Influence of a Chicken Transport Cage-Washing System on Wastewater Characteristics and Bacteria Recovery from Cage Flooring[1]

J. K. Northcutt[2] and M. E. Berrang

USDA-Agricultural Research Service, PO Box 5677, Athens, GA 30604-5677

Primary Audience: Researchers, Plant Managers, Live Production Managers

SUMMARY

A study was conducted to determine the effectiveness of an automated commercial washing system designed to clean chicken transportation cages. Surface swabs of flooring in chicken transport cages were collected before and after washing and again after sanitizer application and evaluated for recovery of bacteria. Cage wash water samples (CWW) were collected and assessed chemically and microbiologically. Washing cages significantly reduced levels of total aerobic bacteria, coliforms, and *Escherichia coli* recovered from flooring by 1.3, 1.6, and 1.5 $\log_{10}$ cfu/cm^2, respectively. Levels of total aerobic bacteria, coliforms, and *E. coli* on flooring were further reduced by 0.7, 0.6, and 0.7 $\log_{10}$ cfu/cm^2 after sanitizer application. Prevalence of *Salmonella* on unwashed flooring (1/27 positive), washed and sanitized flooring (0/27 positive), and in the CWW (1/9 positive) was low. Prevalence of *Campylobacter* (7/27 positive) on unwashed flooring decreased significantly when cages were washed and sanitized (2/27 positive). Counts of total aerobic bacteria, coliforms, and *E. coli* in CWW ranged from 2.0 to 4.0 $\log_{10}$ cfu/mL, and 1 of 9 CWW was positive for *Campylobacter*. Although the CWW collected from the second washing station appeared darker than the CWW collected from the first washing station, there was no statistical difference in total solids, total suspended solids, total dissolved solids, and chemical oxygen demand. The present study demonstrates that washing and sanitizing chicken transport cages reduces, but does not completely eliminate, bacterial contamination on the flooring surface.

Key words: chicken transport cage, cage contamination, bacteriology

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DESCRIPTION OF PROBLEM

Prior to processing, chickens are typically caught by hand, loaded into cages or crates, and transported on over-the-road trailers to the processing plant where they are held until slaughter [1]. The length of time that birds spend in cages depends upon the transportation distance (grow-out house to processing plant), the feed withdrawal program, and the plant's operating conditions, but it usually ranges from 3 to 12 h for broilers [2] and can be as long as 36 h for spent hens. During loading, transportation, and holding, cages become contaminated with feces, ingesta, dirt, feathers, litter, and other debris that may be carried into the processing plant on the birds' feet, feathers, and skin. The processing plant must then work to remove these

[1]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

[2]Corresponding author: jnorthcutt@saa.ars.usda.gov

contaminants. Although bacterial counts on poultry tend to decrease as carcasses progress through the processing plant, the initial microflora of live birds plays an important role in the microbiology of the final product [3, 4, 5, 6]

In 2004, Northcutt and Jones [7] conducted a survey of chicken processing plants across the United States and found that only 28.4% of the survey respondents wash and sanitize transportation cages and trucks between each use. Some of the respondents reported washing cages by hand, whereas others indicated that they have an automated system. Although there is no standard commercial procedure for washing chicken transportation cages, research has shown that unwashed or improperly cleaned transportation cages or crates can harbor pathogenic bacteria and then transfer these microorganisms to subsequent flocks [2, 8, 9, 10, 11, 12, 13, 14]. Rigby et al. [8] evaluated plastic transport crates before loading during 2 experimental trials and found that 98/99 (99%) and 46/52 (88.5%) were positive for *Salmonella*. Stern et al. [9] reported that transportation in plastic crates resulted in a 2.5 $\log_{10}$ increase in *Campylobacter* contamination of ceca. Berrang et al. [2] found that commercial transportation cages (5 compartments high/3 doors across, referred to as 5 high/3 door type) loaded with *Campylobacter*-positive broilers for 8 h became contaminated and transferred bacteria to a subsequent flock that had previously been *Campylobacter* negative. These researchers reported that more than 50% of the defeathered broiler carcasses from the previously negative flock had detectable levels of *Campylobacter* after 2, 4, and 6 h of exposure to contamination in the cages. Hansson et al. [15] reported similar findings for plastic crates in which 42% (11/26) of the on-farm *Campylobacter*-negative broilers became *Campylobacter* positive at slaughter (cloacal and neck skin samples) after exposure to contaminated crates. Conversely, Berrang et al. [2] and Jacobs-Reitsma and Bolder [11] showed that flocks testing negative for *Campylobacter* remained negative during transportation if they were confined in uncontaminated cages or crates.

In another study, Berrang and Northcutt [16] evaluated the bacteriological impact of spray washing and chemical immersion on sections of fiberglass flooring from commercial transportation cages. Counts of bacteria (*Campylobacter*, coliforms, and *Escherichia coli*) on flooring were reduced by 1.5 to 2.0 $\log_{10}$ cfu/25 cm^2 after spray washing with tap water, and further reductions in counts did not occur when the flooring was immersed in a chemical treatment after the tap water washing [16]. Several other researchers have evaluated various experimental techniques for decontamination of poultry transportation cages [11, 12, 14, 16]; however, there have been no reports documenting the effectiveness of commercial cage washing systems for multilevel "dump" cages [17]. A field study was therefore conducted to examine the effects of a commercial transportation cage-washing system on wastewater characteristics and bacteria recovery from cage flooring.

MATERIALS AND METHODS

Transport Cage Sampling

During each of 3 visits to a commercial poultry processing plant, 3 cages (5 high/3 door type) containing chickens from the same flock were tagged for identification and their flooring was evaluated for bacteria recovery [17]. During sampling, flooring in the top, middle, and bottom compartments in the center section of each transport cage was swabbed using a sterile sponge. For sampling, sponges were placed in sterile aluminum clamps with handles that were 0.9 m long. A 15.2 cm × 15.2 cm (232 cm^2) sterile stainless steel template was placed on the flooring surface and samples were collected by swabbing the entire area within the template using a sponge. The aluminum clamps and stainless steel template with soaked with 95% ethanol and flamed between each use.

The following procedure was used to sample each compartment within the center section of the transport cages: unwashed (**UW**) samples were collected from left side of the compartments; washed (**W**) samples were collected from the center area of the compartments; and sanitized (**WS**) samples were collected from the right side of the compartments. For the UW samples, the compartments were swabbed while the chickens were still in the cage. The cages were then sent to an automated unloading area at the processing plant where the chickens were dumped out onto a conveyor belt. After un-

loading, cages were moved via conveyor belt to a 2-stage washing area. The same top, middle, and bottom compartments on each cage were sampled again after washing using the above procedure. Washed cages were put back on the trailer and moved to the end of the live hold area where sanitizer was applied. The marked cages were removed from the truck, placed on the ground, and sprayed with sanitizer. After the sanitizer had been applied, the same top, middle, and bottom compartments in each cage were sampled using the procedure above.

Microbiological Analysis

Cage flooring was sampled as described above by wiping sterile Speci-Sponges [18] across the surface of the flooring. After sampling, the sponge was returned to the same bag, and 50 mL of PBS was added to the bag. Bags were sealed and placed in a cooler on ice for transportation back to the laboratory.

At the laboratory, sponges containing the cage flooring samples were mixed in a stomacher for 30 s, and serial dilutions were prepared in PBS using the rinsate from the bags. Serial dilutions of the cage wash water (**CWW**) were also prepared in PBS. Sponge diluent and CWW were analyzed for total aerobic bacteria, coliforms, *E. coli*, *Salmonella*, and *Campylobacter* using standard microbiological protocols as identified in the *Bacteriological Analytical Manual* [19].

For *Salmonella* detection, all samples were first preenriched in buffered peptone water (**BP**) [20]. Twenty-five milliliters of the sponge diluent was removed from each bag after stomaching and added to 25 mL of double-strength BP to result in single-strength preenrichment broth. For CWW samples, a 100-mL subsample was collected and added to 100 mL of double-strength BP for preenrichment. After 24 h of preenrichment at 35°C, the BP was hand shaken and each sample was analyzed using standard procedures [19]. Suspect *Salmonella* colonies were biochemically and serologically confirmed [20].

Prevalence of *Campylobacter* in the sponge diluent and CWW were determined by plating samples on to Campy-Cefex agar [21]. After a 48-h incubation, each colony type counted as *Campylobacter* was confirmed as belonging to the genus by examination of cellular morphology and motility on a wet mount under phase-contrast microscopy. Colonies were further identified as species *jejuni*, *coli*, or *lari* by a positive reaction on a latex agglutination test kit [22].

Bacteriological data were reported as $\log_{10}$ colony forming units per cm^2 (231 cm^2) of transportation cage flooring or $\log_{10}$ cfu/mL of CWW for total aerobic bacteria, coliforms, and *E. coli*. Prevalence (number of samples testing positive out of total number of samples) was reported for *Salmonella* and *Campylobacter*.

Cage Washing and Sanitation System

As the cages exited the unloading area, they were conveyed to the first of 2 washing stations. At both stations, cages were sprayed on the left and right sides with stationary nozzles while an L-shaped bank of 30 nozzles made 2 passes (10 s/pass) over the cage. The stationary nozzles at both stations were oriented in such a way that 2 nozzles were directed at each compartment. The processing plant had 2 separate collection tanks located below the washing stations for CWW. Both tanks had side exit drains making it possible to collect CWW that corresponded to a specific cage. In addition to the automated cage washing system, a plant employee sprayed the transport cages with a high-pressure hose as cages exited each washing station.

After the cages had been washed, they were removed from the conveyor belt by a forklift and returned to the trailer. When the trailer was full, it was moved to the end slot of the live hold area where sanitizer [23] was applied to the cages using a 5.6-L hand sprayer.

Cage Wash Water

Cage wash water was collected separately from the 2 washing stations when marked cages were washed so that CWW could be compared with samples collected from cage flooring. Approximately 2 L of CWW from the 2 washing stations was collected in 2 sterile 1-L bottles. One liter of water was also collected in a sterile bottle directly from the stationary nozzles. The water samples were placed in a cooler on ice and returned to the laboratory for analyses.

Cage wash water was evaluated for pH, chlorine, total solids (**TS**), total dissolved solids

Table 1. Chemical analyses of commercial cage wash water (CWW) collected before and after (first and second washing station) contacting transportation cages

	CWW[2]		
Water analyses[1]	New	First	Second
pH	8.24 ± 0.34	7.10 ± 0.14	7.12 ± 0.06
Chlorine (mg/L)	102 ± 89.5	0.54 ± 0.19	0.99 ± 0.46
TKN (mg/L)	NA[3]	78.9 ± 5.4[b]	113.3 ± 20.3[a]
TS (mg/L)	NA	1936 ± 106	1903 ± 343
TSS (mg/L	NA	907 ± 88.0	730 ± 184
TDS (mg/L)	NA	959 ± 55.4	793 ± 92.4
COD (mg/L)	22.0 ± 26.2	753 ± 118	1193 ± 244

[a,b]Means ± standard error in a row without common superscripts are significantly different ($P < 0.05$).
[1]TKN = total Kjeldahl nitrogen; TS = total solids; TSS = total suspended solids; TDS = total dissolved solids; and COD = chemical oxygen demand.
[2]CWW was collected from the spray nozzles before (New) contacting the transportation cages, and after washing from the 2 tanks located below the first (First) and second (Second) washing stations.
[3]NA indicates that the measurement was not applicable.

(**TDS**), total suspended solids (**TSS**), total Kjeldahl nitrogen (**TKN**), chemical oxygen demand (**COD**), total aerobic bacteria, coliforms, *E. coli*, *Campylobacter*, and *Salmonella*. The pH was measured by a handheld probe [24]. Total chlorine was determined on every sample using a colorimetric reaction with N, N-diethyl-*p*-phenylenediamine as recommended by the American Public Health Association [25]. N, N-diethyl-*p*-phenylenediamine was introduced using self-filling vacu-vials [26] and total chlorine was measured in milligrams per liter [27].

Total solids, TSS, TDS, TKN, and COD were determined using the methods recommended by the American Public Health Association [25] and data were reported as milligrams per liter. An additional step was added to the TKN analyses and included distilling the water samples before digestion to evolve any nitrogen gas from cleaning chemicals and urine or fecal material. A handheld HACH DR 870 colorimeter [28] was used for measuring COD.

Statistical Analysis

Data were analyzed by the ANOVA option of the GLM procedure of the SAS/STAT program. The CWW data were analyzed using cage, sampling time (UW, W, and WS), and replication as the main effects. The microbiological data were analyzed after logarithmic transformation using cage, sampling time, compartment (top, middle, and bottom) and replication as the main effects. Because there was no significant compartment, replication, or compartment by replication interaction effects, the data were pooled by compartment and replication. All first-order interactions were tested for statistical significance ($P < 0.05$) using the residual error mean squares. Means were separated using the least squares means option and reported along with the standard error [29].

RESULTS AND DISCUSSION

The chemical analyses of CWW collected from the commercial cage washing system before contacting the cages ("new") and after the first and second washing stations ("first" or "second" washing stages) are shown in Table 1. New CWW had an average pH of 8.24, but the pH varied from 7.86 to 8.52 during the field study. In addition, the new CWW had an average chlorine level of approximately 102 mg/L, and levels ranged from 48 to 205 mg/L. The COD for the new CWW was higher on the first sampling day (51 mg/L) than on the other 2 sampling days (0 and 15 mg/L), and the higher COD value corresponded to the elevated pH and chlorine (pH 8.52, chlorine 205 mg/L). Employees of the facility indicated that water used to wash transportation cages was a blend of fresh water and water from the chiller overflow to which extra chlorine was added before cage washing. This explains the wide variation in chlorine levels and suggests that procedures for blending water and chlorine are not closely monitored.

Table 2. Counts of total aerobic bacteria, coliforms, and *Escherichia coli*[1] and prevalence[2] of *Salmonella* and *Campylobacter* found in the cage wash water (CWW) collected from the first and second washing stations

Sample	Total aerobic bacteria	Coliforms	*E. coli*	*Salmonella*	*Campylobacter*
First CWW	3.1 ± 0.9[b]	2.2 ± 0.3[b]	2.5 ± 0.3[b]	0/9	0/9
Second CWW	4.0 ± 0.2[a]	3.0 ± 0.3[a]	3.3 ± 0.3[a]	1/9	1/9

[a,b]Means ± standard error in a column without common superscripts are significantly different ($P < 0.05$).
[1]Log cfu/mL.
[2]Prevalence (number of samples testing positive out of the total number of samples) for CWW.

There was no statistical difference ($P > 0.05$) in pH, chlorine, TS, TSS, TDS, and COD for CWW collected from the first and second washing stages (Table 1). Chlorine concentration in the CWW decreased to a level below 1 mg/L after contacting the cages (first and second CWW). Total Kjeldahl N for the first CWW (78.9 mg/L) was significantly lower ($P < 0.05$) than that for the second CWW (113.3 mg/L). The difference in TKN may be related to the fact that the first CWW contained feathers (keratin) that were not visibly present in the second CWW. The second CWW was also darker in color than the first CWW, suggesting that it contained more fecal material or soil. These observations could explain the higher TKN in the second CWW. It is also interesting to note that the CWW contained lower TKN, TSS, and COD than broiler processing wastewater [30] and lower COD than egg processing wastewater [31]. Merka [30] reported that broiler processing wastewater typically contains 130 mg/L of TKN, 1,440 mg/L of TSS, and 770 mg/L of COD, whereas Northcutt et al. [31] found that egg wash water contained 80 to 300 mg/L of TKN, 300 to 800 mg/L of TSS, and 1,800 to 7,300 mg/L of COD. Total suspended solids and TKN levels found in CWW were similar to those observed in egg wash water [31].

Table 2 shows levels of total aerobic bacteria, coliforms, and *E. coli* and the prevalence of *Salmonella* and *Campylobacter* found in first and second CWW. Second CWW contained levels of bacteria that were 0.9, 0.8, and 0.8 $\log_{10}$ cfu/mL higher than the levels of bacteria found in first CWW for total aerobic bacteria, coliforms, and *E. coli*, respectively. This agrees with the observation that more fecal material was removed from the cages at the second washing stage. Only 1 out of 9 CWW samples tested positive for *Salmonella* or *Campylobacter*. These positive samples were not from the same replication, but both occurred in the second CWW.

Counts for total aerobic bacteria, coliforms, and *E. coli* were highest on the unwashed flooring and decreased with washing and sanitizer application (Table 3). Washing the cages decreased the level of bacteria recovered from flooring by 1.3, 1.6, and 1.5 $\log_{10}$ cfu/cm^2 for total aerobic bacteria, coliforms, and *E. coli*, respectively. Application of a chlorine-based sanitizer [23] resulted in an additional reduction in counts recovered from flooring by 0.7, 0.6, and 0.7 $\log_{10}$ cfu/cm^2 for total aerobic bacteria, coliforms, and *E. coli*, respectively. It is important to note that before the sanitizer was applied, the cages were removed from the truck

Table 3. Counts of total aerobic bacteria, coliforms, and *Escherichia coli*[1] and prevalence[2] of *Salmonella* and *Campylobacter* recovered from the flooring of chicken transportation cages after 3 stages of cleaning

Cage flooring	Total aerobic bacteria	Coliforms	*E. coli*	*Salmonella*	*Campylobacter*
Unwashed	9.1 ± 0.1[a]	7.8 ± 0.1[a]	7.4 ± 0.1[a]	1/27	7/27
Washed	7.8 ± 0.2[b]	6.2 ± 0.2[b]	5.9 ± 0.2[b]	2/27	4/27
Washed and sanitized	7.0 ± 0.2[c]	5.6 ± 0.2[c]	5.2 ± 0.2[c]	0/27	2/27

[a–c]Means ± standard error in a column without common superscripts are significantly different ($P < 0.05$).
[1]Log cfu per cm^2.
[2]Prevalence (number of samples testing positive out of the total number of samples) for cage flooring.

and placed on the ground to make it easier to collect the microbiological sample. This was not routine procedure, and consequently these cages may have received a heavier coat of sanitizer than cages that remained on the trailer.

A low prevalence of *Salmonella* was found on the surface of the cages before washing (1/27), after washing (2/27) and after sanitizing (0/27; Table 3). Prevalence of *Campylobacter* decreased by approximately 11% with washing (7/27 vs. 4/27) and 19% (7/27 vs. 2/27) when washing was followed by the application of a sanitizer. Slader et al. [14] reported finding *Campylobacter* in crate wash water during 2 out of 5 visits to a processing plant, and *Salmonella* was found in the crate wash water during 4 out of 5 visits. Simultaneously, those authors isolated *Campylobacter* and *Salmonella* from the crates during 4 of 5 and 2 of 5 visits, respectively. Slader et al. [14] concluded that the status of the crate wash water did not necessarily reflect the microbial condition of crates if organic material was left on crates after washing. These authors and others suggest that residual organic material left on surfaces after washing and sanitizing may provide a protective layer for bacteria [12, 14, 32]. It is for this reason that washing and sanitizing transportation cages has come into question. Inadequate cleaning may be microbiologically inferior to no cleaning because the surfaces are wetted [2, 16].

The results of the present study confirm those reported by Berrang and Northcutt [16] in which a significant reduction in bacterial contamination occurred on cage flooring during washing, followed by only minimal reduction when sanitizer was applied. Washing and sanitizing transportation cages did not completely eliminate bacterial contamination.

CONCLUSIONS AND APPLICATIONS

1. During sample collection, there was wide variation in the cage washing procedure.
2. Analyses of the CWW showed that overall it contained less organic contaminates (lower TKN, TSS, and COD) than commercial broiler processing wastewater, but level of TSS and TKN were similar to those found in egg wash water.
3. Commercial cage washing and sanitation reduced, but did not completely eliminate bacterial contamination on the fiberglass flooring.
4. If washing and sanitizing chicken transportation cages becomes common industry practice, care should be taken to develop and follow standard protocols, such as Sanitation Standard Operating Procedures (SSOP).

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