

Incidence of *Salmonella* in processed broilers following transportation in contaminated coops

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To determine the influence of contaminated live-haul cages (coops) on subsequent contamination of broilers prior to and during processing, broilers subjected to feed withdrawal were transported in sanitized and *Salmonella*-contaminated coops, processed under simulated commercial conditions, and analyzed for incidence of *Salmonella*. At 5, 6, or 7 wk of age, 128 broilers from the same research flock were subjected to 4 h feed withdrawal, and placed (8 birds/coop) into pre-assigned plastic coops with solid flooring. Coops were loaded onto a trailer as 2 groups, each consisting of 2 stacks of 4 coops. Group 1, placed on the front of the trailer, consisted of sanitized coops while Group 2 placed on the rear of the trailer had 4 sanitized coops alternating with 4 coops containing feces (0.4 g/cm²) from colonized broilers knowingly shedding *Salmonella* (~10⁶ cfu/g). Broilers were transported 6 h, held 2-3 h, and processed by coop number. Half of the broilers from Group 1 were processed first, all of Group 2 second, and the remaining group 1 broilers last. The latter 2 processing Groups were chilled in a common slush ice bath. From each of 4 birds/coop, incidence of *Salmonella* in crop, ceca, post-pick carcass rinse and post-chill carcass rinse samples was determined. *Salmonella* incidence ranged from 10-20% in these samples from broilers transported in the sanitized coops (i.e., first half of Group 1). In contrast, *Salmonella* incidence ranged from 70-100% in samples from broilers transported in the contaminated coops (i.e., Group 2). *Salmonella* incidence ranged from 40-90% in samples from broilers transported in the sanitized coops that were processed after the birds from the contaminated coops (i.e., second half of Group 1). This latter observation indicates that significant cross contamination occurred during processing; to the extent that there was no difference ($p>0.05$) in *Salmonella* incidence on chilled carcasses from Group 2 (95%) vs second half of Group 1 (90%). Coop position had little effect on *Salmonella* incidence.

Keywords: *Salmonella*; broilers; transportation; processing; contamination

Introduction

Salmonellosis continues to be a common foodborne illness in developed countries, and poultry is implicated in ~10% of the foodborne outbreaks reported in the United States (Bryan and Doyle, 1995). Controlling *Salmonella* in poultry production and processing has proven difficult. Implementation of *Salmonella* performance standards in 1996 caused the US poultry industry to focus on pathogen reduction in its products. For broilers, the *Salmonella* performance standard is a 23.6% positive rate (12) for each set of carcasses (51) using tests by the USDA Food Safety Inspection Service (FSIS, 1999). This value was established based on the FSIS baseline data collected in 1994-1995 (FSIS, 1996). From 1998-2001, FSIS reported a *Salmonella* incidence of 10.7% for broilers; however, the percentage of *Salmonella* positive tests incidence has numerically increased from 2002-2005. In 2005, FSIS reported a *Salmonella* incidence of 16.3 % for broilers (FSIS, 2006).

In order to control *Salmonella* contamination, it is important to identify the potential sources of contamination, and assess the risks associated with each source of contamination. Feed and water contamination, feed withdrawal periods, live haul stress and contaminated live haul equipment are factors that contribute to *Salmonella* contamination in broilers prior to processing (Conner *et al.*, 2001; Cox *et al.*, 1983; Dougherty, 1976; Hargis *et al.*, 1995; MacKenzie and Bains, 1976; Moran and Bilgili, 1990).

One potential risk is the *Salmonella* contamination of broilers transported in coops contaminated with feces (Rigby *et al.*, 1980a). Once the feathers and skin of the broilers are contaminated, *Salmonella* can quickly and easily spread to the feathers, skin and ceca of broilers within coops (Waldroup *et al.*, 1992).

A relationship between *Salmonella* on the finished product and *Salmonella* in the growout environment has been established (Jones *et al.*, 1991; Lahellec and Colin, 1985). Previous studies demonstrate that flocks with low numbers of *Salmonella*-contaminated broilers are subjected to cross contamination during processing, and leave the plant with significantly higher numbers of contaminated carcasses (Lillard, 1989; D'Aoust, 1989). Thus, the contamination of broilers before entry into the processing facility that can serve as a prevalent source of subsequent cross contamination must be fully assessed for poultry processors to develop effective pathogen reduction strategies. The purpose of this study was to determine the influence of pre-slaughter transportation on subsequent contamination of broilers prior to and during processing.

Materials and methods

Day old Hubbard x Avian chicks were placed in a broiler house, reared under standard management conditions, and processed at 5, 6, or 7 weeks of age. Birds from the same research flock were subjected to 4 h feed withdrawal, and placed (8 birds/coop) into pre-assigned plastic coops with solid flooring. Four birds in each coop were wing banded for identification for sampling purposes. Coops were loaded onto a live-haul trailer as two groups, each consisting of two stacks of four coops (Figure 1). Group 1, placed on the front of the trailer, consisted of sanitized coops while Group 2 placed on the rear of the trailer had four sanitized coops alternating with four coops containing feces (0.4 g/cm²) from broilers knowingly shedding nalidixic acid resistant (NR) salmonellae (10⁶ cfu/g).

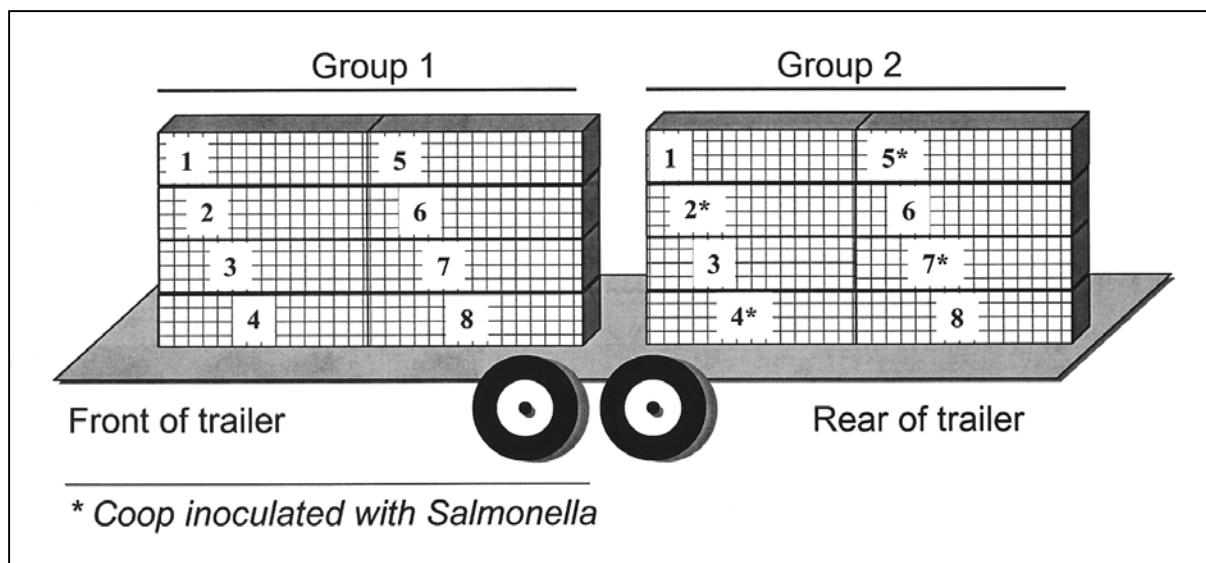


Figure 1 Arrangement of coops during live-haul and holding of broilers.

Three serotypes of NR *Salmonella enterica* were used: Enteritidis, Mission, and Typhimurium (provided by N.A. Cox, USDA, Athens, GA, USA). Each serotype was individually grown in brain heart infusion at 37 C, and combined to provide a composite mixture with equal proportions of each serotype and a final total population of ~10⁹ cfu/ml. Broilers used for shedding were orally gavaged weekly (0.1 ml) using the composite suspension. Feces were collected, verified for *Salmonella* shedding by enumeration on brilliant green sulfa agar (BGSA), and used to contaminate half of the coops used in the study as described above.

After the initial feed withdrawal, the broilers were transported 6 h, held 2-3 h, and continuously processed by coop number. Maximum pre-slaughter fasting time was 14 h.

General processing conditions were as follows. Shortly after being placed on the kill line, all birds were stunned with 20 mA (100 Hz AC, rectangular wave, 50% duty cycle, 8 sec). Birds were then killed by manually neck cutting (one carotid – one jugular), allowed to bleed for 75 sec, scalded (55-57 C for 82 sec), defeathered by one-stage feather picking (1.44m, 42 sec), open-flame gas singed (5 sec), washed with a multinozzle-multidirectional spray (15 sec), and subjected to pneumatic removal of neck and separation of feet from the carcass at the hock (Moran and Bilgili, 1996).

Half of the birds from each coop in Group 1 (4 birds/coop; 32 total) were processed first, all of Group 2 second, and the remaining Group 1 birds last. The latter two processing groups were chilled in a common slush ice bath. From each of 4 birds/coop, post-pick carcass rinse, crop, ceca, and post-chill carcass rinse samples were taken. Buffered peptone water was used for the initial rinse samples and pre-enriched for 24 h at 37 C. Tetrathionate broth was used as the enrichment for 24 h at 42 C. Samples were streaked on BGSA containing 200 ppm nalidixic acid, incubated for 24 h at 37 C, and examined for NR-*Salmonella*. Positive results were recorded and expressed as percentages.

Arc sine transformation was used for all percentage data. Analysis of variance was calculated using the General Linear Model of SAS using a 3 x 8 factorial arrangement of processing time and coop order (SAS, 1989). Turkey's Studentized range test was used to separate the means significant (SAS, 1989). The level of significance was $p < 0.05$.

Results

The incidence of *Salmonella* in crops and ceca from broilers, which were transported-held in the sanitized coops and processed first, was 23 and 10%, respectively (Figure 2). Overall, the highest percentage of positive *Salmonella* crop (71%) and ceca (70%) samples occurred in broilers processed second, which were from *Salmonella* contaminated coops. Birds processed third, which were from sanitized coops and processed after broilers from *Salmonella* contaminated coops, demonstrated a dramatic increase in *Salmonella* contamination of the crop (40%) and ceca (44%) when compared to broilers from the same group that were processed first (Figure 2), which suggests that *Salmonella* was dispersed throughout both groups of broilers.

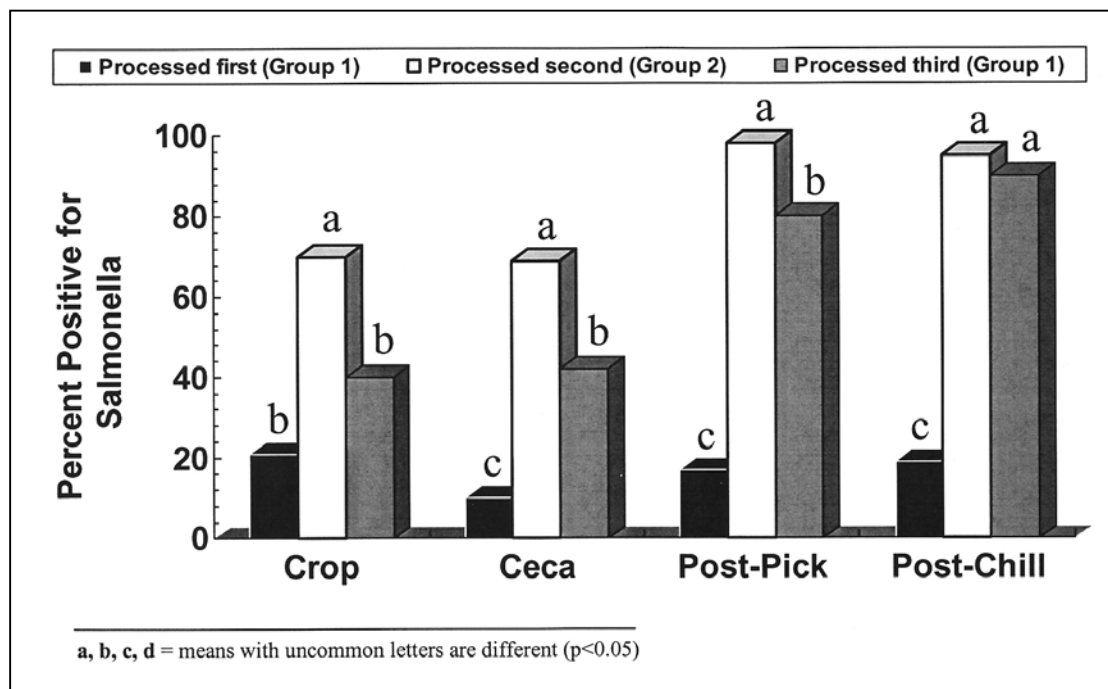


Figure 2. Incidence of *Salmonella* in crop, ceca, post-pick carcass rinse, and post-chill carcass rinse samples as affected by processing sequence.

External contamination of broilers also occurred. When sampled after picking 16%, 99%, and 81%, of broilers processed first, second, and third, respectively were positive for *Salmonella* (Figure 2). The significantly higher contamination of broilers processed third compared to those processed first indicates that the source of contamination was primarily processing, and represented cross contamination originating from the broilers processed second. Extent of contamination of broilers processed first remained approximately the same at post chill as compared to post-pick (Figure 2). However, contamination was equally high (> 90%) in broilers processed second and third.

While the spread of *Salmonella* among most coops was evident, coop position did not affect ($p \geq 0.05$) percentage of positive samples, except for post-pick samples (*data not shown*). Percentage of *Salmonella* positive crop samples from specific coops ranged from 28-65%. Numerically lowest percentages occurred in coops 1 (36%) and 8 (28%) and highest (67%) in coop 7. Numerically high percentages of *Salmonella* positive crop samples occurred in coops 4 (53%), 6 (53%), and 7 (67%). Trends observed for ceca results were similar to those results observed for crop. Percent of positive ceca samples from specific coops ranged from 19% to 50%. Numerically lowest percentages occurred in coops 1 (19%) and 8 (31%), and highest in coops 2 (50%) and 5 (50%). Coops in the middle arrangement (2, 3, 4, 5, 6 and 7) exhibited the highest percentage of *Salmonella* positive samples, which suggests horizontal spread of *Salmonella*. The lowest percent ($p \leq 0.05$) of positive broiler carcasses for *Salmonella* after picking occurred in coops 1 (46%) and 8 (50%) with the broilers in middle coops being equally contaminated (65-78%). At post chill, high contamination rates (>60%) were seen for broilers from all coop positions, which reflects cross contamination during processing and chilling.

Discussion

Contaminated live-haul cages increased cecal and crop carriage of *Salmonella*, and led to contamination of processed carcasses. Heavy contamination was evident following post-pick and post-chill indicating significant exterior contamination, which originated from contaminated coops via cross contamination prior to and during processing.

Overall, there was a lower percent of *Salmonella* positives in the broilers processed first from the sanitized coops. Broilers processed second from the *Salmonella* contaminated coops had the highest percentage of positives. Broilers processed from the sanitized coops had a significant increase in the percent of positives for *Salmonella* when processed following broilers from the contaminated coops.

The crop has been implemented as potential source of *Salmonella* contamination during processing (Hargis *et al.*, 1995). In agreement with this finding, broilers processed first exhibited a higher percentage of positives for *Salmonella* colonization of the crop and a lower percentage of positives for *Salmonella* colonization of the ceca. This increase did not occur in broilers processed third, primarily because of a higher percentage of crop contamination, indicating there is a rate of passage factor that must be considered. Cross-contamination ensues because of direct contact of broilers with cecal excreta and coprophagy (Moran and Bilgili, 1990). The time between broilers processed first and those processed second and third was not extensive, but may have been significant in terms of contributing to the differences in colonization of the crop and ceca between processing times. Carrier *et al.* (1999) observed that *Salmonella* crop contamination increases with feed withdrawal.

The slaughtering of flocks early in the processing day can contaminate birds processed later that day and in subsequent days (D'Aoust, 1989). As Rigby *et al.* (1980b) reported earlier, this study also indicates that cross-contamination during transport and inadequately cleaned crates are all sources of *Salmonella* contamination of processed carcasses. No matter how clean or uncontaminated the birds are initially, if processed following flocks contaminated with *Salmonella* these flocks become contaminated as well.

Salmonella can survive in feces alone for several days (Wakefield and Conner, 1997). Based on our results, colonized birds do excrete feces with 10^4 - 10^6 cfu/g. Broilers in this study were exposed to feces from birds shedding *Salmonella*, long live haul periods, and time off feed. Although the present study was a model system, conditions are similar to those frequently encountered in the poultry industry. As a result of this study, processing plants struggling to meet sanitation and food safety

performance standards should consider the transportation of broilers to the processing plant an important control point. Subjecting broilers to feed withdrawal and live haul predispose them to crop and cecal contamination and retention of *Salmonella* (Moran and Bilgili, 1990; Ramirez *et al.*, 1997; Corrier *et al.*, 1999). Also, Bailey *et al.* (2001) found that *Salmonella* from transport coops contributed to contamination in the processing plant, which is in agreement with the results of the present research.

Therefore, control of factors such as proper food withdrawal times and sanitizing crates and transportation vehicles between flocks should be followed when possible to aid in decreasing bacterial loads of broilers entering the processing plant. Without proper control during transportation, the potential for contamination during live haul and subsequent in-plant cross contamination can increase.

References

- BAILEY, J.S., STERN, N.J., FEDORKA-CRAY, P., CRAVEN, S.E., COX, N.A., COSBY, D.E., LADELY, S. and MUSGROVE, M.T.** (2001) Sources and movement of *Salmonella* through integrated poultry operations: a multistate epidemiological investigation. *Journal of Food Protection* **64**: 1690-1697.
- BRYAN, F.L. and DOYLE, M.P.** (1995) Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. *Journal of Food Protection* **58**: 326-344.
- CONNER, D.E., DAVIS, M.A., and ZHANG, L.** (2001) Poultry-borne pathogens: plant considerations. Pages 137-158 in *Poultry meat processing*, editor A.R. Sams, CRC Press, Boca Raton, FL, USA.
- CORRIER, D.E., BYRD, J.A., HARGIS, B.M., HUME, M.E., BAILEY, R.H. and STANKAR, L.H.** (1999) Presence of *Salmonella* in the crop and ceca of broiler chickens before and after pre-slaughter feed withdrawal. *Poultry Science* **78**: 45-49.
- COX, N.A., BAILEY, J.S. and THOMSON, J.E.** (1983) *Salmonella* and other *Enterobacteriaceae* found in commercial poultry feed. *Poultry Science* **62**: 2169-2175.
- D'AOUST, J.** (1989). *Salmonella*. Pages 327-445 in: *Food Microbiology*, editor M.P. Doyle, Marcel Dekker, Inc. New York, New York.
- DOUGHERTY, T.J.** (1976) A study of *Salmonella* contamination in broiler flocks. *Poultry Science* **55**: 1811-1815.
- FSIS** (1996) Nationwide broiler chicken microbiological baseline data collection program. United State Department of Agriculture, Washington, DC
- FSIS** (1999) One-year progress report on *Salmonella* testing for raw meat and poultry products. March 9. United State Department of Agriculture, Washington, D.C.
- FSIS** (2006) Progress report on *Salmonella* testing of raw meat and poultry products, 1998-2005. posted 23 Feb. at http://www.fsis.usda.gov/Science/Progress_Report_Salmonella_Testing/index.asp
- HARGIS, B.M., CALDWELL, D.J., BREWER, R.L., CORRIER, D.E. and DELOACH, J.R.** (1995). Evaluation of the chicken crop as a source of *Salmonella* contamination for broiler carcasses. *Poultry Science* **74**: 1548-1552.
- JONES, F.T., AXTELL, R.C., RIVES, S.E., SCHEIDELER, F.R., TARVER, JR., WALKER, R.L., and WINELAND, M.J.** (1991) A survey of *Salmonella* contamination in modern broiler production. *Journal of Food Protection* **54**: 502-507.
- LAHELLEC, C., and COLIN, P.** (1985) Relationship between serotypes of *Salmonella* from hatcheries and rearing farms and those from processed poultry carcasses. *British Poultry Science* **26**: 179-186.
- LILLARD, H.S.** (1989) Factors affecting the persistence of *Salmonella* during the processing of poultry. *Journal of Food Protection* **52**: 829-832.
- MACKENZIE, M.A., and BAINS, B.S.** (1976) Dissemination of *Salmonella* serotypes from raw feed ingredients to chicken carcasses. *Poultry Science* **55**: 957-960.
- MORAN, E.T. and BILGILI, S.F.** (1990) Influence of feeding and fasting broilers prior to marketing on cecal access of orally administered *Salmonella*. *Poultry Science* **53**: 205-207.

- MORAN, E.T.** and **BILGILI, S.F.** (1996) Carcass yield and weep loss from fast-food cuts after processing of broilers using extremes in stunning and current and slush-ice chilling. *Poultry Science* **75**: 186-190.
- RAMIREZ, G.A., SARLIN, L.L., CALDWELL, D.J., YEZAK, C.R., HUME, M.E., CORRIER, D.E., DELOACH, J.R.** and **HARGIS, B.M.** (1997) Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. *Poultry Science* **76**:654-656.
- RIGBY, C.E., PETTIT, J.R., BAKER, M.F., BENTLEY, A.H., SALMONS, M.O.** and **LIOR, H.** (1980a) Flock infection and transport as sources of *Salmonella* in broiler chickens and carcasses. *Canadian Journal of Comparative Medicine* **44**: 328-337.
- RIGBY, C.E., PETTIT, J.R., BAKER, M.F., BENTLEY, A.H., SALOMONS, M.O.,** and **LIOR, H.** (1980b) Sources of *Salmonella* in an uninfected commercially-processed broiler flock. *Canadian Journal of Comparative Medicine* **44**: 267-274.
- SAS** (1989) SAS users guide: 6th edition. SAS Institute, Inc., Cary, NC, USA.
- WAKEFIELD, C.B.** and **CONNER, D.E.** (1997) Survival of salmonellae in broiler feces. *Poultry Science* **76** (supplement 1): 30.
- WALDROUP, A.L., SKINNER, J.T., HIERHOLZER, R.E., KOPEL, J.M.,** and **WALDROUP, P.L.** (1992) Effects of bird density on *Salmonella* contamination of prechill carcasses. *Poultry Science* **71**: 844-849.