

Evaluation of Logistic Processing To Reduce Cross-Contamination of Commercial Broiler Carcasses with *Campylobacter* spp.

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ABSTRACT

Cross-contamination of broiler carcasses with *Campylobacter* is a large problem in food production. Here, we investigated whether the contamination of broilers carcasses from *Campylobacter*-negative flocks can be avoided by logistic scheduling during processing. For this purpose, fecal samples were collected from several commercial broiler flocks and enumerated for *Campylobacter* spp. Based on enumeration results, flocks were categorized as *Campylobacter* negative or *Campylobacter* positive. The schedule of processing included the testing of *Campylobacter*-positive flocks before or after the testing of *Campylobacter*-negative flocks. During processing, flocks were also sampled for *Campylobacter* spp. before and after chilling. *Campylobacter* strains were identified with multiplex PCR and analyzed for relatedness with pulsed-field gel electrophoresis. Our results show that *Campylobacter*-negative flocks were indeed contaminated with *Campylobacter* strains originating from previously processed *Campylobacter*-positive flocks. *Campylobacter* isolates collected from carcasses originating from different farms processed on the same day showed similar pulsed-field gel electrophoresis patterns, confirming cross-contamination. These findings suggest that a simple logistic processing schedule can preserve the *Campylobacter*-negative status of broiler carcasses and result in products with enhanced food safety.

Campylobacteriosis continues to be an important diarrheal disease in humans worldwide (1, 2). A recent report from the European Food Safety Authority highlighted *campylobacteriosis* as the most reported zoonosis in the European Union (6). Mishandling of raw poultry and consumption of undercooked poultry are important risk factors (3, 29) and potential contamination sources (18) in human *campylobacteriosis*. In the United States, a high percentage of commercial broiler chickens are positive for *Campylobacter* at the time of slaughter (28), which results in a high incidence of *Campylobacter* spp. in retail products (5). The contamination that occurs at processing is believed to originate from the viscera of processed chickens (3). Cross-contamination of carcasses between different flocks occurs during processing (19, 27). Although reports suggest that a decline in the number of *Campylobacter* occurs during scalding, cross-contamination is still important during defeathering, evisceration, washing, and chilling of broiler carcasses (9). Therefore, there is considerable interest in reducing the incidence and numbers of *Campylobacter* contamination during the processing of broilers to achieve a safer final product.

There is a positive correlation between the contamination of live broilers at the end of rearing and the contamination of the carcasses after processing (10). Rivoal et al.

(27) reported that the same genotypes of *Campylobacter jejuni* isolated from intestinal contents were found in carcasses of successive flocks, which suggested a cross-contamination of flocks by *Campylobacter* strains. Miwa et al. (17) conducted a study to determine the source of *C. jejuni* contamination on broiler carcasses, and found that the intestinal contents of previously processed *C. jejuni*-positive flocks were the primary contamination source for *C. jejuni*-negative flocks that were processed later on the same processing day. Therefore, if *Campylobacter*-negative flocks are scheduled for processing first at the starting of processing, there may be an opportunity to maintain the negative status of these carcasses throughout processing and retail. On the other hand, if *Campylobacter*-negative flocks are processed after *Campylobacter*-positive flocks, then the negative status of the product will not be preserved due to cross-contamination. The reason to maintain the negative status of *Campylobacter*-negative flocks relates to the preparation of high-risk products, such as breaded tenders or fillets that may be sold ready to eat.

The objectives of our studies were to test these assumptions by (i) determining the colonization status of the flocks before slaughter, (ii) developing a logistic processing where *Campylobacter*-negative or *Campylobacter*-positive flocks were processed earlier on the processing day, and (iii) characterizing *Campylobacter* isolates using pulsed-field gel electrophoresis (PFGE) to determine any correla-

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tion between isolates from live chickens and isolates from processed carcasses.

MATERIALS AND METHODS

Organization of trials. Two different commercial broiler companies ("A" and "B") were included in the study. Company A was sampled four times, and Company B was sampled three times. Each trial consisted of the collection of samples at the farms, the isolation of *Campylobacter* spp. from farm samples, the scheduling of the processing of each flock, and the sampling of the flocks during processing. Trials were given roman numerals (I, II, III, etc.).

Sample collection from farms. Broiler fecal samples were collected in independent trips to commercial broiler farms. In each visit, three flocks from three different farms scheduled to be processed at the same processing plant and on the same day were sampled to determine the number of *Campylobacter* spp. colonizing the birds. Flocks were sampled a week before the actual processing day. At the farm, 10 fecal samples per flock were collected, with 30 samples per visit. Each sample consisted of 1 g of feces that was immediately mixed with 9 ml of Preston broth. Samples were kept in anaerobic jars under a microaerobic gas mixture (10% CO₂, 5% O₂, and 85% N₂) generated with CampyGen (Oxoid, Ltd., New York, N.Y.). Samples were transported to our laboratory in refrigerated ice boxes and analyzed within 4 h of collection. At the laboratory, samples were serially diluted in phosphate-buffered saline and plated onto modified charcoal cefoperazone deoxycholate (mCCDA) agar plates (12) and modified Campy-Cefex (mCC) agar plates (23) in duplicates. Plates were incubated at 42°C for 48 h under microaerobic conditions (Airgas, Radnor, Pa.) in sealed plastic bags (23) or in anaerobic jars gassed with the MACSmics Jar Gassing System (Microbiology International, Frederick, Md.). Samples were also enriched in Preston broth under microaerobic conditions at 42°C for 24 h. Enriched samples were swabbed (~0.1 ml) on mCC plates, which were incubated under microaerobic conditions at 42°C for 48 h.

Sample collection from processing plants. During processing, 10 carcasses were collected immediately before the chiller (prechilled samples) and 10 immediately after the chiller (postchilled samples) from each flock sampled at the farm a week before processing. Samples were collected using the carcass rinse method (30) and 400 ml of buffered peptone water. Each carcass rinse was plated onto modified mCC agar and mCCDA agar plates. For each medium, two plates were each spread with 0.1 ml of the carcass rinse, and four plates were each spread with 0.25 ml of the rinse. The plates were incubated at 42°C for 48 h under a microaerobic gas mixture. In addition, 30 ml of the rinse was enriched in 30 ml of 2× Bolton broth under a microaerobic atmosphere at 42°C for 24 h. Enriched samples were swabbed (~0.1 ml) on mCC plates, which were then incubated under microaerobic conditions at 42°C for 48 h.

Identification of *Campylobacter* isolates. Typical *Campylobacter* colonies were considered presumptive positive if they showed typical morphology and motility under phase contrast microscopy (Optiphot-2, Nikon, Tokyo, Japan). Isolates were stored at -80°C in tryptic soy broth (Becton Dickinson, Sparks, Md.) supplemented with 30% glycerol (vol/vol) and 5% blood. The speciation of the isolates was done with the hippurate test (Hardy Diagnostics, Santa Maria, Calif.) and a multiplex PCR assay that identifies *C. jejuni* and *Campylobacter coli*. Isolates were also analyzed with PFGE. *Campylobacter* isolates were identified with

TABLE 1. *Campylobacter* counts for fecal material from the farm and for pre- and postchilled samples

Company	Feces (log CFU/g)	Prechilled (log CFU/ml)	Postchilled (log CFU/ml)
A	5.5 ± 0.23 A ^a	1.9 ± 0.36 A	0.4 ± 0.08 B
B	5.0 ± 0.51 A	1.7 ± 0.14 A	1.0 ± 0.11 A

^a Values are mean ± standard error of the mean. Means in a column followed by different letters are statistically different ($P < 0.05$, Duncan's test).

a multiplex PCR assay. Strains were grown on mCC agar plates from stock cultures under microaerobic conditions at 42°C for 24 h. Bacterial DNA was extracted using PrepMan Ultra (Applied Biosystems, Foster City, Calif.). DNA samples were also tested with a multiplex PCR assay that is based on three sets of primers targeting the aspartokinase gene (15), the hippuricase gene, and the 16S rDNA genes (25). PCR reactions were performed in 25- μ l aliquots as described elsewhere (24). Amplicons were detected by standard gel electrophoresis in 1.5% agarose (Ultra Pure DNA Grade Agarose, Bio-Rad Laboratories, Hercules, Calif.) in Tris-borate-EDTA buffer at 70 V for 1 h. Gels were stained with ethidium bromide and visualized using a UV transilluminator (Gel-Doc System) with a molecular analyst computer program (Bio-Rad Laboratories).

PFGE analyses of isolates. The suggested protocol by the Centers for Disease Control and Prevention (<http://www.cdc.gov/pulsenet/protocols.htm>) was followed (26). *Sma*I was the restriction endonuclease used in the analysis. Pair comparison and cluster analysis was done using the Dice correlation coefficient and the unweighted pair group mathematical average (UPGMA) clustering algorithm of BioNumerics version 4.50 (Applied Maths, Austin, Tex.). The optimization and position tolerance for band analysis were set at 1%. A cutoff of 90% was used to resolve the different PFGE patterns (4).

Statistical analysis. *Campylobacter* counts were converted to log CFU/g of fecal material. Means were analyzed for differences ($P \leq 0.05$) using Duncan's test (PROC GLM of SAS), and the standard error calculated with PROC MEANS (SAS Institute Incorporated, Cary, N.C.).

RESULTS

From 21 sampled flocks, 12 flocks (57%) tested positive for *Campylobacter* at the farm level (Table 1). In these flocks, the average log CFU of *Campylobacter* spp. per gram of fecal material was 5.4 (minimum, 3.5; high, 7.1). The average log CFU of *Campylobacter* spp. per gram of fecal and prechilled samples from companies A and B were similar ($P > 0.05$). There was a decrease in the number of *Campylobacter* counts prechilled versus postchilled for all trials. However, there was a significant difference ($P < 0.05$) between the postchilled carcass counts for company A versus company B (Tables 1 and 2). Company A had a reduction of 1.5 log CFU/ml, while company B had only a reduction of 0.7 log CFU/ml. From all *Campylobacter*-positive flocks, 100% of the fecal samples were positive, and all samples that were negative after direct plating were negative after the enrichment of the fecal material in Preston broth for 24 h under microaerobic conditions.

Multiplex PCR assays performed in 93 isolates from

TABLE 2. *Campylobacter* counts from commercial broiler samples taken at the farm and in the processing plants^a

Company A					Company B				
Trial	Farm ^b	Feces (log CFU/g)	Prechilled (log CFU/ml)	Postchilled (log CFU/ml)	Trial	Farm	Feces (log CFU/g)	Prechilled (log CFU/ml)	Postchilled (log CFU/ml)
I	1	N	N	N	V	1	5.6	2.2	1.4
	2	5.5	2.4	0.5		2	N	1.7	1.1
	3	7.1	NC	NC		3	N	2.1	0.6
II	1	4.8	N	N	VI	1	5.7	1.9	1.0
	2	5.7	2.9	0.5		2	N	1.8	0.9
	3	5.8	2.3	NC		3	N	1.6	0.8
III	1	6.1	2.3	0.6	VII	1	N	N	N
	2	N	0.9	0.2		2	3.5	2.0	1.4
	3	N	NC	NC		3	5.2	0.9	NC
IV	1	N	N	N					
	2	5.3	0.7	N					
	3	4.3	NC	NC					

^a N, negative for *Campylobacter* after enrichment; NC, samples not collected.

^b The order of the farms corresponds to the order of processing.

all 12 positive flocks showed that 77 isolates were *C. jejuni*, 7 were *C. coli*, and 9 showed a mixed culture of both *C. jejuni* and *C. coli*. Further identification of these cultures resulted in five *C. jejuni* and four *C. coli* isolates. PFGE analysis of selected isolates showed that in most cases there was a predominant isolate from fecal samples that was also collected from the carcasses of the broilers from that farm during processing (Fig. 1). In three trials (III, V, and VI), two flocks representing two independent farms were *Campylobacter* negative, while the remaining flock was *Campylobacter* positive. In those trials, the *Campylobacter*-positive flock was processed first to evaluate if strains from the *Campylobacter*-positive flock contaminated the subsequent negative flocks. PFGE analysis of *Campylobacter* isolates collected from carcasses from flocks that were originally *Campylobacter*-negative showed that the carcasses were indeed contaminated with the isolates from *Campylobacter*-positive flocks (Table 2). Carcasses were positive for both prechilled and postchilled samples (Fig. 2).

In three other trials (I, IV, and VII), one flock per trial was *Campylobacter* negative. The negative flock was processed first on each processing day, and the *Campylobacter*-positive flocks were processed subsequently. The carcasses

from negative birds remained negative throughout processing, while the carcasses from the first *Campylobacter*-positive flock that was processed immediately after the *Campylobacter*-negative flock were positive for *Campylobacter* (Table 2). In trial II, all three farms were positive for *Campylobacter*. During processing, flock 1, with lower *Campylobacter* contamination, was processed first at the starting or processing. The prechilled and postchilled carcass samples from that flock were negative for *Campylobacter* (Table 2). PCR and PFGE analyses also showed that *C. jejuni* and *C. coli* and/or more than one isolate from each species could be cultured from one flock, or from carcass samples belonging to one flock.

DISCUSSION

Campylobacter spp. are present in the intestinal tract of birds at high numbers, which allows for a direct counting using serial dilution of fecal material and direct plating on selective agar plates. It is known that the main source of contamination for broiler flocks is related to horizontal transmission during the rearing of the broilers, and farms with positive flocks are exposed to the presence of *Campylobacter* spp. in the surrounding environment (10). There

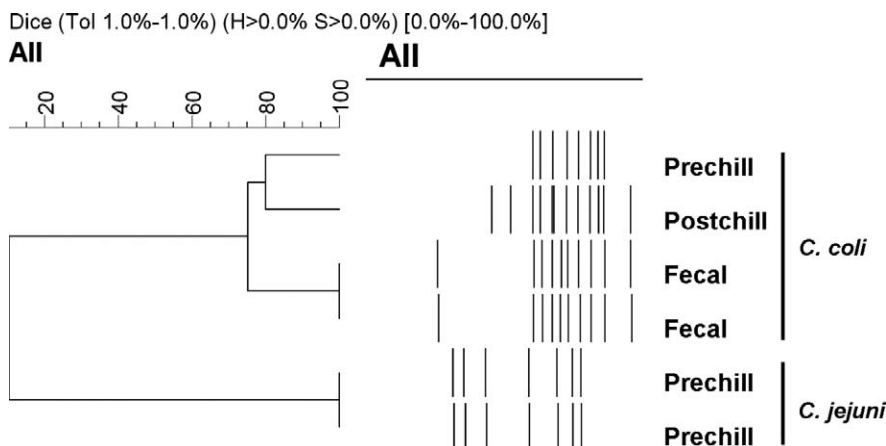
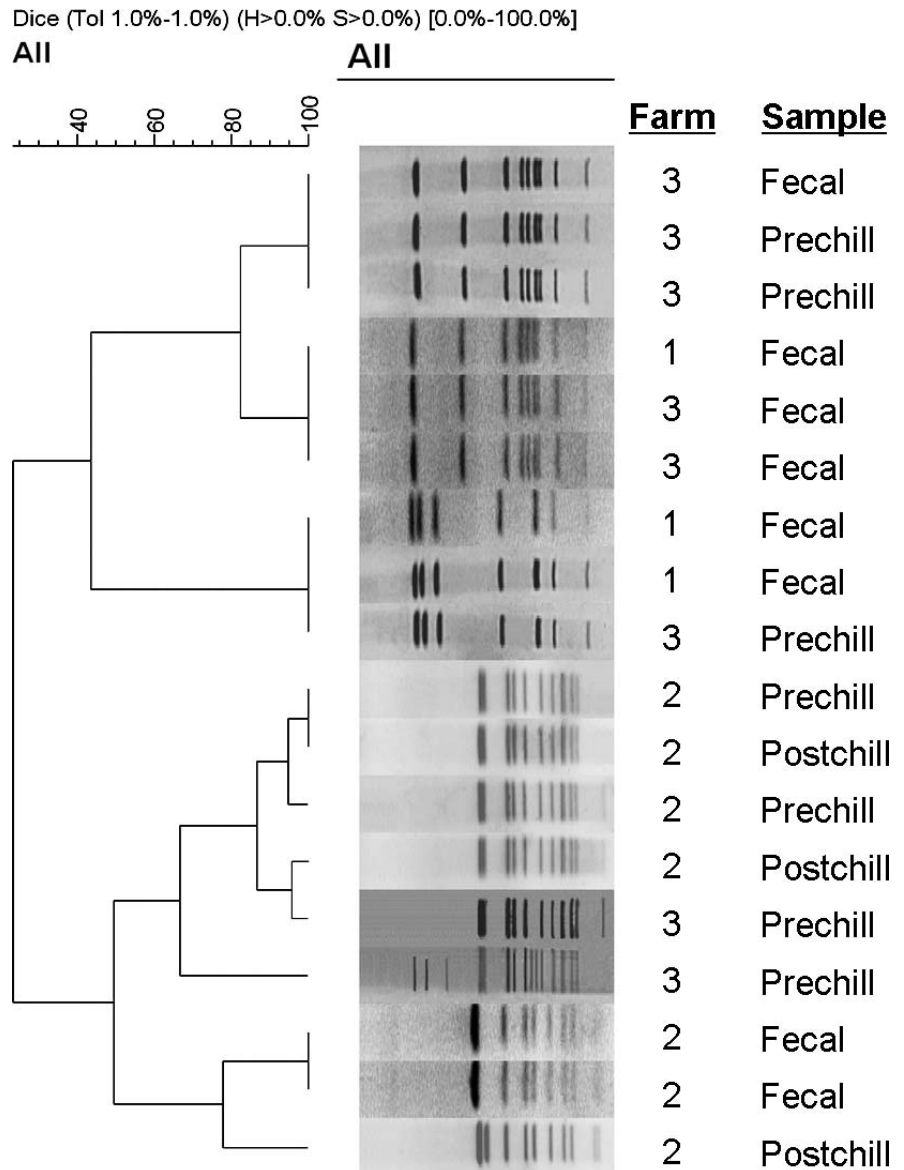


FIGURE 1. PFGE profiles of *C. jejuni* and *C. coli* isolates collected from the same farm. The DNA percentage relatedness was calculated using the UPGMA analysis by BioNumerics.

FIGURE 2. Diversity of PFGE profiles of *C. jejuni* isolates collected from three farms that were processed on the same day. The DNA percentage relatedness was calculated using the UPGMA analysis by BioNumerics.



is a positive correlation between the contamination of live broilers at the end of rearing and the contamination of the carcasses after processing (10). Our results suggest that a logistic scheduling system may reduce the cross-contamination of *Campylobacter*-negative flocks during processing. *Campylobacter*-negative flocks processed after *Campylobacter*-positive flocks become contaminated with *Campylobacter* strains originating from previously processed *Campylobacter*-positive flocks. The analysis of the restriction endonuclease profiles of the isolates using PFGE, a powerful technique for fingerprinting, helped us infer the origin of the contaminating strains.

A previous study has found that sampling four birds from each quarter of the broiler house (total = 16 birds) allows for more than 97% probability of detecting *Campylobacter* infection in a flock containing at least 5% infected birds (7). Therefore, our protocol based on the collection of 10 random samples in a house appears to be satisfactory to detect the presence of *Campylobacter* in a broiler house. The number of cells enumerated per gram of fecal material in our study correlated to previous reports on

the carriage of *Campylobacter* in the intestine of broiler chickens (20). Once *Campylobacter* has colonized the intestinal tract of chickens, it will remain in the intestine at high numbers. Flocks that were negative on direct plating were also negative after enrichment. If a low number of cells are carried by some birds, then our current methodology may not be able to detect them. Yet, the food safety threat of such a low number of *Campylobacter* cells in the intestine of broiler chickens may be arguable.

Surprisingly, the number of negative flocks found in our study was lower than it was in previous surveys of commercial broiler flocks in the United States (28) and the United Kingdom (8). In another study, Herman et al. (10) found that only 39% of the flocks tested ($n = 18$) were *Campylobacter* positive at the end of the rearing period, and that six *Campylobacter*-negative flocks yielded contaminated carcasses due to contamination that may have occurred during transportation and/or during processing. Under current commercial processing activities, it is extremely difficult to avoid contamination of carcasses when *Campylobacter*-positive birds are randomly delivered (10).

It has been suggested that efforts within the slaughterhouse to improve hygiene to reduce *Campylobacter* contamination may have a limited effect on the risk to consumers (16), and that logistic processing brings little change in the calculated risk for consumers (9). These conclusions have an underlying assumption that most of the flocks arriving at the processing plant are contaminated with *Campylobacter*. However, we found that the actual number of commercial broiler flocks that are *Campylobacter* negative is higher than expected, and therefore a logistic processing may have a larger impact in preserving the negative status of these flocks throughout processing. The differences in the counts postchill clearly exemplify the variability found in the management of the chiller tank in commercial processing plant. Although the reduction of *Campylobacter* was in the range of commercial production systems (22), the reduction in the number of *Campylobacter* counts in company A showed that the correct managing of key processing steps impacts the number of *Campylobacter* spp. in the final product, and that those key steps should not be underestimated when performing risk assessment studies (9). Keeping an effective chlorine concentration and low temperature on a continuous basis in the chiller tank is challenging in broiler processing plants. Indeed, these differences among companies should be further explored to determine the actual impact on *Campylobacter* reduction by a well-managed chill tank.

It is known that *C. jejuni* subtypes found in broiler chickens, cattle, and milk are represented within the domestically acquired cases of campylobacteriosis, an indication that *C. jejuni* from these reservoirs are likely sources of human infections (21). In addition, we know that multiple genotypes of *C. jejuni* colonize commercial broiler chickens, and more than one genotype may be found in the gastrointestinal tracts of an individual bird (11). In general, we found that one particular genotype was dominant in a given flock, and that the same genotype was the most frequently isolated one from carcasses during processing. These results are similar to the situation in Germany, where the clone that is most frequently found in primary production is also found during processing (13). In Swedish chicken carcasses, an average of 1.5 genotypes have been found per carcass, with genotypes present on carcasses that were, in some cases, not found in the corresponding cloacal samples but were present in cloacal samples of the preceding slaughter group (14).

In our study, PFGE restriction profiles of isolates collected from *Campylobacter*-negative flocks matched the PFGE profiles of previously processed, *Campylobacter*-positive carcasses. This is a strong association of a contamination that may have occurred during processing. We do not know if both positive and negative flocks were transported to the processing plant on the same truck. However, the high number of cells that were counted on the negative flock implied that an actual contamination of the equipment during processing may be responsible for these results.

Carcass contamination of *Campylobacter* during processing ultimately results in the contamination of end products, a serious hazard for public health. This study showed

that *Campylobacter*-negative flocks can acquire *Campylobacter* strains from previously processed *Campylobacter*-positive flocks, and that a logistic processing system is a relatively simple system to implement to guarantee the preservation of the negative status of the flocks. The simplicity of the system can be further improved by reducing to a minimum the number of samples collected from live birds before the actual processing day. Most of the processing facilities in the southeastern United States process boilers from an average of two to four farms per day. Therefore, our calculation based on the sampling of three farms shows that negative flocks are more frequent than expected.

In addition to efficient cleaning and disinfection and the implementation of various reduction strategies, logistic processing can also be employed by processors to avoid the contamination of *Campylobacter*-free carcasses. Preserving the *Campylobacter*-negative status of a flock may not be a high priority in current poultry operations, but the actual cost of decontamination strategies may be higher and less efficient than the implementation of simple logistic processing.

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