

Campylobacter Recovery from External and Internal Organs of Commercial Broiler Carcass Prior to Scalding

M. E. Berrang,¹ R. J. Buhr, and J. A. Cason

USDA-ARS, Poultry Processing and Meat Quality Research Unit, Russell Research Center, Athens, Georgia 30604-5677

ABSTRACT *Campylobacter* is a human pathogen commonly found on live broilers and processed carcasses. To plan effective intervention strategies, it would be helpful to know which *Campylobacter* populations are associated with the external and internal organs of broilers. Six carcasses were collected after exiting the bleed tunnel at a commercial broiler plant on each of three visits (n = 18). Carcasses were placed individually into sterile plastic bags, sealed, and covered with ice for transport to the laboratory. Five locations were sampled aseptically from each carcass: breast feathers (hand picked from the sternal tracts); breast skin, including the sternal tracts; crop; ceca; and colon. Samples included adhering contamination or lumen contents and were covered with phosphate-buffered saline and blended. Serial dilutions were made for examination of *Campylobacter*, coliform, *Escherichia coli*, and total aerobic bacterial populations. Average sample

weights (grams) were as follows: feathers, 1.5; skin, 6.5; crop, 5.1; ceca, 7.8; and colon, 3.1. *Campylobacter* populations (mean log₁₀ colony-forming units per gram of sample) found were feathers, 5.4; skin, 3.8; crop, 4.7; ceca, 7.3; and colon, 7.2. Coliform/*E. coli* populations observed were feathers, 6.4/6.0; skin, 5.3/4.9; crop, 4.3/3.7; ceca, 6.6/6.2; and colon, 5.8/5.3. Total aerobic bacterial populations found were feathers, 7.9; skin, 7.1; crop, 5.8; ceca, 6.8; and colon, 6.4. On a per gram basis, ceca and colon are the internal organs that if ruptured could cause the highest number of *Campylobacter* to be leaked onto the carcass. The crop also contained more *Campylobacter* per gram than did the skin, and if compromised may increase the numbers on the surface of the carcass. However, even with no contamination from an internal organ, a substantial population of *Campylobacter* is already resident on broiler skin as the carcass enters the early stages of processing.

(Key words: *Campylobacter*, ceca, colon, crop, feathers)

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INTRODUCTION

Campylobacter is a common foodborne pathogen of humans that has been associated with poultry carcasses and further processed poultry products (White *et al.*, 1997; Saleha *et al.*, 1998). It is generally thought that *Campylobacter* flows into commercial processing facilities on and within the live birds and is disseminated during the various processing procedures (Saleha *et al.*, 1998). *Campylobacter* can be recovered from broiler carcasses prior to entering the scald tank by rinsing feathered carcasses (Stern *et al.*, 1995; Berrang and Dickens, 1999) or by excising or swabbing the skin (Izat *et al.*, 1988; Kotula and Pandya, 1995).

Despite the presence of *Campylobacter* on the outside of broilers, emphasis is commonly placed on the presence and level of *Campylobacter* and other human pathogens in the alimentary tract. This interest is fueled by the concern that ruptured organs, such as crop or ceca, may spill

contents rich in *Campylobacter* onto the carcass. It has been reported (Hargis *et al.*, 1995) that the crop can be broken during processing. Byrd *et al.* (1998) reported that *Campylobacter* is evident in the majority (62%) of crop samples examined on the farm just prior to catching and transport to the plant. Oosterom *et al.* (1983) found that *Campylobacter* is commonly recovered in high numbers, more than log₁₀ 6.0 cfu/g in the ceca and colon. Musgrove *et al.* (1997) reported that plugging the vent prior to killing resulted in a lower number of *Campylobacter* on carcasses, indicating that cloacal contents voided during processing can contribute to skin counts.

Campylobacter has also been found on carcass skin samples. Berndtson *et al.* (1992) found 89% of skin samples from processed carcasses were positive for *Campylobacter* at about log₁₀ 3.0 cfu/g, which is lower than that found in intestinal samples (Oosterom *et al.*, 1983; Musgrove *et al.*, 1997). However, Kotula and Pandya (1995) found high levels of *Campylobacter* on defeathered skin prior to scalding. Before scalding, breast skin had higher *Campylobacter* populations (log₁₀ 6.9 cfu/g) than did drum or thigh skin. Likewise, feathers over the breast were more heavily contaminated with *Campylobacter* (counts of log₁₀ 7.5 cfu/g) than were feathers over the thigh and drum (Kotula and

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¹To whom correspondence should be addressed: mberrang@ars.usda.gov.

Pandya, 1995). Stern *et al.* (1995) found that following transport, the levels of *Campylobacter* on a feathered carcass rinse increased significantly, reaching levels from 6.8 to 8.7 log₁₀ cfu per carcass. These feather and skin *Campylobacter* populations are close to those often reported from the ceca and colon (Oosterom *et al.*, 1983; Musgrove *et al.*, 1997). Nevertheless, interest remains focused on the possibility of alimentary tract contents further contaminating the surfaces of broiler carcasses.

Little research has been published on the levels of *Campylobacter* recovered from external and internal sites of the same commercial broiler as it proceeds through the processing procedure. Therefore, this study examined the levels of *Campylobacter* associated with broiler chickens after entering the processing plant. Because food safety standards related to *Campylobacter* may be introduced in the United States, this information will be helpful in designing and maintaining effective sanitation programs. The approach of this study was to enumerate *Campylobacter* from breast feather, breast skin, crop, ceca, and colon samples from the same commercial broiler carcasses at the end of the blood tunnel prior to scalding.

MATERIALS AND METHODS

Broiler Carcasses

Six broiler carcasses were removed from shackles at the end of the blood tunnel in a commercial processing plant on each of three visits (n = 18 carcasses). All samples were collected during January of 1999. All birds had been without feed for approximately 12 h. By timing collection such that all carcasses were from the same transport trailer, each sample day represented a different flock. All carcasses were individually placed in sterile plastic bags that were sealed and covered with ice for transport to the laboratory. Each carcass was kept on ice until dissection and removal of samples.

Samples

Five samples were collected from each bird. Feathers from the sternal tracts over the breast were picked by hand with new latex gloves. Breast skin from the defeathered area was collected by aseptic removal with sterile forceps and scalpel. Crop, ceca, and colon were each aseptically removed with the contents intact. Tissue clamps were used to contain the contents of each organ. New latex gloves were worn to manually hold cloacal contents in the colon, preventing escape prior to placement of the tissue clamp. The colon sample included that portion of the intestine from the ileo-cecal junction to within 0.5 to 1 cm of the vent.

All samples, including surface contamination or contents, were placed into sealable plastic bags and weighed. Phosphate-buffered saline was used to dilute each sample according to weight with 10 times the weight for feather samples and three times the weight for all other samples. Feather samples were massaged by hand for 30 s, whereas the other organ samples were mixed in a stomacher² for 30 s.

Culture Methods

Serial dilutions were made in phosphate-buffered saline, and *Campylobacter* was enumerated by plating in duplicate onto the surface of Campy-Cefex agar (Stern *et al.*, 1992). One-tenth mL was spread on the surface of each plate with a sterile plastic inoculating loop, after which plates were incubated at 42 C for 36 h in a microaerophilic environment (5% O₂, 10% CO₂, and 85% N₂). Colony-forming units characteristic of *Campylobacter* were counted. Each colony type counted as *Campylobacter* from each sample was confirmed as a member of the genus by examination of cellular morphology and motility on a wet mount under phase contrast microscopy. Each colony type was further characterized by a positive reaction on a latex agglutination test kit.³ Total aerobic bacterial populations were enumerated on plate count agar.⁴ One-tenth mL from a serial dilution of the stomached sample was plated in duplicate on the surface of the agar, spread, and incubated at 35 C for 18 to 24 h prior to counting the resulting colony-forming units. Coliform and *E. coli* counts were made by plating 1 mL from a serial dilution of the stomached sample onto duplicate *E. coli* petrifilm^{TM,5} plates. PetrifilmTM plates were incubated at 35 C for 18 to 24 h, and colony types characteristic of coliforms and *E. coli* were counted.

Statistical Analyses

Bacterial counts were converted to log₁₀ cfu/g (or cfu/sample) before conducting an analysis of variance. No replication by sample interaction was found for *Campylobacter*, *E. coli*, or coliform populations; therefore, means from each sample type were compared with other samples by Tukey's honest statistical difference (HSD) test. A replication by sample interaction was detected for total aerobic bacterial population; therefore, these populations were examined within replications by Tukey's honest statistical difference. Pearson correlations were used to determine relationships between populations measured for the five sample types. All statistical analyses were conducted using Statistica, Release 5, 1997 Edition.⁶

RESULTS

Campylobacter results are presented in Table 1. Overall there was a significant replication effect ($P \leq 0.01$) uncovered by analysis of variance, indicating that the three different flocks did not have the same level of *Campylobacter* contamination. Therefore, replications are shown

²Seward Medical, London, SE1 1PP, UK.

³Integrated Diagnostics, Inc., Baltimore, MD 21227.

⁴Difco, Detroit, MI 48232.

⁵3M Health Care, St. Paul, MN 55144.

⁶Statsoft, Tulsa, OK 74104.

TABLE 1. *Campylobacter* counts recovered from external and internal organs of precald broiler carcasses from a commercial processing plant

Replication	Feathers ¹	Skin ²	Crop	Ceca	Colon
	(Mean log ₁₀ cfu/g of sample)				
1	4.6	3.1	4.7	6.9	6.8
2	5.5	3.9	4.5	7.7	7.7
3	6.1	4.5	5.0	7.3	7.2
Mean ³	5.4 ± 0.5 ^b	3.8 ± 0.7 ^c	4.7 ± 0.3 ^b	7.3 ± 0.4 ^a	7.2 ± 0.4 ^a

^{a-c}Means with no common superscript are different at $P \leq 0.05$ by Tukey's honest statistical difference; n = 6 per replication.

¹Feathers collected from sternal feather tracts over breast.

²Skin collected from defeathered area over breast muscle.

³± 95% confidence interval.

individually along with the means. Because there was no replication by sample interaction, the relationship between sample type and *Campylobacter* populations was further examined by applying a Tukey's honest statistical difference test to means for the three replications. Ceca and colon were found to have the highest *Campylobacter* populations of any organ tested. Our results showed that on a per gram basis feathers had significantly ($P \leq 0.05$) more *Campylobacter* than skin. *Campylobacter* populations on feathers were positively correlated with those recovered from skin ($r = 0.83$, $P \leq 0.01$). One gram of crop and contents had more *Campylobacter* than did each gram of skin ($P \leq 0.05$) but not significantly more than 1 g of feathers.

Escherichia coli and coliform results are presented in Table 2, and these two populations mirror each other closely. The crop and contents had the lowest level of *E. coli* of all organs tested, whereas feathers, ceca, and colon had the highest levels. Therefore, it appears that the exterior of the carcass (feather + skin) is as contaminated with *E. coli* as the crop, ceca, or colon. No significant correlation was found between *E. coli* or coliform populations and *Campylobacter* using data from any sample type.

Total aerobic bacterial populations measured from broiler organs are presented in Table 3. With these data, an interaction between sample site and replication was noted; comparisons within replication are presented in the table. Although in general, the highest mean total counts were recorded from feathers and skin, these numbers were not higher than those from other sites in every replication. Nevertheless, the precald carcass was at least

as contaminated with aerobic bacteria on the exterior as within the crop, ceca, or colon. No significant correlations were noted between aerobic plate counts and *Campylobacter* populations for any of the sample types tested.

Mean weights of each organ collected are shown in Table 4. These values have been used to calculate populations present for entire organs as sampled. It is interesting to note that, on average, 1 g of ceca or colon with contents had more *Campylobacter* (log₁₀ 7.3 and 7.2, respectively) than that found on the entire 1.5-g breast feather sample (log₁₀ 5.5 cfu) or 6.5-g breast skin sample (log₁₀ 4.6 cfu). However, 1 g of crop with contents did not have more *Campylobacter* than breast feathers on either a per gram or total basis. Furthermore, 1 g of crop and contents had about the same number of *Campylobacter* as the total found on the whole 6.5-g breast skin sample.

DISCUSSION

Campylobacter levels found on feather samples from the breast area were lower than those reported by Kotula and Pandya (1995) by about a 2 log₁₀ magnitude. *Campylobacter* populations on skin sampled by stomaching were also found to be lower than those previously reported when skin was sampled by homogenization (Kotula and Pandya, 1995). One possible reason for the difference between these two studies is that the Kotula and Pandya study (1995) was conducted in the winter on the Delmarva peninsula, whereas the current study was conducted in northern Georgia during the winter. The difference in results points to the variation that may be expected with

TABLE 2. Mean *Escherichia coli* and coliform counts recovered from external and internal organs of precald broiler carcasses from a commercial processing plant

Population	Feathers ²	Skin ³	Crop	Ceca	Colon
	(Mean ¹ log ₁₀ cfu/g of sample)				
Coliform	6.4 ± 0.2 ^a	5.3 ± 0.3 ^b	4.3 ± 0.6 ^c	6.6 ± 0.4 ^a	5.8 ± 0.5 ^{a,b}
<i>E. coli</i>	6.0 ± 0.2 ^a	4.9 ± 0.3 ^b	3.7 ± 0.6 ^c	6.2 ± 0.4 ^a	5.3 ± 0.4 ^b

^{a-c}Means within a population with no common superscript are different at $P \leq 0.05$ by Tukey's honest statistical difference; n = 18.

¹± 95% confidence interval.

²Feathers collected from sternal feather tracts over breast.

³Skin collected from defeathered area over breast muscle.

TABLE 3. Total aerobic bacteria counts recovered from external and internal organs of prescald broiler carcasses from a commercial processing plant

Replication	Feathers ¹	Skin ²	Crop	Ceca	Colon
Mean log ₁₀ cfu/g of sample					
1	7.8 ^a	6.6 ^{bc}	6.1 ^c	7.3 ^{ab}	6.8 ^{bc}
2	8.2 ^a	7.4 ^{ab}	6.0 ^c	6.4 ^{bc}	6.3 ^{bc}
3	7.8 ^a	7.3 ^{ab}	5.4 ^c	6.7 ^{abc}	6.0 ^{bc}
Mean ³	7.9 ± 0.2	7.1 ± 0.2	5.8 ± 0.4	6.8 ± 0.4	6.4 ± 0.5

^{a-c}Means within a replication with no common superscript are different at $P \leq 0.05$ by Tukey's honest statistical differences; n = 6 per replication.

¹Feathers collected from sternal feather tracts over breast.

²Skin collected from defeathered area over breast muscle.

³± 95% confidence interval.

different flocks, management styles, and sampling methods.

Campylobacter populations that were encountered in the crop in this study differ from those recently reported by Byrd *et al.* (1998). In that study, the crop was examined for presence or absence of *Campylobacter*. Overall, they found 38% of crops sampled on the farm following feed withdrawal just prior to transport were negative for *Campylobacter*. However, in that report, incidence increased with lengthened feed withdrawal. Eight hours of feed withdrawal was the closest to the 12 h feed withdrawal used in the current study. At 8 h, flocks ranged from 75 to 100% *Campylobacter* positive using an enrichment technique (Byrd *et al.*, 1998). The current study found that in the plant just prior to scalding, all crop samples (100%) were positive for *Campylobacter* at levels that did not require enrichment. The difference in results is even more pronounced when one considers the ceca samples. Byrd *et al.* (1998) reported that overall only 4% of ceca samples were positive (highest incidence being 15% at 8 h feed withdrawal), whereas the current results show 100% of ceca samples were positive at very high levels. These differences could be due to a host of factors including geographic location and season. The current study reports on the *Campylobacter* populations associated with these organs when the bird is ready to be scalded, taking into account the cooping and transport process, which can increase populations (Stern *et al.*, 1995) and as such may provide more representative information for

the plant sanitation program. The data for ceca and colon *Campylobacter* incidence and levels in this report are in line with other published data (Oosterom *et al.*, 1983; Musgrove *et al.*, 1997). Based on these results, because the crop contains fewer *Campylobacter* than the ceca or colon, crop breakage would contribute less to the spread of *Campylobacter* than would ceca or colon breakage. Arguably the colon-cloaca is the most likely organ to contain *Campylobacter* that is spread onto the carcass. Contents of the colon-cloaca may escape during processing and result in adding contamination to the skin of the carcass. The colon was found to have very high levels of *Campylobacter*; therefore, the transfer of even a small amount of contents could be expected to cause an increase in the number of *Campylobacter* on the carcass. However, it is important to note that even if the carcass is not contaminated with organ contents during processing, on average, skin had close to 10,000 *Campylobacter* cfu/g.

The lack of correlation between *Campylobacter* populations and aerobic bacteria has been previously reported for defeathered carcasses (Cason *et al.*, 1997). The present study shows a further lack of correlation with *E. coli*, coliforms, and aerobic bacteria on carcasses before scalding. The finding of high levels of aerobic bacteria and members of the family Enterobacteriaceae, which includes coliforms, on feathers and skin confirms data previously reported (Geornaras *et al.*, 1997). Geornaras *et al.* (1997) found that both before and after scalding, feathers had higher levels of aerobic bacteria than skin. The

TABLE 4. Mean weights and bacterial recovery from entire external and internal organ samples of broilers carcasses from a commercial processing plant

Organ	Weight (g) ¹	<i>Campylobacter</i>	Coliform	<i>Escherichia coli</i>	Total aerobes
(Cumulative mean log ₁₀ cfu/sample)					
Feathers ²	1.5 ± 0.4	5.5 ^b	6.5 ^b	6.2 ^b	8.0 ^a
Skin ³	6.5 ± 0.7	4.6 ^c	6.1 ^b	5.7 ^b	7.9 ^a
Crop	5.1 ± 0.4	5.4 ^{bc}	5.0 ^c	4.4 ^c	6.5 ^b
Ceca	7.8 ± 1.3	8.2 ^a	7.6 ^a	7.1 ^a	7.7 ^a
Colon	3.0 ± 0.8	7.7 ^a	6.2 ^b	5.7 ^b	6.8 ^b

^{a-c}Means within a population (column) with no common superscript are different at $P \leq 0.05$ by Tukey's honest statistical difference; n = 18.

¹± 95% confidence interval.

²Feathers collected from sternal tracts over breast.

³Skin collected from defeathered area over breast muscle.

makeup of this population has been reported to change from predominantly Gram-positive bacteria before scald to Gram-negative bacteria after scald (Geornaras *et al.*, 1998). Nevertheless, the population of *E. coli*, coliforms or total aerobic bacteria cannot be used to make a judgement regarding the likelihood that a prescald carcass harbors *Campylobacter* or at what level.

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