Journal of Food Protection, Vol. 79, No. 6, 2016, Pages 896–901
doi:10.4315/0362-028X.JFP-15-486

Transfer of *Campylobacter* from a Positive Batch to Broiler Carcasses of a Subsequently Slaughtered Negative Batch: A Quantitative Approach

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MS 15-486: Received 27 October 2015/Accepted 31 January 2016

ABSTRACT

The present study was conducted to quantify *Campylobacter* cross-contamination from a positive batch of broiler chicken carcasses to a negative batch at selected processing steps and to evaluate the duration of this cross-contamination. During each of nine visits conducted in three broiler slaughterhouses, *Campylobacter* levels were determined on broiler carcasses originating from *Campylobacter*-negative batches processed immediately after *Campylobacter*-positive batches. Data were collected after four steps during the slaughter process (scalding, plucking, evisceration, and washing) at 1, 10, and 20 min after the start of the slaughter of the batches. *Campylobacter* levels in ceca of birds from *Campylobacter*-positive batches ranged from 5.62 to 9.82 log CFU/g. When the preceding positive batch was colonized at a low level, no (enumerable) carcass contamination was found in a subsequent negative batch. However, when *Campylobacter* levels were high in the positive batch, *Campylobacter* was found on carcasses of the subsequent negative batch but at levels significantly lower than those found on carcasses from the preceding positive batch. The scalding and the evisceration process contributed the least (<1.5 log CFU/g) and the most (up to 4 log CFU/g), respectively, to the *Campylobacter* transmission from a positive batch to a negative batch. Additionally, the number of *Campylobacter* cells transferred from positive to negative batches decreased over the first 20 min of sampling time. However, the reduction was slower than previously estimated in risk assessment studies, suggesting that pathogen transfer during cross-contamination is a complex process.

Key words: *Campylobacter*; Cross-contamination; Poultry; Slaughterhouse; Transfer

Campylobacter is considered an important cause of bacterial zoonotic infections in humans worldwide *(29)*. In the European Union (EU), the number of reported human campylobacteriosis cases exceeded 200,000 in 2013 *(11)*, but the true infection rate for all EU member states might be even 46 times higher *(14)*. The *Campylobacter* reservoir is warm-blooded animals, including broiler chickens, which can be colonized by *Campylobacter* at more than 10^8 CFU/g in their ceca at the end of the rearing period *(13, 25)*. Slaughter of *Campylobacter*-positive batches of broilers results in contamination of carcasses *(2, 4, 7, 8, 22–24)* and of the slaughterhouse environment *(16, 21)*. During the slaughter of subsequent broiler batches, transmission of *Campylobacter* can occur; thus, *Campylobacter* carcass contamination can be influenced by the *Campylobacter* status of the previously processed batch of carcasses.

Cross-contamination is of particular concern when *Campylobacter*-negative batches are processed immediately after colonized batches. To avoid contamination of negative carcasses via the slaughterhouse environment, logistic slaughter (i.e., the slaughter of *Campylobacter*-positive batches at the end of the day after batches that have tested negative) has been proposed *(10)*. However, based on quantitative risk assessment models, the public health benefit of logistic slaughter has been estimated as minimal *(12, 19)*. This estimation was supported by an analysis of carcass contamination after chilling, which revealed that limited transmission of the pathogen occurred from a positive to a subsequent negative batch *(15)*. However, in a more recent study *(8)* carcasses from a *Campylobacter*-negative batch processed immediately after a *Campylobacter*-positive batch became contaminated at levels similar to those found on carcasses in the positive batch. As suggested by *Salmonella* transfer during the grinding process, a tailing phenomenon of cross-contamination can occur *(17)*. These findings indicate that the significance of cross-contamination

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TABLE 1. *Differences between three slaughterhouses (A through C)*

Process parameter	A	B	C
Line speed	11,000	12,000	6,000
Unloading system	Drawers	Containers	Containers
Stunning method	Electrical	Gas	Electrical
Scalding water temp (°C)	52–53	54	50.5–52.5
Scalding time (s)	150	145	210
Plucking time (s)	35	42	60
Ruptured intestines after evisceration (%)	15	7	44

from *Campylobacter*-positive to *Campylobacter*-negative batches of broilers should be revaluated. Because probably not all processing stages contribute equally to cross-contamination with regard to the actual level of *Campylobacter* being transferred between batches, those steps during which the transfer of *Campylobacter* numbers is most likely should be identified. Such knowledge can lead to dedicated interventions at defined processing steps, resulting in reduced cross-contamination of *Campylobacter*-negative batches. The aim of the present study was to quantify *Campylobacter* cross-contamination from a positive to a negative batch of broilers at selected processing steps.

MATERIALS AND METHODS

Sample collection. This study was conducted in three Belgian slaughterhouses (A, B, and C). The main differences between these slaughterhouses are summarized in Table 1, although the impact of these differences on *Campylobacter* contamination levels was not addressed in this study. Each slaughterhouse was visited three times, and samples were collected from a *Campylobacter*-positive batch and a subsequently processed *Campylobacter*-negative batch. During each visit, six intestinal packages from each of the first four batches of the process day were collected directly after evisceration to quantify the cecal colonization level. From the second, third, and fourth batches, two carcasses were removed from the line after each of four slaughter operation steps (scalding, plucking, evisceration, and washing) at 1, 10, and 20 min after the first carcass of the respective batch. In slaughterhouse B, practical limitations hampered the sampling of carcasses after scalding. All samples were collected aseptically, placed in sterile plastic bags, stored at 8°C during the slaughterhouse visit, and transported to the laboratory under cooled conditions. Intestinal packages were analyzed on the sampling day, whereas carcass samples were stored overnight at 2°C until analysis.

***Campylobacter* status of sample batches.** Cecal contents from the first batch of the processing day were analyzed as a pooled sample, but the cecal contents from the second, third, and fourth batches were analyzed separately. Cecal contents were spread on modified cefaperazone-charcoal-desoxycholate agar (mCCDA; Oxoid, Basingstoke, England) to evaluate the *Campylobacter* status of the sampled batches. Plates were incubated under microaerobic conditions at 41.5°C for 24 h, and the identity of presumptive *Campylobacter* colonies was confirmed by Gram staining and microscopic observation. This analysis allowed the selection of two subsequently processed batches per visit: the first batch was *Campylobacter* positive (i.e., *Campylobacter* was recovered on mCCDA plates) and the second batch was *Campylobacter* negative (i.e., *Campylobacter* was not detected on mCCDA plates).

Enumeration of *Campylobacter* in cecal contents. Approximately 1 g of the contents of each cecum was aseptically collected and homogenized with 0.1% peptone water (Bio-Rad Laboratories, Hercules, CA) at a ratio of 1:10. Homogenates were plated on Campy Food agar (bioMérieux, Marcy-l'Étoile, France) and incubated under microaerobic conditions at 41.5°C for 48 h. After incubation, colonies with typical *Campylobacter* morphology were counted, and at least four presumptive *Campylobacter* colonies per sample were confirmed by PCR assay *(27)*.

Enumeration of *Campylobacter* on carcass samples. Carcass samples from two subsequently processed batches were selected for analysis. From each carcass, approximately 10 g of breast skin was removed as the sample *(3)*. For carcasses after scalding, feathers were aseptically removed and only breast skin was analyzed. *Campylobacter* was enumerated in carcass samples with the same methodology used for cecal contents.

Data analysis. All *Campylobacter* counts were expressed as CFU per gram of breast skin or cecal contents. The analyses were conducted using negative binomial regression in STATA SE/13.0 (StataCorp, College Station, TX) with a significance level of 5%. Both slaughterhouse and batch were initially included in a multilevel mixed-effects model. Because the multilevel model was not significantly different from the model including batch only, the simplest model (with batch as a random effect, if applicable) was retained. Bonferroni adjustments were applied for multiple testing. Differences in *Campylobacter* counts between subsequently slaughtered batches were evaluated for each sampling site. Differences in *Campylobacter* counts on carcasses collected at different times (1, 10, and 20 min) at each sampling site also were evaluated. *Campylobacter* counts at different sampling sites on the carcasses also were compared. Numerical results were log transformed for descriptive statistics.

RESULTS

Our study presents results from nine visits during which a *Campylobacter*-positive batch of broilers was slaughtered directly before a *Campylobacter*-negative batch. The average cecal level in *Campylobacter*-positive batches was 5.62 to 9.82 log CFU/g (Table 2). The slaughter of these batches resulted in quantifiable *Campylobacter* contamination in carcasses along the processing line (Table 2).

Campylobacter contamination was detected in carcasses from *Campylobacter*-negative batches that were processed immediately after *Campylobacter*-positive batches (Table 3). However, *Campylobacter* counts on carcass samples collected during the slaughter of *Campylobacter*-negative batches were significantly lower ($P < 0.05$) than those from the previously processed positive batches at all sampling sites (Fig. 1). With regard to sampling site, *Campylobacter* counts on carcasses from both *Campylobacter*-positive and -negative batches were lowest after scalding and highest after evisceration (Fig. 1).

Enumeration of *Campylobacter* on carcasses collected at defined time points (1, 10, and 20 min) from the start of processing of a *Campylobacter*-negative batch revealed a decreasing trend in counts over time when a negative

TABLE 2. Campylobacter *counts in broiler cecal contents and on carcass breast skin samples collected from* Campylobacter-*positive batches*

		Mean (SD) *Campylobacter* count (log CFU/g)				
			Carcass			
Slaughterhouse	Visit no.	Cecum	After scalding	After plucking	After evisceration	After washing
A	1	8.90 (1.03)	ND[a]	3.23 (0.18)	4.13 (0.32)	3.07 (0.46)
	2	8.73[b]	NC[c]	NC	NC	NC
	3	5.62 (0.89)	ND	2.01 (0.48)	2.75 (0.75)	2.07 (0.36)
B	4	9.12 (0.69)	NC	2.95 (0.26)	3.76 (0.41)	3.69 (0.47)
	5	8.39 (0.34)	NC	2.28 (0.55)	3.98 (1.22)	2.62 (0.91)
	6	8.67 (0.30)	NC	2.09 (0.36)	2.51 (0.72)	2.21 (0.68)
C	7	8.83 (0.86)	2.60 (1.14)	3.36 (0.60)	4.29 (0.42)	4.26 (0.43)
	8	7.90[b]	NC	NC	NC	NC
	9	9.82[b]	NC	NC	NC	NC

[a] ND, not detected (enumeration limit = 10 CFU/g).
[b] Pooled sample.
[c] NC, no samples collected. Practical limitation hampered carcass collection after scalding in slaughterhouse B. Carcass samples were not collected from the first batch of the processing day.

batch was processed after a positive batch (Table 3). After plucking, evisceration, and washing, *Campylobacter* counts were significantly higher ($P < 0.05$) on carcasses collected during the minute 1 than those on carcasses collected at later time points (10 and 20 min; Fig. 2). However, no significant differences in *Campylobacter* counts were observed between carcasses from 10 and 20 min (Fig. 2).

TABLE 3. Campylobacter *counts on broiler carcass breast skin samples collected from* Campylobacter-*negative batches processed immediately after* Campylobacter-*positive batches*

			Mean (SD) *Campylobacter* count (log CFU/g)			
Slaughterhouse	Visit no.	Time (min)	After scalding	After plucking	After evisceration	After washing
A	1	1	ND[a]	2.73 (0.06)	3.66 (0.14)	ND
		10	ND	1.95 (0.07)	2.91 (0.46)	1.35 (0.92)
		20	ND	1.27 (0.81)	1.99 (0.06)	1.60 (0.05)
	2	1	ND	1.00 (0.43)	2.68 (0.79)	1.69 (0.12)
		10	ND	ND	2.33 (0.40)	1.42 (0.60)
		20	ND	ND	1.77 (0.66)	0.85 (0.21)
	3	1	ND	ND	ND	ND
		10	ND	ND	ND	ND
		20	ND	ND	ND	ND
B	4	1	NC[b]	1.30 (0.24)	4.05 (0.75)	2.83 (0.18)
		10	NC	1.15 (0.64)	2.38 (0.05)	2.06 (0.51)
		20	NC	1.62 (0.87)	1.39 (0.12)	1.48 (0.03)
	5	1	NC	1.15 (0.21)	2.49 (0.58)	2.44 (0.09)
		10	NC	ND	1.20 (0.71)	1.24 (0.76)
		20	NC	ND	1.23 (0.34)	1.00 (0.43)
	6	1	NC	1.08 (0.55)	2.08 (0.34)	2.05 (0.49)
		10	NC	1.15 (0.21)	1.60 (0.03)	1.00 (0.43)
		20	NC	ND	1.57 (0.38)	0.85 (0.21)
C	7	1	ND	1.96 (0.16)	3.45 (0.24)	2.69 (0.09)
		10	ND	1.94 (0.34)	2.54 (0.10)	1.96 (0.38)
		20	ND	1.63 (0.21)	2.46 (0.06)	2.02 (0.03)
	8	1	ND	1.74 (0.62)	2.39 (0.49)	1.07 (0.12)
		10	ND	ND	1.76 (0.40)	ND
		20	ND	0.85 (0.21)	0.85 (0.21)	ND
	9	1	0.85 (0.21)	2.08 (0.05)	3.02 (0.35)	2.02 (0.25)
		10	1.09 (0.55)	2.01 (0.15)	2.15 (0.21)	1.66 (0.26)
		20	ND	1.20 (0.70)	2.57 (0.14)	1.50 (0.28)

[a] ND, not detected (enumeration limit = 10 CFU/g).
[b] NC, no samples collected. Practical limitation hampered carcass collection after scalding.

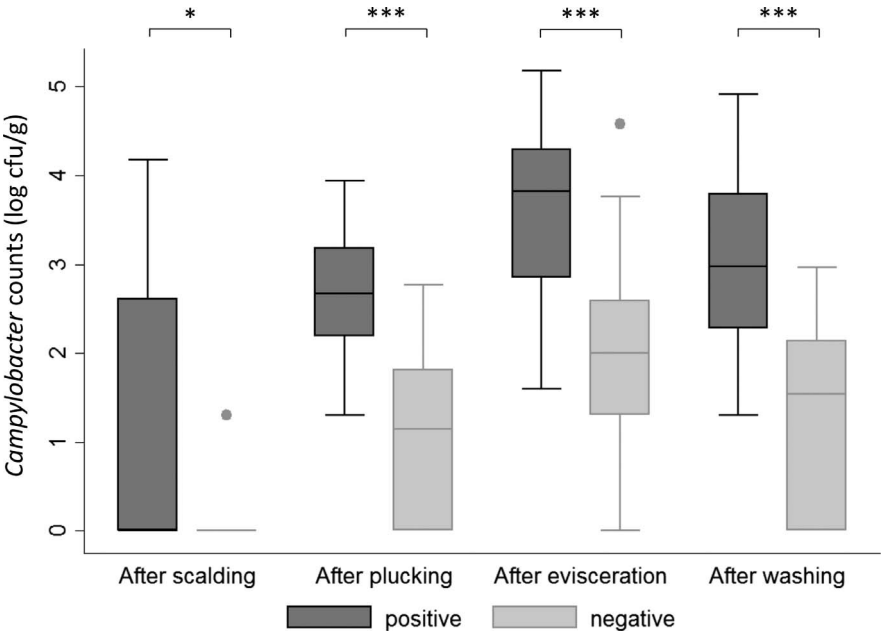

FIGURE 1. Campylobacter *counts on broiler carcass breast skin samples collected during various processing steps from* Campylobacter-*positive broiler batches and after processing of* Campylobacter-*negative batches. Asterisks indicate significant differences (*$P < 0.05$; ***$P < 0.001$).*

When a *Campylobacter*-positive batch with a low levels was slaughtered (slaughterhouse A, visit 3), *Campylobacter* was not detected at levels exceeding 10 CFU/g on carcasses collected from the subsequently processed *Campylobacter*-negative batch (Table 3).

DISCUSSION

The *Campylobacter* prevalence in broiler chickens in Belgium has been estimated at approximately 30% *(9)*. Therefore, the probability of slaughtering a *Campylobacter*-negative batch immediately after a *Campylobacter*-positive batch is relatively high, which results in frequent cross-contamination between these positive carcasses and those of negative batches *(6)*. Because the within-flock *Campylobacter* prevalence and level in positive flocks is high *(13, 25)*, we considered a broiler batch as negative when *Campylobacter* was not detected using the direct plating method (cecal content limit of 100 CFU/g) in any of six cecal content samples or in the pooled cecal sample.

Currently, the relationship between *Campylobacter* levels on broiler meat and the health risk for consumers is established *(5, 20)*. In the present study, *Campylobacter*

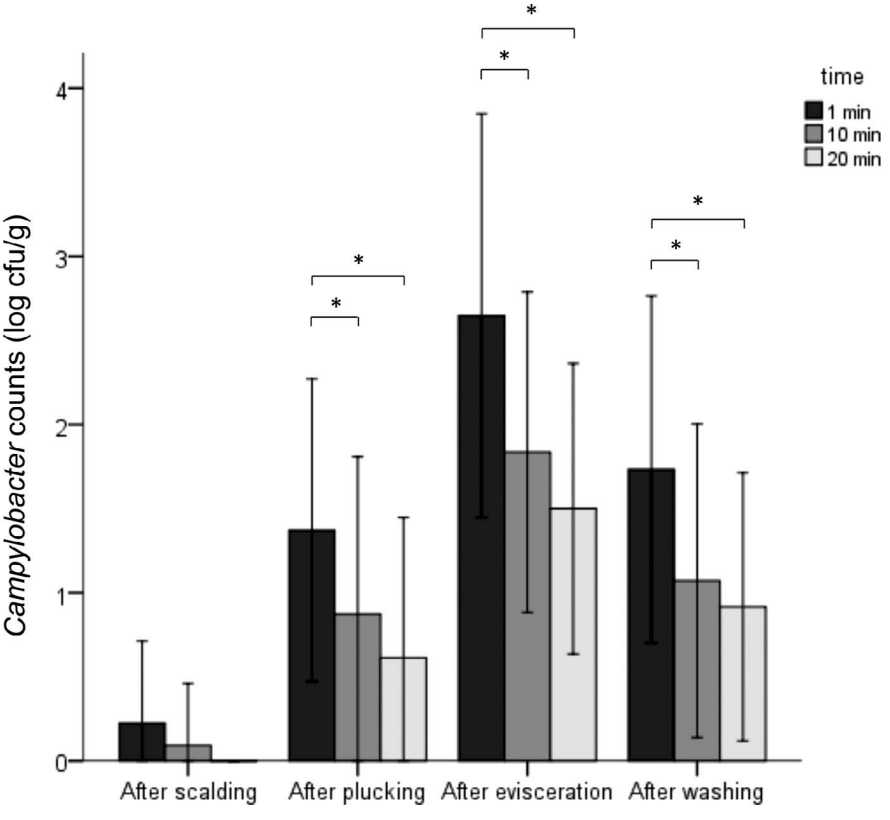

FIGURE 2. Campylobacter *counts on broiler carcass breast skin samples collected during various processing steps at 1, 10, and 20 min after the start of the slaughter of* Campylobacter-*negative batches processed directly after* Campylobacter-*positive batches. Asterisks indicate significant differences (*$P < 0.05$).

cross-contamination between positive and successively slaughtered negative batches was assessed quantitatively. The lowest *Campylobacter* cross-contamination between batches occurred during scalding, which can be explained by the rapid reduction of *Campylobacter* due to the temperature of the scalding water (50 to 54°C; *(30)*) and a simple dilution effect (counter-flow scalding tanks in the slaughterhouses). However, the highest *Campylobacter* cross-contamination occurred through the evisceration process. During the processing of *Campylobacter*-positive batches, the evisceration equipment may get highly contaminated due to leakage of the intestinal content, resulting in extensive cross-contamination of *Campylobacter*-negative carcasses passing through the evisceration machine.

To avoid cross-contamination from *Campylobacter*-positive to -negative batches, logistic slaughter has been suggested as a possible intervention strategy *(10)*. In our study, *Campylobacter* counts were lower on carcasses from negative batches than on those from positive batches and declined over time when negative batches were processed. This general trend agrees with the observations made by Elvers et al. *(8)* and Johannessen et al. *(15)* about *Campylobacter* cross-contamination between positive and negative broiler batches. However, the generalized concept that a limited number of carcasses from a negative batch became contaminated by *Campylobacter* from preceding positive broilers *(10, 15, 18)* is debatable. In the present study, during 20 min of operation, approximately 3,500, 4,000, and 2,000 carcasses from consecutive *Campylobacter*-negative batches passed the slaughter line in slaughterhouses A, B, and C, respectively. However, on average, *Campylobacter* levels decreased only to 1.60, 1.48, and 2.02 log CFU/g on carcasses after washing in slaughterhouses A, B, and C, respectively. The observed slow decline in *Campylobacter* transfer over time might be explained by the distinct tailing phenomenon during cross-contamination observed previously for other pathogens *(1, 17, 26, 28)*.

In addition to logistic slaughter, interventions aiming at reducing *Campylobacter* counts in cecal contents may decrease the level of *Campylobacter* transmission from a positive to a subsequently processed negative batch. In the present study (slaughterhouse A, visit 3), when the *Campylobacter* level in the cecal content of a positive batch was low (5.62 ± 0.89 log CFU/g), cross-contamination to the subsequently processed *Campylobacter*-negative broilers was limited.

In conclusion, carcasses from *Campylobacter*-negative batches can become contaminated when they are processed immediately after *Campylobacter*-positive birds. This cross-contamination decreases over time but is slower than was previously estimated. Consequently, risk assessment models should consider the observed prolonged occurrence of cross-contamination to facilitate realistic risk predictions. *Campylobacter* counts on carcasses from negative batches also are influenced by the levels in previously slaughtered broilers, and the evisceration step is where most cross-contamination occurs between *Campylobacter*-positive and *Campylobacter*-negative broiler batches.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the slaughterhouses for their kind cooperation. We also thank Carine Van Lancker, Martine Boonaert, Sandra Vangeenberghe, Jeroen Vandenheuvel, Tommy Van Limbergen, and Cinzya De Frenne for their assistance in media preparation, sample collection, and sample analysis. This study was funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment (contract RF 11/6241 CAMPYTRACE).

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