sampled; for the drain, an area of 366 cm^2 was sampled; and for the rollers, an area of 96 cm^2 was sampled. Samples were taken at the following eight time points: 1) precleaning; 2) postcleaning; 3) postinoculation (Time = 0); 4–7) machine total running (water and rolling) times of 30, 60, 120, and 300 s; and 8) postrun sanitizing.

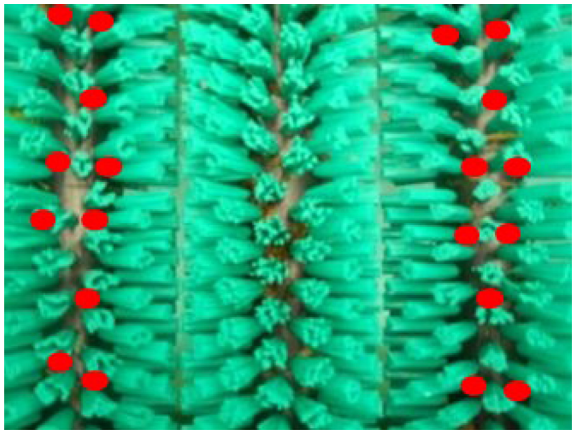

Figure 3. Detail of Brush Rollers (Partial). Red annotations indicate approximate inoculation points (Source: Author)

While testing the chlorine concentration treatment studies, two rollers on the machine were inoculated with an average of 5.9 log CFU of *E. coli* per third of each roller (a sampling area of 96 cm^2). The machine was rinsed by running with water from the internal sprayers for 30 s and then the sanitizing effects of different chlorine concentrations were tested by pouring five gallons of 200, 100, 50, and 25 ppm chlorine bleach solutions inside the chamber until the rollers and contact surfaces were completely doused. These solutions were prepared by diluting Clorox germicidal bleach (75,500 ppm sodium hypochlorite) into municipal water at the farm site, and each concentration was confirmed using a chlorine test strip. After dousing the inside chamber with the specified chlorine concentration, the machine was run for 30 s to distribute the chlorine solution and then switched off for two minutes to allow the chlorine to take effect. Rollers were swabbed before inoculation, after inoculation, after the 30-second water rinse, after dousing with chlorine and running for 30 s, and after the two-minute decontamination to quantify any *E. coli* remaining on the machine. Swabs were taken of a third of each roller sampled and reported as log CFU/96 cm^2 (the swabbed surface area). The first two sample time points were collected to gauge how many bacteria were present on the machine in each site before any intervention and to then remove those bacteria. The last time point served to measure the successful removal of all bacteria, especially the bacteria that were intentionally applied. Samples were transported on ice in a cooler back to the laboratory, kept at 4°C, and processed within twenty-four hours. Each entire procedure was repeated a total of three times on three separate days.

To prevent introduction of the target bacteria into the environment, the water exiting the machine was collected and treated with 400 ppm free chlorine. The water was allowed to sit for 30 min, according to recommendations for water treatment before being disposed of down the drain (LeChevallier et al., 1988; LeChevallier & Au, 2004).

Microbial Plating. Samples were serially diluted in buffered peptone water dosed with 20 μg streptomycin per 9 mL and plated in duplicate onto *E. coli*/Coliform Count Petrifilm (3M). Preliminary data (not shown) confirmed that this method yielded similar counts and selection of target bacteria to the agar plate method, so it presented a faster and more user-friendly alternative. Films were allowed to incubate at 37°C for 24 h and then counted for coliforms. According to the manufacturer's direction, films were allowed to incubate further for 24 h before counting for *E. coli*. Counts were recorded as CFU/cm^2 in the area sampled.

Data Analysis. For each treatment used in this study, three trials were performed on different days and microbial enumeration results were averaged to determine the bacterial reductions at each site for each treatment. Statistical analysis was performed using Statistical Analysis Software (SAS); a one-way ANOVA was used to determine if there was any significant ($p < 0.05$) difference between means, and statistical groupings were determined using a Waller-Duncan Test ($\alpha < 0.05$).

Results

The first sanitation method evaluated was the water-only rinse procedure. Here, Rollers 1 and 3 were purposefully inoculated with

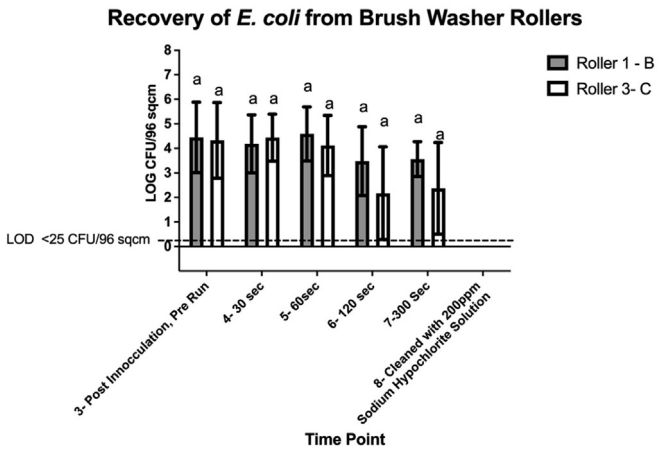

Figure 4. Recoveries of *E. coli* from Rollers of Brush Washer During Water Rinse. Error bars indicate standard deviations of the mean from the analysis. Means sharing a letter are not significantly different ($\alpha = 0.05$, Waller-Duncan comparisons). There was no statistically significant difference between the means of both rollers at each time point and the mean amount initially recovered after inoculation as determined by a one-way ANOVA ($p = 0.348$). The Waller-Duncan Test revealed only one statistical grouping for this data set (SAS).

8.17 log CFU of *E. coli* per section, and the recoveries of bacteria were measured at seven different sites on the brush washer after running the machine for 30, 60, 120, and 300 s (Fig. 4). This allowed for evaluation of the effect of water-only rinse on microbial load as well as the potential for microbial transfer from the rollers to other parts of the machine. There was no statistically significant difference between the amounts recovered from the two rollers over the time period tested and the amount initially recovered as determined by a one-way ANOVA ($p = 0.348$); a Waller-Duncan Test revealed only one statistical grouping, further indicating no statistical differences among treatment times. Plate counts at all time points from Input Chute, Output Chute, Roller 9, and Wall were below counting limits (data not shown); therefore, microbial loads at these sites were below the lowest quantifiable limit for the films (<25 CFU/mL). Mean recoveries of bacteria at the drain after 30, 60, 120, and 300 s respectively were 0.79, 0.93, 1.69, and 1.04 log CFU/366 cm^2. No apparent trend was observed for this site.

Mean recoveries of *E. coli* from Roller 1 in the machine are displayed in Fig. 5. In addition, 8.17 log CFU of *E. coli* were applied to each section of the roller, 4.47 log CFU were recoverable in the same area by sponge sampling, and after 300 s (5 min) of water-only processing, 3.56 log CFU remained, indicating that this roller achieved almost a 1 log CFU or 90% reduction using water alone. A one-way ANOVA yielded no statistical difference among means from the time points ($p = 0.707$). Statistical analysis revealed one statistical grouping for the means.

The recoveries of *E. coli* from the third roller in the machine are displayed in Fig. 6. In addition, 8.17 log CFU of *E. coli* were applied to each section of the roller, 4.33 log CFU were recoverable in the same area by sponge sampling, and after 300 s (5 min) of water-only processing, 2.37 log CFU remained, indicating that this roller achieved a 1.96 log CFU reduction over the five minutes of treatment. A one-way ANOVA yielded no statistical difference among means from the time points ($p = 0.254$) While some time points have large standard deviations, the Waller-Duncan Test revealed only one statistical grouping for this data set (SAS).

In contrast to water-only rinsing, treatment of rollers with chlorine bleach sanitizer solutions resulted in significant reductions of the inoculated *E. coli* levels in all trials (25, 50, 100, and 200 ppm). The levels of *E. coli* remaining on rollers throughout the experiment are shown in

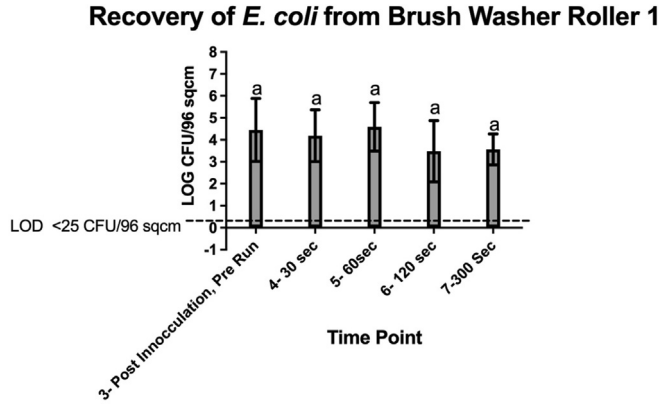

Figure 5. Recoveries of *E. coli* from Roller 1 During Water Rinse. Error bars indicate standard deviations of the mean from the analysis. Means sharing a letter are not significantly different ($\alpha = 0.05$, Waller-Duncan comparisons). There was no statistically significant difference in *E. coli* counts between each time point as determined by a one-way ANOVA ($p = 0.707$). The Waller-Duncan Test revealed only one statistical grouping for this data set (SAS).

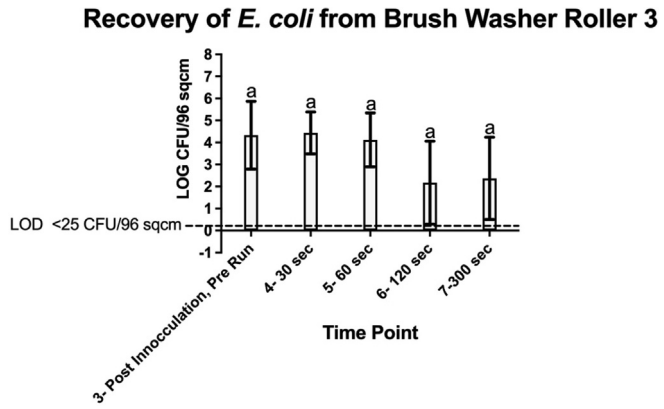

Figure 6. Recoveries of *E. coli* from Roller 3 During Water Rinse. Error bars indicate standard deviations of the mean from the analysis. Means sharing a letter are not significantly different ($\alpha = 0.05$, Waller-Duncan comparisons). There was no statistically significant difference in *E. coli* counts between each time point as determined by a one-way ANOVA ($p = 0.254$). The Waller-Duncan Test revealed only one statistical grouping for this data set (SAS).

Figure 7. All chlorine treatments showed statistical difference from postinoculation and water-rinse time points. This shows that all treatments significantly decreased the amount of bacteria on the rollers, even at just 25 ppm chlorine. The 200 ppm and 100 ppm chlorine treatments yielded bacterial reductions that were statistically similar to the levels at postprocess decontamination, meaning these are the most effective at killing bacteria of all the chlorine concentrations tested. Chlorine concentrations of 50 ppm and 25 ppm left levels that were statistically different from the postprocess decontamination results, meaning there was a significant number of bacteria left on the brush washer after treatment.

Reductions of *E. coli* for each chlorine treatment are enumerated in Figure 8. Mean *E. coli* reductions after 200 ppm, 100 ppm, 50 ppm, and 25 ppm chlorine treatments were 4.08, 3.95, 3.51, and 1.79 log CFU per third of roller, respectively, compared to the water-rinse time point. Treatment with 25 ppm chlorine was statistically significantly different from the other treatments, meaning it was the least effective in reducing bacterial levels on the brush washer. Treatment with 50 ppm chlorine was statistically similar on average to the higher chlorine level treatments but showed greater variability in log reduction

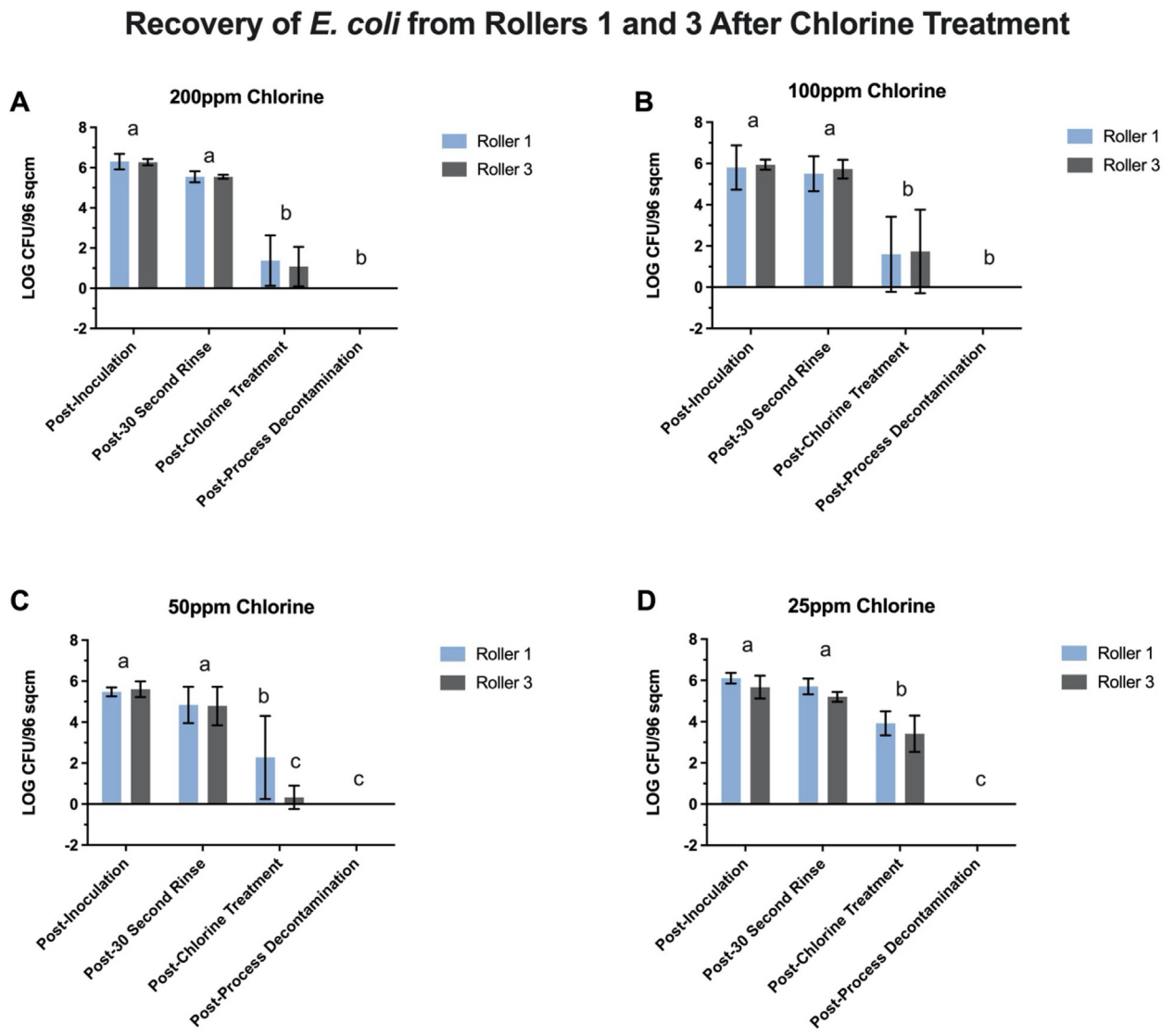

Figure 7. Recoveries from Rollers 1 and 3 of *E. coli* in each Chlorine Treatment. Letters to the left of each graph represent the chlorine treatment concentration applied: A: 200 ppm, B: 100 ppm, C: 50 ppm, and D: 25 ppm. Letters above the graph columns indicate statistical significance groupings by Duncan test (SAS) relative to the same treatment's time points; different letters indicate statistically different results.

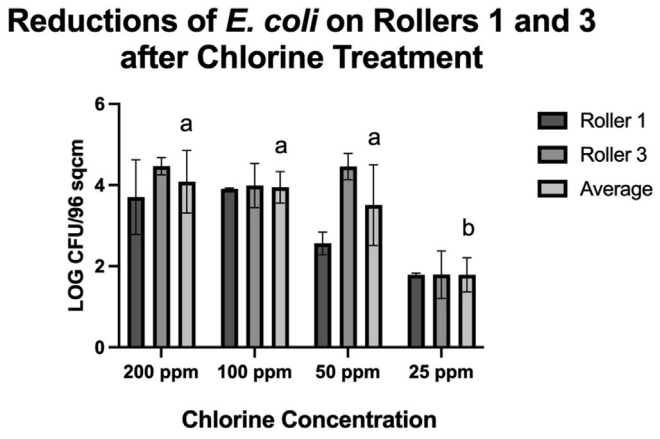

Figure 8. Reductions in *E. coli* Levels After Chlorine Treatments. Letters above the graph columns indicate statistical significance groupings by Duncan test (SAS) relative to the same treatment's time points; different letters indicate statistically different results.

across different rollers. Overall, these data suggest that a 100 ppm minimum chlorine solution would be most effective to reduce bacterial levels on a machine like this brush washer.

Discussion

If a piece of equipment will be used in direct contact with food, there is a need for strong sanitation practices and SOPs that counter the complexity and unpredictability of bacterial transfer. Washers such as the brush washer studied here have the potential to cross-contaminate batches of produce if not cleaned effectively and regularly; unfortunately, cleaning this machine is particularly challenging because it cannot be disassembled easily. Disassembly for cleaning is therefore not a common practice, which is problematic as the majority of food contact surfaces are on the inside of the machine. Defining parameters for this study demonstrated how the design of this machine is not conducive for quick and easy sanitation. Some research focusing on toothbrushes has found larger bacterial loads on open versus closed brushes because of the increase in the number of harborage points (Morris et al., 2014). In this vein, the brush rollers present the highest

risk because the bristles have numerous harborage points, which contribute to an unpredictable transfer rate. For this reason, our study focused on the reduction of microbial load on the brush rollers. This was further supported by a study on dish washing utensils, which found that bacteria could remain on kitchen brushes for at least seven days with only a ~2 log CFU reduction from initial levels, demonstrating that bacteria can persist on brush surfaces for long periods without proper sanitation (Møretrø et al., 2021).

To evaluate the efficacy of the water-only sanitation SOP used at this particular small farm, two sites on the brush washer unit were inoculated with nonpathogenic *E. coli* bacteria. This particular strain was previously used in a juice processing plant for a sanitation validation study and was selected to serve a similar role in this study (Annous et al., 2001). Seven sites on the brush washer were swabbed at eight different time points and analyzed for recovery of the bacteria. There were four sites from which the target bacteria were not recovered, indicating that there is a low frequency of transfer or splatter from the inoculated sites to the other parts of the machine during this procedure. This does not eliminate the potential for transfer, but shows that under the prevailing conditions, there are insignificant levels of transfer from the rollers to the rest of the machine.

The orientation of water nozzles over the roller arrangement causes changes in direction and pressure of water as the produce travels through the machine to aid in cleaning produce; however, this also exposes each roller to a different treatment of water. For this reason, two rollers at different locations in the machine were analyzed. Roller 1 at the front of the machine sees only a mist of water, while Roller 3 is more directly under a nozzle and sees a different treatment. This may be the explanation for why some load reductions were numerically higher on Roller 3 than on Roller 1. The fixed nozzle orientation and water pressure present a limitation of this equipment, further reinforcing the need for changes to this standard sanitation operating procedure to ensure that consistent reductions are achieved across the machine.

In addition to the variance in water spray, variations in reduction levels of the chlorine-treated tests are most likely due to variation of the application of the chlorine solution. The machine's build does not allow for clear visibility into the chamber, making it somewhat challenging to apply the five gallons of solution evenly. Replication of the trials served to help minimize this error and other possible experimenter errors. Another potential source of variation in the results was the lack of pH adjustment in the chlorine solutions prior to treatment. Current guidance for the use of chlorine-based sanitizers dictates that the solution pH should be adjusted to 6.5–7.5 prior to application, since this range gives the greatest concentration of hypochlorous acid, which is the active sanitizing agent in chlorine bleach (McGlynn, 2016). However, this was not performed in this study. This is because the aim of this study was to inform guidance for small- and medium-scale produce growers and processors, who may not have the resources and equipment to perform pH adjustment on their sanitizer solutions before treating their equipment. Though the lack of pH adjustment may reduce sanitizer efficacy and increase variability in the results, it gives a more realistic reflection of the practices that would be used in an on-farm setting. Future extension education programs should also note, however, that pH adjustment of chlorine solutions to the 6.5–7.5 range should be performed whenever feasible for best results. In this vein, the use of municipal water rather than purified water may have also limited the efficacy of the bleach solutions due to the potential presence of organic load and other interfering components, but it was important to use the same resources that would actually be available to small- and medium-scale growers.

Total reductions of 1–2 log CFU with water rinse were not statistically significant but show the baseline reduction that can be provided by a basic SOP. This lack of reduction is consistent with other published studies using only water (Goode et al., 2013; Wirtanen et al., 1996). The lack of effectiveness of a water-only cleaning regimen found in this and other studies is problematic because a water-only rinse is a common method of "cleaning" machinery by farmers who may be pressed for time, leaving the resulting "washed" produce at risk for bacterial contamination from and across the equipment.

Considering that bacterial load can be as high as 10^7 CFU/mL on this machine, the reductions indicate that water washing alone as well as treatment with 25 ppm chlorine do not significantly reduce bacteria. The most effective treatments tested in this study were 200 ppm and 100 ppm chlorine solutions, with reduction averages of 4.08 and 3.96 log CFU *E. coli,* respectively. Compared to these, moderate reductions were found from 50 ppm and 25ppm chlorine treatments, with reduction averages of 3.51 and 1.79 log CFU *E. coli,* respectively. This was consistent with previous studies, which found that 100–200 ppm chlorine treatments were effective against *Salmonella* Enteritidis and *Listeria monocytogenes* biofilms on a range of common food contact surfaces, including stainless steel, plastic, and rubber (Byun et al., 2021; Hua et al., 2019). Another study also found that immersion in a chlorine solution was highly effective for the sanitation of kitchen brushes, which further corroborates the results presented in this study (Møretrø et al., 2021).

The data from this study make a compelling case for the use of chlorine sanitizer to reduce bacterial populations on machines like this brush washer. Therefore, the use of sanitizers such as 100–200 ppm chlorine bleach should be considered when implementing a standard sanitation operating procedure for produce washers, equipment, and other food contact surfaces. It should also be noted that studies of other farms which sprayed produce with chlorinated water (50–100 ppm) still found the brush beds to be a significant source of microbial cross-contamination (Wang et al., 2021). This shows that the brush washer itself must be cleaned thoroughly between produce batches to ensure adequate disinfection.

This project aimed to provide the empirical basis for extension support for a brush washer when used at a small scale, which is currently limited. It identified the strengths and limitations of current SOPs, highlighting the need for a stronger SOP. This research is a powerful, direct demonstration for extension educators to show farmers that 1) an SOP provides consistent results, even if reductions are limited, 2) a water-only cleaning regimen gives limited bacterial reductions on this machine and would be recommended as a last resort for a sanitation plan, 3) a mitigating strategy such as the use of 100–200 ppm chlorine bleach more effectively manages the risk.

Declaration of Competing Interest

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 paper.

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