

Effect of Different Cleaning Regimens on Recovery of *Clostridium perfringens* on Poultry Live Haul Containers

B. A. McCrea* and K. S. Macklin[†]

*Animal Science Department, University of California, Davis 95616-8521; and [†]Department of Poultry Science, Auburn University, Auburn, AL 36849-5416

ABSTRACT *Clostridium perfringens* is important to both poultry producers and humans. The excretion rate of pathogenic foodborne bacteria increases after live haul; however, the majority of research into flock cross-contamination has been performed on *Salmonella* and *Campylobacter*. Research into the sources of *C. perfringens* in poultry operations have implied that dirty transport containers do harbor this organism and, therefore, can potentially contaminate subsequent flocks. The objectives of this study were to examine both small plastic crates and large dump coops to determine which cleaning regimens were most effective in reducing *C. perfringens* contamination. Additionally, 2 different holding periods for small crates were compared to determine whether holding time influences *C. perfringens* recovery before and after cleaning. Two experiments were performed. One involved small plastic crates; the other involved large dump coops. Four small crate cleaning and disinfection treatments consisted of pressure washing, pressure washing and sun-drying, pressure washing with a (5%, vol/vol) sodium hypochlo-

rite dip, and pressure washing with a quaternary ammonium dip. The second experiment involved dump coops. The 5 dump coop cleaning and disinfection treatments consisted of pressure washing, pressure washing with a (5%, vol/vol) sodium hypochlorite spray, pressure washing with a quaternary ammonium spray, 48-h drying after the sodium hypochlorite spray, and 48-h drying after the quaternary ammonium spray. The recovery of *C. perfringens* from small and large dirty transport containers averaged 1.94 and 4.43 log₁₀ cfu/mL, respectively. There was no significant difference in *C. perfringens* recovery based on holding time for small crates. With small crates, pressure washing provided a significant decrease in the amount of *C. perfringens* recovered. The greatest bacterial reduction in dump coops, 2 to 3 log₁₀ cfu/mL, was observed after 48 h of drying. This information provides solutions to poultry operations to reduce the cross-contamination of this food safety pathogen via transport containers.

Key words: *Clostridium perfringens*, live haul, crate, dump coop, cleaning

2006 Poultry Science 85:909–913

INTRODUCTION

The stress of live haul has been proven to increase the shedding of foodborne pathogens by poultry (Stern et al., 1995; Whyte et al., 2001). Both large- and small-scale producers face problems in the efficacy of cleaning transport containers, which may or may not be cleaned by producers prior to loading another flock. Cleaning is time-consuming, and washing equipment for dump coops is expensive. However, dirty crates have the potential to spread bacteria, such as *Salmonella* and *Campylobacter*, from colonized flocks to birds that are free of the organism (Corry et al., 2002; Slader et al., 2002; Berrang et al., 2003). Dirty containers used to load *Salmonella*- and *Campylobacter*-negative flocks have yielded isolates that were then

found on the final product (Newell et al., 2001; Slader et al., 2002). This indicates a lack of or ineffectual cleaning.

Cleaning regimens for large dump coops have been investigated with regard to the elimination of *Salmonella* and *Campylobacter*. Effective combinations of heat and chemical sanitizers were investigated by Ramesh et al. (2002, 2003). Their research found that a 7-log reduction in *Salmonella* was possible, and the most effective sanitizers were 0.05% sodium hypochlorite solution or a 1% alkaline peroxide solution. Not all organisms were removed in the initial pass through a prototype washing system, but rather several passes were necessary to decrease the bacterial load (Ramesh et al., 2003). Additionally, it has been shown that if a crate cleaning system is not well maintained, then it may add to cross-contamination between flocks (Carr et al., 1999). Two days of drying or down time has also been examined as an effective solution to crates contaminated with *Campylobacter* (Berrang et al., 2004).

Clostridium perfringens is a foodborne pathogen that can be found on dirty transport containers. This anaerobic

©2006 Poultry Science Association, Inc.

Received September 17, 2005.

Accepted January 9, 2006.

¹Corresponding author: macklks@auburn.edu

organism is capable of forming a spore for survival once exposed to oxygen, making it difficult to eliminate. The organism has a broad distribution and is a part of the natural gut flora of warm-blooded animals. It is the causative agent of necrotic enteritis and gangrenous dermatitis in poultry (Songer, 1997) and foodborne gastroenteritis in humans (McClane, 1997). Immerseel et al. (2004) provides a review of *C. perfringens* in poultry production with particular emphasis on virulence factors, epidemiology, predisposing factors for necrotic enteritis, clinical signs, subclinical forms, preventions and control in flocks, and human foodborne pathogen in poultry meat.

In several outbreaks of foodborne illness, ribotyping of patient stool samples was able to identify poultry products as the source of infection (Schalch et al., 1997). *Clostridium perfringens* is capable of traversing an integrated poultry operation from the breeder flocks, through the hatchery, into growout, and onto the final product (Craven et al., 2003). Sources of contamination in the poultry-rearing environment have also been examined, and positive transport containers have been noted (Craven et al., 2001).

Our objective was to determine optimum cleaning regimens for small crates and large dump coops with regard to effectiveness against *C. perfringens*. Additionally, the effect of long and short holding periods on the recovery of *C. perfringens* in small crates before and after cleaning was examined. Both direct plating and enrichment of samples were performed to ensure recovery.

MATERIALS AND METHODS

Experimental Design

The *C. perfringens* colonization status of birds was assessed and confirmed through litter grab samples prior to loading and transport. Birds were loaded into containers and transported for 1 h to simulate transport conditions. After birds were unloaded, transport containers were held under a shelter to mimic commercial conditions. All birds in the study were 42-d-old broilers.

Small Crates. Six straight-run, 4-d-old broilers were randomly placed in 1 of 24 cleaned, plastic transport crates (88.9 × 59.7 cm; Pakster Poultry Products, Athens, TN). Birds were held for 2 different lengths of time. The a.m. and p.m. groups were held for 2 and 8 h, respectively. Three crates were used for each cleaning regimen within each holding period. Three randomly selected crates per holding period were swabbed immediately after bird removal to determine the *C. perfringens* load on crates prior to cleaning. In both holding periods, all crates were pressure-washed, and 3 randomly selected crates were swabbed. The pressure-washed crates were then randomly distributed into the sun, chlorine, or quaternary ammonium treatments. The entire crate floor surface was swabbed with sterile 12-layer 4 × 4 in. gauze (~10 g).

Treatments were selected based on industry protocols and prior research (Corry et al., 2002; Ramesh et al., 2002, 2003). Treatment 1 consisted of pressure washing (1,000

psi) the crates until no feces were visible. Treatment 2 consisted of pressure washing the crates and then placing them in a sunny location for 2 h prior to swabbing. Treatment 3 consisted of a 5% (vol/vol) sodium hypochlorite dip. Crates were pressure-washed and then agitated in this solution for 1 min. The crates were allowed to dry for 10 min and were then rinsed with a hose and swabbed. Treatment 4 consisted of a 0.4% (vol/vol) quaternary ammonium dip (Synergize, Preserve International, product no. 340352, QC Supply LLC, Schuyler, NE). A commercially available quaternary ammonium solution was prepared according to the manufacturer's recommendations. Crates were dipped, handled, and swabbed as described in treatment 3.

Dump Coop. Twenty birds, 10 male and 10 female, were placed into each compartment of a cleaned 3-column, 6-tier commercial dump coop (Bright Coop, Inc., Nacogdoches, TX). The steel-frame dump coop contained fiberglass floors in each compartment and hinged doors. A 74.9 × 78.7-cm area of the fiberglass floor in each compartment was swabbed with sterile 12-layer, 4 × 4-in. gauze (~10 g). Nine compartments for each treatment, including 9 randomly selected dirty compartments, were swabbed after cleaning and disinfection. Two replicates of the large dump coop study were performed.

Treatments were selected in the same manner as small crates with a few modifications. Instead of dipping the crates in a chlorine or quaternary ammonium solution, these disinfectants were sprayed onto the fiberglass floors of randomly selected compartments within the dump coop. The sun-drying treatment was omitted and replaced by a 48-h drying treatment similar to that described by Berrang et al. (2004). The compartments that were subjected to a chlorine or quaternary ammonium spray were swabbed again after 48 h of drying under a shelter.

Microbiological Analysis

Each swab was immediately placed in an individual, sterile sample bag with 90 mL of sterile buffered peptone water (Berrang et al., 2004). Samples were transported on ice, stomached for 1 min, followed by serial dilution in sterile 0.8% saline. Samples were then spread-plated onto tryptose sulfite cycloserine (TSC; product no. OXCM0587B, Fisher Scientific, Pittsburgh, PA) agar plates in duplicate. Plates were incubated anaerobically (5% CO₂, 5% H₂, 90% N₂) for 24 h at 42°C. Plates yielding characteristic colonies were counted, and 4 colonies from each sample were subcultured to tryptic soy agar (TSA; product no. B21239, Fisher Scientific) with 5% sheep's blood and incubated anaerobically for 24 h at 42°C. Isolates yielding double zone hemolysis on TSA blood were confirmed as *C. perfringens* using gram stain and cell morphology.

Each sample was selectively enriched by placing 1 mL of sample into cooked meat media tubes (product no. OXCM0081B, Fisher Scientific). Enriched samples were then incubated anaerobically for 24 h at 42°C. Samples were then streaked for isolation in duplicate onto TSC

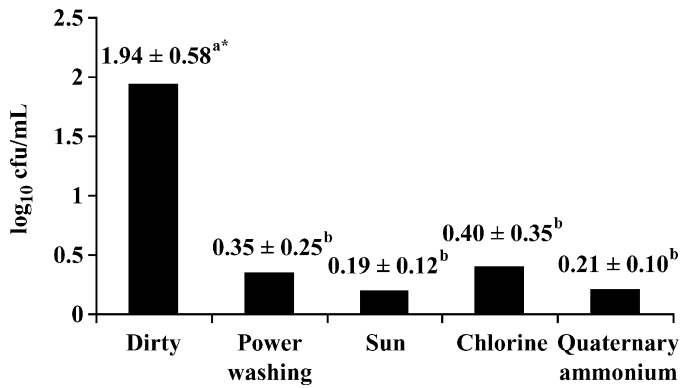


Figure 1. Small crate summary of *Clostridium perfringens* recovery from both holding periods by treatment. ^{a-c}Superscripts indicate significant differences ($P < 0.05$) between treatments. *Means \pm SE.

agar and incubated anaerobically for 24 h at 42°C. Up to 4 colonies were subcultured to TSA with 5% sheep's blood if there were characteristic colonies. Blood agar plates were then incubated anaerobically for 24 h at 42°C. Isolates yielding double zone hemolysis on TSA blood were confirmed as *C. perfringens* using gram stain and cell morphology.

Statistical Analysis

Clostridium perfringens counts were converted to log₁₀ values and subjected to ANOVA (SPSS for Windows, release 9.0, SPSS, Inc., Chicago, IL). A Tukey's test for significance was used to compare the cleaning treatment mean for both the large and small crate studies. Additional analyses of holding time and cleaning treatment \times holding time were performed for the small crate study. A Tukey's test for significance was also used to compare direct plating with enrichment for both large and small containers.

RESULTS

Small Crates

There were no significant differences in the recovery of *C. perfringens* between the 2- and 8-h holding periods; therefore, the small crate results were pooled for the 2 time periods. The dirty small crates averaged 1.94 log₁₀

cfu/mL. All cleaning and disinfection treatments resulted in a significant ($P < 0.05$) reduction of *C. perfringens*. The recovery from the different treatments did not exceed 0.4 \pm 0.35 log₁₀ cfu/mL (Figure 1). Although not statistically significant, the greatest reduction was 1.75 log₁₀ cfu/mL. This result was observed in crates that were pressure-washed and exposed to the sun for 2 h. After enrichment, all treatments yielded the same or an increase in the percentage of crates that were positive. The number of positive crates determined by enrichment of crate samples was not significantly different from the number of positive crates as determined by direct plating (Table 1).

Dump Coop

The recovery of *C. perfringens* from dirty dump coops averaged 4.43 log₁₀ cfu/mL. Regardless of the replicate, allowing crates to dry for 48 h produced a significant log reduction in *C. perfringens*. The treatment that performed the best was the quaternary ammonium spray followed by 48 h of drying. Unlike small crates, pressure washing did not significantly reduce *C. perfringens* when compared with dirty compartments (Figure 2). With the exception of the drying treatments, the treatment that produced the greatest log reduction in *C. perfringens* recovery was pressure washing followed by a chlorine spray (1.84 log₁₀ cfu/mL; Table 2). The majority of treatments yielded a significant increase in the percentage of positive crates after samples were enriched. The only exception was observed with the pressure-washing treatment (Table 3).

DISCUSSION

Poultry operations that use small plastic crates may wash crates with water and perhaps set them in the sun to dry after transporting birds. The efficacy of these methods was examined, as well as the addition of sanitizers, for the reduction of *C. perfringens*. The simple act of pressure washing was sufficient to reduce the number *C. perfringens*. Direct plating of crate samples did provide adequate detail as to the levels of contamination by *C. perfringens*. Direct plating was found to be equivalent to enrichment as a method of recovery.

Berrang et al. (2004) studied the recovery of *Campylobacter* from dump coops that were stored for 48 h. Their results indicated that *Campylobacter* numbers were re-

Table 1. Direct plating and enrichment recovery of *Clostridium perfringens* from small crates from both holding times¹

Treatment	Direct plating	Enrichment
	— n positive/n of samples (%) —	
Dirty	6 / 6 (100%)	6 / 6 (100%)
Pressure washing	2 / 6 (33.3%)	3 / 6 (50.0%)
Sun	1 / 6 (16.7%)	4 / 6 (66.7%)
Chlorine dip	2 / 6 (33.3%)	2 / 6 (33.3%)
Quaternary ammonium dip	3 / 6 (50.0%)	3 / 6 (50.0%)

¹No significant differences between direct plating and enrichment at $P \leq 0.05$.

Table 2. Direct plating and enrichment recovery of *Clostridium perfringens* from dump coops from both trials¹

Treatment	Direct plating	Enrichment
	— n positive/n of samples (%) —	
Dirty	17 / 18 (94.4%)	18 / 18 (100%)
Pressure washing	17 / 18 (94.4%)	16 / 18 (88.9%)
Chlorine	13 / 18 (72.2%)	16 / 18 (88.9%)
Quaternary ammonium	14 / 18 (77.8%)	16 / 18 (88.9%)
48 h + chlorine	9 / 18 (50.0%)	16 / 18 (88.9%)
48 h + quaternary ammonium	7 / 18 (38.9%)	15 / 18 (83.3%)

¹No significant differences between direct plating and enrichment at $P \leq 0.05$.

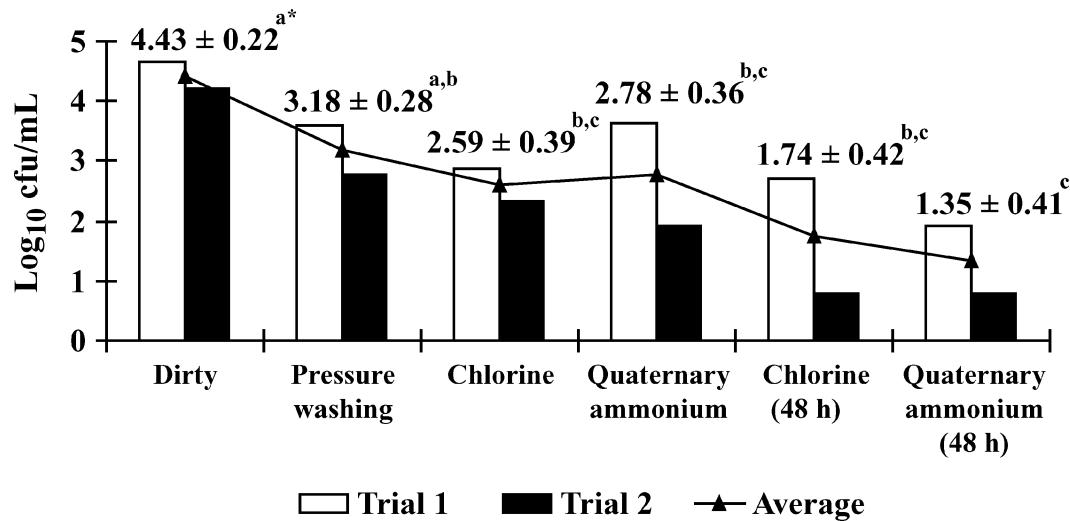


Figure 2. Large crate summary of *Clostridium perfringens* recovery from both trials and the averages for each treatment. ^{a-c}Superscripts indicate significant differences ($P < 0.05$) between treatments. *Means \pm SE.

duced by extended storage. Results presented here also indicate that *C. perfringens* numbers are reduced by 48 h of drying following sanitizer use. The 2 drying treatments indicated that 48 h of drying following a quaternary ammonium spray or chlorine spray was capable of producing a 3- and 2-log reduction (Table 3), respectively. If drying for 48 h is not an option, which is often the case for many commercial companies (Berrang et al., 2004), then a chlorine spray after pressure washing can produce a 1.84-log₁₀ reduction in *C. perfringens*. Unlike small crates, pressure washing alone was not sufficient to significantly reduce *C. perfringens* levels on the fiberglass floors of the dump coops. This difference may lie in the container material and bird contact time, both of which may play a role in the recovery of bacteria from feathers before carcasses are picked (Buhr et al. 2000). The ability to clean a large dump coop thoroughly is difficult and too time-consuming to be performed regularly. Enrichment permitted the further identification of positive compartments beyond those identified by direct plating, although direct plating alone performed well in indicating a significant reduction of bacteria.

If pressure washing were performed, cross-contamination of *C. perfringens* between flocks would be diminished.

Table 3. Mean log₁₀ reduction in *Clostridium perfringens* from dirty dump coops in both trials using different cleaning and disinfection regimens

Treatment	Small crate		Dump coop	
	log reduction			
Pressure washing	1.59 ^a	1.26 ^b	1.59 ^a	1.26 ^b
Sun	1.75 ^a	NA ¹	1.75 ^a	NA ¹
Chlorine	1.54 ^a	1.84 ^a	1.54 ^a	1.84 ^a
Quaternary ammonium	1.73 ^a	1.65 ^a	1.73 ^a	1.65 ^a
48 h + chlorine	NA	2.69 ^a	NA	2.69 ^a
48 h + quaternary ammonium	NA	3.08 ^a	NA	3.08 ^a

^{a,b}Superscripts within columns indicate significant differences ($P < 0.05$) between treatments.

¹NA = not applicable.

Both experiments presented herein show that pressure washing alone is an effective means for reducing *C. perfringens* numbers; however, its effectiveness on large dump coops is reduced. The addition of a sanitizer or drying greatly aids in *C. perfringens* reduction when it is used in conjunction with pressure washing. The government currently supports controls of pathogens such as *Salmonella*. Given the nature of *C. perfringens* as a human food-borne pathogen and consumer concerns for food safety, we can consider the cleaning of transport containers to be a control point for cross-contamination between flocks.

ACKNOWLEDGMENTS

The authors thank John Blake, Auburn University Poultry Science Department, for the use of his flocks and for the review of this manuscript.

REFERENCES

- Berrang, M. E., J. K. Northcutt, and J. A. Cason. 2004. Recovery of *Campylobacter* from broiler feces during extended storage of transport cages. *Poult. Sci.* 83:1213–1217.
- Berrang, M. E., J. K. Northcutt, D. L. Fletcher, and N. A. Cox. 2003. Role of dump cage fecal contamination in the transfer of *Campylobacter* to carcasses of previously negative broilers. *J. Appl. Poult. Res.* 12:190–195.
- Buhr, R. J., J. A. Cason, J. A. Dickens, A. Hinton, Jr., and K. D. Ingram. 2000. Influence of flooring type during transport and holding on bacteria recovery from broiler carcass rinses before and after defeathering. *Poult. Sci.* 79:436–441.
- Carr, L., C. Rigakos, G. Carpenter, G. Berney, and S. Joseph. 1999. An assessment of livehaul poultry transport container decontamination. *Dairy, Food Environ. Sanitation* 19:753–759.
- Corry, J. E. L., V. M. Allen, W. R. Hudson, M. F. Breslin, and R. H. Davies. 2002. Sources of *Salmonella* on broiler carcasses during transportation and processing: Modes of contamination and methods of control. *J. Appl. Microbiol.* 92:424–432.
- Craven, S. E., N. A. Cox, J. S. Bailey, and D. E. Crosby. 2003. Incidence and tracking of *Clostridium perfringens* through an integrated broiler chicken operation. *Avian Dis.* 47:707–711.

- Craven, S. E., N. J. Stern, J. S. Bailey, and N. A. Cox. 2001. Incidence of *Clostridium perfringens* in broiler chickens and their environment during production and processing. *Avian Dis.* 45:887–896.
- Immerseel, F. V., J. De Duck, F. Pasmans, F. Huyghebaert, and R. Ducatelle. 2004. *Clostridium perfringens* in poultry: An emerging threat for animal and public health. *Avian Pathol.* 33:537–549.
- McClane, B. A. 1997. *Clostridium perfringens*. Pages 305–326 in *Food Microbiology: Fundamentals and Frontiers*. M. P. Doyle, L. R. Beuchat, and T. J. Montville, ed. ASM Press, Washington, DC.
- Newell, D. G., J. E. Shreeve, M. Toszeghy, G. Domingue, S. Bull, T. Humphrey, and G. Mead. 2001. Changes in the carriage of *Campylobacter* strains by poultry carcasses during processing in abattoirs. *Appl. Environ. Microbiol.* 67:2636–2640.
- Ramesh, N., S. W. Joseph, L. E. Carr, L. W. Douglass, and F. W. Wheaton. 2002. Evaluation of chemical disinfectant for the elimination of *Salmonella* biofilms from poultry transport containers. *Poult. Sci.* 81:904–910.
- Ramesh, N., S. W. Joseph, L. E. Carr, L. W. Douglass, and F. W. Wheaton. 2003. Serial disinfection with heat and chlorine to reduced microorganism population on poultry transport containers. *J. Food Prot.* 66:793–797.
- Schalch, B., J. Bjorkroth, H. Eisgruber, H. Korkeala, and A. Stolle. 1997. Ribotyping for strain characterization of *Clostridium perfringens* isolates from food poisoning cases and outbreaks. *Appl. Environ. Microbiol.* 63:3992–3994.
- Slader, J., G. Domingue, F. Jorgensen, K. McAlpine, R. J. Owen, F. J. Bolton, and T. J. Humphrey. 2002. Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. *Appl. Environ. Microbiol.* 68:713–719.
- Songer, J. G. 1997. Clostridial diseases of animals. Pages 153–182 in *The Clostridia: Molecular Biology and Pathogenesis*. J. I. Rood, B. A. McClane, J. G. Songer, and R. W. Titball, ed. Acad. Press, San Diego, CA.
- Stern, N. J., M. R. Clavero, J. S. Bailey, N. A. Cox, and M. C. Robach. 1995. *Campylobacter* spp. in broilers on the farm and after transport. *Poult. Sci.* 74:937–941.
- Whyte, P., J. D. Collins, K. McGill, C. Monahan, and H. O'Mahony. 2001. The effect of transportation stress on excretion rates of *Campylobacters* in market-age broilers. *Poult. Sci.* 80:817–820.