# Use of Water Spray and Extended Drying Time to Lower Bacterial Numbers on Soiled Flooring from Broiler Transport Coops

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**ABSTRACT** Broiler transport coops soiled with *Campylobacter*-positive feces have been shown to facilitate cross-contamination of broilers. Washing and sanitizing coop surfaces do not always effectively eliminate bacteria. The objective of this study was to examine drying as a means of lowering bacterial numbers on transport coop flooring. Small squares (5 × 5 cm) of fiberglass flooring from transport coops were intentionally contaminated with 1 g of *Campylobacter*-positive broiler gut contents. Soiled floor squares were sprayed with water and allowed to dry for 15 min, 24 h, or 48 h. Unsprayed squares were examined at each time period as controls. All squares were sampled by cotton-tipped applicators that were cultured for *Campylobacter*, coliforms, and *Escherichia coli.* Sampling of unsprayed squares at 15 min yielded 7.3 log cfu of *Campylobacter,* 6.2 log cfu of coliforms and 5.9 log cfu of *E. coli* per floor square. Water spray alone resulted in a significantly lower number of organisms recovered: 4.1 log cfu *Campylobacter,* 3.6 log cfu coliform, and 3.2 log cfu *E. coli* per floor square. When water spray was followed by a 24-hour drying period, no *Campylobacter,* coliforms, or *E. coli* were detected on the floor surface. However, allowing unsprayed soiled flooring to simply dry for 24 or 48 h also resulted in no recovery of *Campylobacter* and very low numbers of coliforms and *E. coli.* A 24- or 48-hour drying period for fecal matter on broiler transport cage flooring may be a viable method to lower bacterial numbers on these surfaces.

(*Key words*: broiler, *Campylobacter, Escherichia coli,* dump coop, transport cage)

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## INTRODUCTION

*Campylobacter* is a human bacterial pathogen that is often associated with poultry and poultry products. A large percentage of broiler flocks become positive for *Campylobacter* during grow-out (Stern et al., 2001); moreover, birds in a positive flock generally carry large numbers of the organism in the gut (Berrang et al., 2000) and shed it in the feces. Transport of broilers under commercial or simulated commercial conditions can result in higher numbers of *Campylobacter* associated with gut contents (Whyte et al., 2001) and the resulting carcass (Stern et al., 1995).

Broiler transportation containers, called coops in the United States and crates in Europe, become soiled with feces during use. In this way, transport containers can become a source of bacteria. *Salmonella* and *Campylobacter* have been recovered from poultry transport containers even when sampled before placement of broilers (Bailey et al., 2001; Newell et al., 2001; Stern et al., 2001). *Campylobacter* associated with fecal matter inside transport coops has been shown to contaminate broilers from a previously *Campylobacter*-free flock (Berrang et al., 2003). *Campylobacter* contamination acquired in transportation coops stays with carcasses during processing and can be recovered by whole carcass rinse after scalding and defeathering (Berrang et al., 2003).

There have been reports in the literature of chemicals, applications, and procedures designed to sanitize transport containers and lower numbers of pathogenic bacteria associated with them (El-Assaad et al., 1995; Ramesh et al., 2002, 2003, 2004a,b). Some of these procedures show considerable promise in experimental situations. However, there are also reports of washing and sanitizing procedures used in commercial settings that are not completely effective at eliminating zoonotic pathogens from transport container surfaces (Corry et al., 2002; Slader et al., 2002; Ramabu et al., 2004). Some of these reports cite a breakdown in washing and sanitizing systems (Corry et al., 2002; Ramabu et al., 2004), and others indicate that the methods used were inadequate to eradicate microorganisms that seem to exhibit remarkable persistence in the face of washing and sanitizing procedures (Slader et al., 2002). The questionable effectiveness of transport container washing techniques may be part of the reason

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**Abbrevation Key:** VNC = viable nonculturable.

that only about 28% of US poultry plants are using a truck or coop washing system (Northcutt and Jones, 2004). Another reason coops are not commonly washed may be that a processor would have to use extra water to do so. Poultry processors use an average of 26.5 L (7 gal) of water per bird during processing (Northcutt and Jones, 2004), but drought conditions and high water costs have resulted in increased interest in saving water. A coop sanitizing technique that is effective and minimizes water use would be of great assistance to the poultry industry.

It is common practice for commercial transport coops to remain empty during periods when processing plants are not being run (i.e., weekends and holidays). Berrang et al. (2004) found that numbers of feces-borne *Campylobacter* in an unwashed transport coop decrease during extended dry storage for 24 or 48 h. In another study, Berrang and Northcutt (2005) showed that a low-pressure water spray can lower the numbers of *Campylobacter* on coop flooring, but following water spray with immersion in sanitizer does not necessarily improve that effectiveness. Our hypothesis for the current study was that a low-pressure water spray followed by extended drying of the floor surface could result in a significant reduction in *Campylobacter* numbers due to a combination of physical removal and desiccation injury. There is, however, a concern relative to the possibility that bacterial numbers lowered because of drying could rebound if dried fecal material becomes remoistened during later use.

Therefore, there were 2 objectives of this study. The first objective was to determine the efficacy of drying, with and without prior water spray, to lower bacterial numbers associated with gut contents on dump coop flooring. The second objective was to examine the effect of subsequent wetting on bacterial numbers associated with dried contamination on coop flooring.

## MATERIALS AND METHODS

### Transport Coop Flooring and Application of Gut Contents

Fiberglass flooring from used transport dump coops (Bright Coop, Nacogdoches, TX) was donated by a commercial broiler processing company. Flooring was cut into squares that measured 5 cm on each side (25 $cm^2$). Each floor square was thoroughly scrubbed, rinsed, and sterilized by autoclaving at 121°C for 15 min. All squares were stored in aseptic conditions until use in the studies as described below.

On each day of the experiment, intestinal tracts were collected from the evisceration line at a commercial broiler processing plant. Gut contents from the colon, ceca, and small intestine were manually expressed and collected in a sterile specimen cup. Gut contents were thoroughly mixed with a sterile lab spatula before application to the floor squares. Then, 1 g ± 0.1 g of gut contents was smeared evenly in a thin layer on each of 10 squares for all treatments within each of 3 replications. Gut contents were allowed to dry at room temperature and humidity for 60 min prior to any further treatment. Room temperature and humidity were monitored by a data logger (Cox Tracer, Cox Recorders, Belmont, NC).

### Experiment 1

Following the initial 60-min drying period, floor squares with gut contents were subjected to a low-pressure water spray wash and then an extended drying time at room temperature. Water spray washing was conducted using a standard laboratory sink fitted with a pressure gauge and a nozzle with an inside diameter of 4 mm. Tap water (average total chlorine of 0.5 ppm) was adjusted to 10 psi by altering the flow rate and used to spray the gut contents on floor squares for 15 s at a distance of about 8 cm. This resulted in approximately 1,500 mL of tap water sprayed onto each square, which removed most but not all of the visible gut contents. Spray-washed floor squares were allowed to dry on a lab bench at room temperature and humidity for 15 min, 24 h, or 48 h prior to sampling. An unsprayed control was included for comparison at each drying time. Room temperature and humidity were monitored hourly by a data logger (Cox Tracer, Cox Recorders, Belmont, NC) for each drying period.

### Experiment 2

In the second experiment, treatment order was reversed; an extended drying time was followed by rewetting the gut contents with a water spray. After the initial 60-min drying time of gut contents, floor squares were rewetted with water immediately or after 24 or 48 h of drying at room temperature and humidity. Room temperature and humidity were monitored hourly by a data logger (Cox Tracer, Cox Recorders, Belmont, NC) for each drying period. Rewetting was done using the same spray technique used in experiment 1 for washing. Floor square sampling was conducted immediately after the water spray. At each drying time, an unsprayed control was included for comparison.

### Floor Sampling

Broilers become contaminated by physical contact with coop flooring; therefore a surface wipe method was chosen for sampling. Each floor square was sampled by rubbing a sterile premoistened cotton tipped swab (Hardwood Products Co. LLC., Guilford, ME) across the surface. Swabs were premoistened by dipping in PBS and rubbed across the entire surface of the square; the square was then rotated 90°, and the same swab was rubbed over the entire surface again. After sampling, each swab was placed into 10 mL of PBS and vigorously mixed, and 10-fold serial dilutions were plated for *Campylobacter, E. coli,* and coliforms.

**Table 1.** Effects of water spray with and without subsequent drying on mean log cfu bacteria recovered from 25-$cm^2$ pieces of broiler transport coop flooring previously contaminated with gut contents

| Treatment, n = 30 | Drying time[1] | *Campylobacter*[2] | Coliform[2] | *Escherichia coli*[2] |
|---|---|---|---|---|
| No wash | 15 min | $7.3^a \pm 0.2$ | $6.2^a \pm 0.1$ | $5.9^a \pm 0.1$ |
| Spray wash[3] | 15 min | $4.1^b \pm 0.4$ | $3.6^b \pm 0.3$ | $3.2^b \pm 0.3$ |
| No wash | 24 h | ND[4,c] | $1.2^c \pm 0.3$ | $0.4^c \pm 0.2$ |
| Spray wash | 24 h | ND[c] | ND[d] | ND[c] |
| No wash | 48 h | ND[c] | $0.9^c \pm 0.2$ | $0.3^c \pm 0.1$ |
| Spray wash | 48 h | ND[c] | $0.02^d \pm 0.05$ | $0.02^c \pm 0.05$ |

[a,b,c,d]Means within the same column with no common superscripts are different by Tukey's honest significant difference test ($P < 0.01$).

[1]Flooring allowed to air dry at room temperature (mean 22.9°C) and humidity (mean 59.7% RH).

[2]Mean ± 95% confidence interval.

[3]Flooring sprayed with tap water for 15 s at 10 psi.

[4]None detected; fewer than 50 cfu of *Campylobacter* or 5 cfu of *E. coli* and coliform cfu recovered per square.

### Bacterial Culture

To enumerate *Campylobacter*, 0.1 mL from a serial dilution in PBS was spread on the surface of duplicate campy-cefex agar plates (Stern et al., 1992). Plates were incubated at 42°C for 48 h in resealable plastic bags flushed with a microaerobic gas mixture containing 5% $O_2$, 10% $CO_2$, and 85% $N_2$ (BOC Gasses, Chattanooga, TN). Colonies characteristic of *Campylobacter* were counted. All colony types from each sample were confirmed as a member of the genus *Campylobacter* by observation of typical morphology and motility in a wet mount under phase-contrast-microscopy. Each colony type was further confirmed as *coli, jejuni* or *lari* by positive reaction to a serological latex agglutination test (Microscreen *Campylobacter*, Microgen Bioproducts, Camberly, UK).

*Escherichia coli* and coliforms were enumerated by plating on Petrifilm *E. coli* and coliform count plates (3M Microbiology Products, St. Paul, MN). One milliliter from a serial dilution in PBS was plated onto duplicate Petrifilm plates. Petrifilm plates were incubated at 35°C for 24 h, and colonies characteristic of *E. coli* and coliforms were counted.

The lowest number of bacteria that could be detected was 1 cfu on 1 of the duplicate plates at the lowest dilution used. The lowest dilution used for *Campylobacter* was 0.1 mL from the sample swab tube direct plated in duplicate onto campy-cefex agar. Therefore, the limit of detection was 1 cfu/0.2 mL or 5 cfu/mL. The lowest dilution used for *E. coli* and coliforms was 1 mL from the sample swab tube plated in duplicate onto Petrifilm, resulting in a limit of detection of 1 cfu/2 mL. For both populations, sample swabs were in 10 mL of PBS; therefore, the detection limit was 50 cfu of *Campylobacter* and 5 cfu of *E. coli* coliforms recovered from each square by swab sampling.

### Statistical Analyses

Three replications of each experiment were conducted using 10 floor squares (n = 30) for each of the 6 treatments. Bacterial counts were transformed to $\log_{10}$ cfu recovered per floor square. Data were analyzed by general linear model using a statistical software package (Statistica 6.0, Statsoft, Tulsa, OK). A randomized complete block design was used with replication as the blocking factor. Thus, replication effect was removed from the analysis and placed into the error term. Means were separated by Tukey's honest significant difference test. Significance was assigned at $P < 0.01$.

## RESULTS AND DISCUSSION

Results from experiment 1 in which water spray was followed by 15 min, 24 h, or 48 h of drying, are shown in Table 1. Spraying the floor squares with water resulted in a significant decrease in numbers of bacteria recovered. However, simply allowing the squares to dry for 24 h under room conditions (mean temperature 22.9°C, mean RH 59.7%) was even more effective. A combination of spraying with water, followed by a drying time of 24 h, further lowered the numbers of *E. coli* and coliforms but was no more effective against *Campylobacter* than simply allowing the flooring to dry. Forty-eight hours of drying was not more effective than 24 h of drying for lowering numbers of bacteria.

Experiment 2 was conducted to determine the effect of remoistening on bacterial numbers previously lowered during extended drying. Data from experiment 2 are shown in Table 2. Data from the controls in which gut contents were not allowed to dry on the surface for 24 or 48 h were similar to those observed in experiment 1; significantly fewer bacteria were recovered from floor squares subjected to water spray than from untreated control flooring.

Also like in experiment 1, allowing gut contents to dry for 24 h (mean temperature 23.0°C, mean RH 45.0%) resulted in the recovery of significantly fewer *E. coli*, coliforms, and *Campylobacter* than found on the control floor squares. However, using a water spray to rewet gut contents that had been allowed to dry on flooring for 24 h resulted in the recovery of more *E. coli* and coliforms from floor squares. No increase in *E. coli* or coliform numbers was noted when gut contents were allowed to dry on floor squares for 48 h and then rewet by water spray.

**Table 2.** Effects of drying with and without subsequent water spray on mean log cfu bacteria recovered from 25-cm$^2$ pieces of broiler transport coop flooring previously contaminated with gut contents

| Drying time[1] | Treatment, n = 30 | *Campylobacter*[2] | Coliform[2] | *Escherichia coli*[2] |
|---|---|---|---|---|
| Control | No wash | 6.8[a] ± 0.4[3] | 6.3[a] ± 0.2 | 6.1[a] ± 0.2 |
| Control | Spray wash[3] | 5.0[b] ± 0.4 | 4.9[b] ± 0.3 | 4.7[b] ± 0.3 |
| 24 h | No wash | ND[4,c] | 1.2[d] ± 0.4 | 0.9[d] ± 0.3 |
| 24 h | Spray wash | ND[c] | 2.7[c] ± 0.2 | 2.0[c] ± 0.5 |
| 48 h | No wash | ND[c] | 0.9[d] ± 0.2 | 0.7[d,e] ± 0.2 |
| 48 h | Spray wash | ND[c] | 1.4[d] ± 0.2 | 0.3[e] ± 0.2 |

[a-e]Means within the same column with no common superscripts are different by Tukey's honest significant difference test ($P < 0.01$).

[1]Flooring allowed to air dry at room temperature (mean 23.0°C) and humidity (mean 45.0% RH).

[2]Mean ± 95% confidence interval.

[3]Flooring sprayed with tap water for 15 s at 10 psi.

[4]None detected; fewer than 50 cfu of *Campylobacter* or 5 cfu of *E. coli* and coliform cfu recovered per square.

Rewetting gut contents did not result in an increase in the numbers of *Campylobacter* recovered regardless of how long the gut contents were allowed to dry; these remained below the limit of detection (50 cfu per floor square). This observation helps relieve some of the concern that fecal-borne *Campylobacter* numbers will decrease during drying only to rebound when the coop is exposed to rain or other moisture during normal use at a later time.

The drying method used in this study resulted in lower numbers of *Campylobacter* than were detected when floor squares were treated with water spray in combination with chemical sanitizers (Berrang and Northcutt, 2005). In the literature, there are reports of transport container sanitizing methods that result in reductions in bacterial numbers of 4 to 8 log cfu per unit sampled (Ramesh et al., 2002, 2003, 2004b). Although direct comparisons are not possible between the current data and other studies, 24 or 48 h of drying did result in a decrease in *Campylobacter* numbers of about 7 log cfu per sample, resulting in none detected. *Campylobacter* sensitivity to drying has been reported previously (Doyle and Roman, 1982). It is unclear whether drying causes bacterial death or change into a viable nonculturable (VNC) state. Viable nonculturable *Campylobacter* have been shown to be able to colonize chicks (Stern et al., 1994) and may pose a health hazard if present on processed poultry. The current study did not include an assay for VNC *Campylobacter*.

Allowing broiler transport container floor surfaces to dry thoroughly between uses could be part of an effective commercial strategy to limit the exposure of uncontaminated flocks to culturable *Campylobacter* during transport and holding prior to processing. However, more work may be required to determine if VNC *Campylobacter* would be a concern on dried coops. At any rate, incorporating coop floor drying into a sanitization program would require a change in thinking relative to coop management or design but not large amounts of water and the costs associated with water use.

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## REFERENCES

Bailey, J. S., N. J. Stern, P. Fedorka-Cray, S. E. Craven, N. A. Cox, D. E. Cosby, S. Ladely, and M. T. Musgrove. 2001. Sources and movement of *Salmonella* through integrated poultry operations: A multistate epidemiological investigation. J. Food Prot. 64:1690–1697.

Berrang, M. E., R. J. Buhr, and J. A. Cason. 2000. *Campylobacter* recovery from external and internal organs of commercial broiler carcass prior to scalding. Poult. Sci. 79:286–290.

Berrang, M. E., and J. K. Northcutt. 2005. Water spray and immersion in chemical sanitizer to lower bacterial numbers on broiler transport coop flooring. J. Appl. Poult. Res. 14:315–321.

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.

Corry, J. E., 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.

Doyle, M. P., and D. J. Roman. 1982. Sensitivity of *Campylobacter jejuni* to drying. J. Food Prot. 45:507–510.

El-Assaad, F. G., L. E. Stewart, E. T. Mallinson, L. E. Carr, S. W. Joseph, and G. E. Berney. 1995. Decontamination of poultry transport cages. Trans. ASAE 38:1213–1220.

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.

Northcutt, J. K. and D. R. Jones. 2004. A survey of water use and common industry practices in commercial broiler processing facilities. J. Appl. Poult. Res. 13:48–54.

Ramabu, S. S., N. S. Boxall, P. Madie, and S. G. Fenwick. 2004. Some potential sources for transmission of *Campylobacter jejuni* to broiler chickens. Lett. Appl. Microbiol. 39:252–256.

Ramesh, N., L. E. Carr, S. W. Joseph, L. W. Douglass, and F. W. Wheaton. 2004a. A prototype poultry transport container decontamination system: 1. Design and construction of the system. Trans. ASAE 47:541–547.

Ramesh, N., S. W. Joseph, L. E. Carr, L. W. Douglass, and F. W. Wheaton. 2002. Evaluation of chemical disinfectants 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 reduce microorganism populations on poultry transport containers. J. Food Prot. 66:793–797.

Ramesh, N., S. W. Joseph, L. E. Carr, L. W. Douglass, and F. W. Wheaton. 2004b. A prototype poultry transport container decontamination system: II. Evaluation of cleaning and disinfection efficiency. Trans. ASAE 47:549–556.

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.

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.

Stern, N. J., P. J. Fedorka-Cray, J. S. Bailey, N. A. Cox, S. E. Craven, K. L. Hiett, M. T. Musgrove, S. Ladely, D. E. Cosby, and G. C. Mead. 2001. Distribution of *Campylobacter* spp. in selected U.S. poultry production and processing operations. J. Food Prot. 64:1705–1710.

Stern, N. J., D. M. Jones, I. V. Wesley and D. M. Rollins. 1994. Colonization of chicks by non-culturable *Campylobacter* spp. Lett. Appl. Microbiol. 18:333–336.

Stern, N. J., B. Wojton, and K. Kwiatek. 1992. A differential selective medium and dry ice generated atmosphere for recovery of *Campylobacter jejuni.* J. Food Prot. 55:514–517.

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.