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Research Paper

Salmonella Contamination of Broiler Chicken Carcasses at Critical Steps of the Slaughter Process and in the Environment of Two Slaughter Plants: Prevalence, Genetic Profiles, and Association with the Final Carcass Status

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ABSTRACT

Salmonella is a foodborne pathogen commonly associated with poultry products. The aims of this work were to (i) estimate the impact of critical steps of the slaughter process on *Salmonella* detection from broiler chicken carcasses in two commercial poultry slaughter plants in Quebec, Canada; (ii) investigate the presence of *Salmonella* in the slaughter plant environment; (iii) describe, using a high-resolution melting (HRM) approach, the HRM *Salmonella* profiles and serotypes present on carcasses and in the slaughter plant environment; and (iv) evaluate whether the HRM flock status after chilling could be predicted by the flock status at previous steps of the slaughter process, the status of previous flocks, or the status of the processing environment, for the same HRM profile. Eight visits were conducted in each slaughter plant over a 6-month period. In total, 379 carcass rinsates from 79 flocks were collected at five critical steps of the slaughter process. Environmental samples were also collected from seven critical sites in each slaughter plant. The bleeding step was the most contaminated, with >92% positive carcasses. A decrease of the contamination along the slaughtering process was noted, with carcasses sampled after dry-air chilling showing ≤2.5% *Salmonella* prevalence. The most frequently isolated serotypes were *Salmonella* Heidelberg, Kentucky, and Schwarzengrund. The detection of the *Salmonella* Heidelberg 1-1-1 HRM profile on carcasses after chilling was significantly associated with its detection at previous steps of the slaughter process and in previously slaughtered flocks from other farms during a same sampling day. Results highlight the importance of the chilling step in the control of *Salmonella* on broiler chicken carcasses and the need to further describe and compare the competitive advantage of *Salmonella* serotypes to survive processing. The current study also illustrates the usefulness of HRM typing in investigating *Salmonella* contamination along the slaughter process.

HIGHLIGHTS

- *Salmonella* contamination of chicken carcasses was the highest after bleeding.
- Most frequent *Salmonella* serotypes were Heidelberg, Kentucky, and Schwarzengrund.
- Carcass status after chilling is associated with flock status at previous steps.
- Results support chilling as being critical in the control of *Salmonella*.

Key words: Carcass rinsate; High-resolution melting; Poultry slaughter plant; Production environment; *Salmonella*

Salmonella enterica is an important zoonotic pathogen, having a significant economic and health impact on animals and humans worldwide *(3)*. In Canada, *Salmonella* remains one of the most burdensome foodborne pathogens, with more than 17.3 cases of salmonellosis per 100,000 inhabitants reported each year, with the number of affected people probably being up to 30 times higher due to underdiagnosis or underreporting *(18, 42)*. More than 2,600 *Salmonella* serotypes have been identified, but a limited number of these serotypes are commonly linked to foodborne diseases in humans *(38)*. Clinical manifestations of salmonellosis are usually characterized by fever, abdominal pain, nausea, and vomiting. Young children, the elderly, and immunocompromised people are at increased risk of complications from the infection *(3)*.

The intestinal tract of farm animals represents the main habitat of zoonotic *Salmonella*. Various sources, namely

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feedstuff, rodents, litter, and visitors, can all contribute to the introduction of the pathogen into farming environments, probably explaining the high prevalence (44 to 59% depending on the year) of contaminated preharvest chicken farms reported in Canada *(22, 24)*. Transmission of the pathogen to humans is most often occurring through the ingestion of contaminated foods, with meat, eggs, dairy products, and vegetables identified as the main contributing vehicles *(24)*. Among foods of animal origin, poultry meat is recognized as a main source of human exposure to *Salmonella (3)*. Several studies have reported that live birds originating from *Salmonella*-positive flocks are responsible for the introduction of the pathogen into poultry processing plants; the introduction can occur through plumage contamination or via damage to the gut during the slaughter activities, resulting in a leakage of *Salmonella*-contaminated intestinal content *(34, 49)*. Although a limited number of *Salmonella* serotypes have been isolated from poultry, different prevalences and within-serotype diversities have been observed between geographic regions and farms *(2, 27, 38)*.

The identification of critical control points to manage the microbiological risk in poultry slaughter plants is a key element of the slaughter process *(49)*. A critical control point is defined as a step at which control measures can be applied to prevent or to control a hazard throughout the operations. Food processors have largely relied on the application of this approach to manage meat product contamination *(9)*. The monitoring of *Salmonella* on broiler chicken carcasses at different steps along the slaughter line by using a carcass rinse approach has helped identifying critical control points impacting the meat product contamination *(4, 25, 26, 39, 49)*.

Few studies have described the dynamics of broiler chicken contamination by *Salmonella* serotypes along the poultry production chain and the influence of critical steps of the poultry slaughter process, from the incoming live birds to the processed carcasses, on these dynamics *(25, 31, 47)*. Although rapid detection and differentiation of *Salmonella* serotypes still represent a challenge for the food industry, recent advances in molecular methods have made the detection of *Salmonella* more accurate and convenient *(48)*. Many typing approaches used for the differentiation of *Salmonella* based on genomic characteristics are proposed *(5, 7, 19, 25, 40, 51, 52)*. The high-resolution melting (HRM)–based *Salmonella* genotyping method is rapid, robust, easy to interpret, and affordable compared with other approaches such as serotyping, pulsed-field gel electrophoresis, and whole genome sequencing *(7)*. Compared with a complete serotyping method, the greater discriminatory power of the HRM approach allows for the generation of subtypes among the serotypes identified. To our knowledge, no study using an HRM approach and aiming at understanding the distribution of *Salmonella* on broiler chicken carcasses along the slaughter process in commercial poultry processing plants has been conducted.

Therefore, the objectives of the present study were to (i) estimate the impact of five critical steps of the slaughter process on *Salmonella* detection from broiler chicken carcasses in two commercial poultry slaughter plants (abattoirs 1 and 2) in Quebec, Canada; (ii) investigate the presence of the pathogen in the environment of the two surveyed plants; (iii) describe the HRM *Salmonella* profiles and serotypes present on broiler chicken carcasses and in the slaughter plant environment; and (iv) evaluate whether, at the flock level, *Salmonella* carcass contamination detected at previous steps of the slaughter process, in the previously slaughtered flock, and/or in the slaughter plant environment can be predictive of the final meat product contamination by specific *Salmonella* HRM profiles after chilling.

MATERIALS AND METHODS

Broiler chicken flock selection. Sampling for this study was carried out in two different commercial poultry processing plants in the province of Québec, Canada. Each plant was visited eight times between February and July 2017. The characteristics of these surveyed abattoirs are presented in Table 1. For both slaughter plants, sampling visits were scheduled according to the number and origin of flocks slaughtered per day, to ensure that at least five broiler chicken flocks, all from different farms, were scheduled for slaughter at the beginning of the first shift of the day, after completion of the sanitation procedures. For each plant visit, these first five flocks slaughtered during a sampling day were sampled.

Carcass sampling. For each sampled flock, one carcass was sampled at each of five critical (C) steps identified as C1, after bleeding, with the feathers still attached to the carcass; C2, at time of transfer between the live receiving and the evisceration departments, before evisceration; C3, before chilling, after evisceration; C4, after water-immersion chilling; and C5, after dry-air chilling (Fig. 1). For each flock, five carcasses were selected from the last one-third of the slaughtered flock by collecting the first carcass arriving on the chain in front of the collection site once the research team was ready to proceed. Only four carcasses were sampled for flocks from which no air-chilled carcasses were available due to the production requirements of the surveyed slaughter plants.

Each sampled carcass was placed in a sterile plastic bag (Nasco poultry rinse sample bag, Fisher Scientific, Ottawa, Ontario, Canada), and a 550-mL volume of buffered peptone water (Biokar Diagnostic, Beauvais, France) was added. The carcass was vigorously shaken for 1 min before being removed from the bag with an approach preventing contamination of the sample. The remaining volume of rinsate was recovered, placed on ice, transported back to the laboratory, and stored overnight at 4°C. Samples were individually processed the next morning.

Environmental sampling. Environmental (E) samplings were carried out at seven critical sampling locations (CSLs) *(23)*. CSLs were defined as follows: E1, the feather-plucking rubber fingers; E2, the conveyor belt between the live-receiving and the evisceration departments; E3, the eviscerating machine; E4, the floor surface in the evisceration department; E5, the conveyor belt before chilling; E6, the conveyor belt after chilling; and E7, a stainless steel equipment surface in contact with the meat product and located in the cut-up room (Fig. 1). Each of the seven CSLs was sampled twice during a same plant visit: after the sanitation procedures, before the slaughter activities (postsanitation [PS]) and at the end of the work shift (postoperation [PO]), for a total of 14 samples on each of the eight visits in each abattoir.

TABLE 1. *Surveyed slaughter plants' processing characteristics, Quebec, Canada, 2017*

Processing description	Abattoir: 1	Abattoir: 2
Line speed (birds/min)	225	230
No. of scalding tanks	3	2
Minimum scalding temp (°C)	53.33	50
Maximum scalding temp (°C)	57.22	61.7
Scalding time	1 min 30 s	1 min 20 s
Plucking time (s)	35	26
Carcass sanitizer during water-immersion chilling	None	Peracetic acid
Type of immersion water chiller	Not counterflow	Counterflow
Immersion water chiller tank temp (°C)	1–3	1
Time in immersion water chiller	1 h 30 min	1 h 50 min
Carcass sanitizer during dry-air chilling	Cetylpyridinium chloride	Cetylpyridinium chloride
Air temp in dry-air chilling room (°C)	−3 to 2	0.6
Time in dry-air chilling room	1 h 30 min	1 h 47 min
Water turnover (L/min)	35	110

Sterile gauzes moistened in a 10-mL volume of neutralizing buffer (DE neutralising broth, Lab M, Ltd., Heywood, UK) were used to rub a surface (10 by 10 cm) on each CSL. After sampling, swabs were put back into their respective sterile bags, placed on ice, transported back to the laboratory, and stored overnight at 4°C. Samples were individually processed the next morning.

Sample treatment and microbiological analysis. Based on the Canadian Food Inspection Agency and Food Safety and Inspection Service requirements to which exporting Canadian poultry processors need to comply, the carcass rinsate volume submitted to a preenrichment step was limited to 50 mL, which represents 9% of the volume rinsate *(15)*. To do so, from each carcass rinsate, a 200-mL volume was centrifuged for 20 min at 15,000 × *g*. The supernatant was removed, and a volume of 4 mL of buffered peptone water was added to the pellet and vortexed until complete resuspension of the pellet. The isolation protocol for *Salmonella* described by Larivière-Gauthier et al. *(30)* was used, with slight modifications. For each sample, 1 mL of the suspension was distributed into 9 mL of sterile buffered peptone

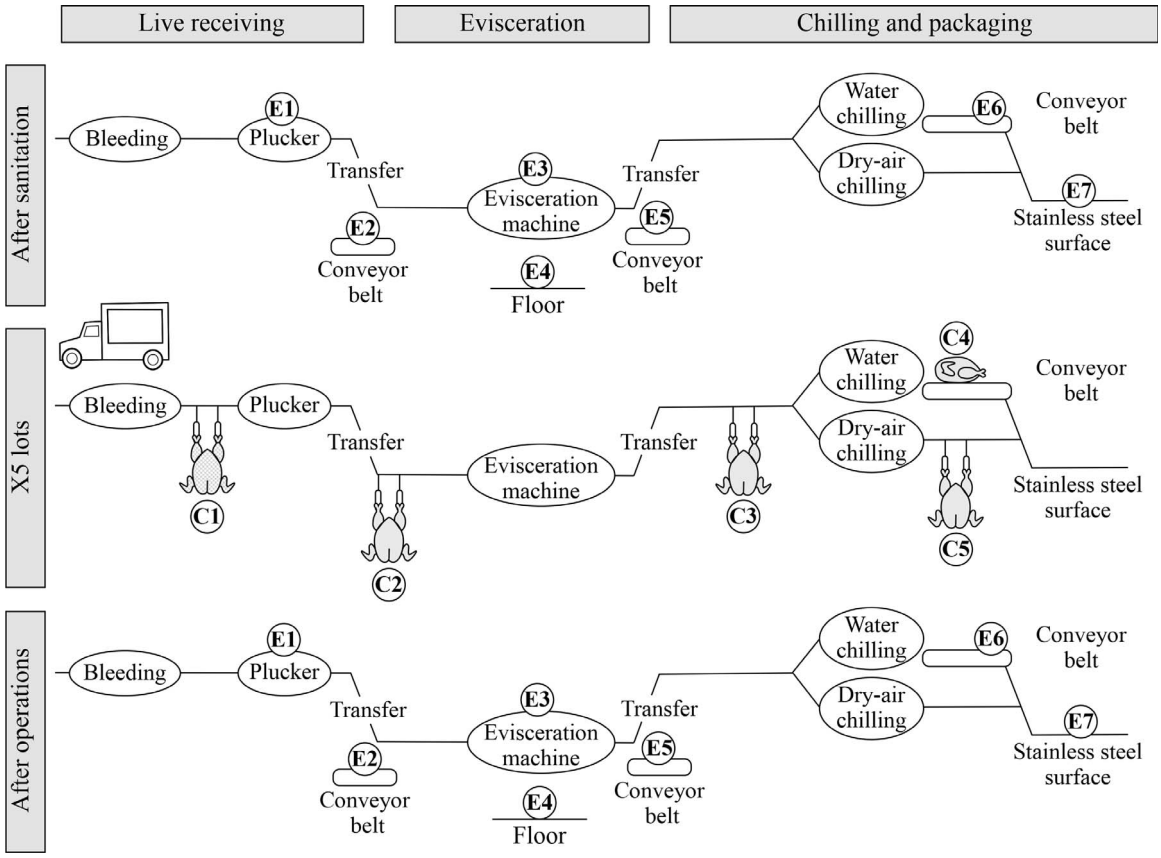

FIGURE 1. *Flowchart illustrating the chicken carcass and environmental sampling on each slaughterhouse visit.*

water before being homogenized. Tubes were incubated at 37°C for 24 h. Three 100-µL equidistant drops of the preenriched culture were inoculated onto the surface of modified semisolid Rappaport-Vassiliadis agar plates (Lab M, Ltd.). Plates were incubated at 42°C for 24 h and then examined for any bacterial growth that was revealed by the development of a white migration zone. When no migration zone was observed, modified semisolid Rappaport-Vassiliadis agar plates were incubated for an additional 24 h and then reassessed. Typical migrations on modified semisolid Rappaport-Vassiliadis medium were subcultured on both brilliant green sulfa (BGS) agar plates (BD, Difco, Franklin Lakes, NJ) and on xylose lysine desoxycholate (XLD) agar plates (Biokar Diagnostics; International Organization for Standardization [ISO] 6579) that were incubated at 37°C for 24 h. When suspect colonies were available on BGS and/or XLD agar plates, one colony per selective agar plate was recovered. Suspect colonies on BGS and XLD plates were confirmed as *Salmonella* by using triple sugar iron agar (Lab M, Ltd.), urea agar (Lab M, Ltd.), followed by sero-agglutination by using *Salmonella* O antiserum Poly A-I C Vi (Statens Serum Institute, Copenhagen, Denmark). Positive cultures were subcultured on sheep blood agar plates (Oxoid, Nepean, Ontario, Canada) and stored at −80°C in a Brucella agar freezing medium (Difco, BD) containing 25% glycerol (Fisher Scientific).

For environmental samples, 20 mL of sterile buffered peptone water was added to each gauze-containing bag. Bags were homogenized for 1 min by using a stomacher and incubated at 37°C for 24 h. The same *Salmonella* detection protocol as described above was applied.

Preparation of genomic DNA. A DNA extraction protocol using a 10% Chelex 100 (Bio-Rad Laboratories, Mississauga, Ontario, Canada) solution in water was applied *(23)*. In brief, three to five colonies of each pure *Salmonella* isolate grown on blood agar plates were suspended in 1 mL of sterile water, vortexed, and centrifuged at 12,000 × *g* for 3 min. After removal of the supernatant, 200 µL of the 10% Chelex 100 solution was added to the bacterial pellet that was vortexed and then incubated in a water bath at 100°C for 20 min. The supernatant (125 µL) was recovered after a second centrifugation at 12,000 × *g* for 3 min, transferred into sterile Eppendorf tubes, and stored at −20°C until used for molecular analysis.

***InvA* PCR-based *Salmonella* confirmation.** The identity of all recovered *Salmonella* isolates was PCR confirmed by amplification of the *invA* gene *(12)*. PCR amplifications were performed using a TProfessional Basic 96 thermocycler (Biometra GmbH, Göttingen, Germany). Each reaction was conducted in a 25-µL volume made of 1 µL of each primer (10 nmol/µL) *(12),* 3 µL of extracted genomic DNA, 2.5 µL of reaction buffer (10× ThermoPol reaction buffer, NEB, Whitby, Ontario, Canada), 2 µL of deoxyribonucleotide triphosphates (10 mM; Bio Basic Inc., Markham, Ontario, Canada), 0.5 µL of *Taq* DNA polymerase (NEB), and 15 µL of sterile water.

Reaction conditions were determined according to the protocol published by Chiu and Ou *(12)*. The PCR program was as follows: an initial denaturation step at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 54.2°C for 30 s, extension at 72°C for 2 min, and final extension step at 72°C for 10 min.

PCR products were visualized and photographed under UV illumination following electrophoresis on a 1% agarose gel containing 0.01% SYBR Safe DNA gel stain (Invitrogen, Burlington, Ontario, Canada). A 100-bp ladder (Track It, Invitrogen) was used as a molecular weight marker.

Real-time PCR and HRM curve analysis. HRM-based genotypic characterization was used on all PCR-confirmed *Salmonella* isolates. DNA templates used for the conduct of the approach were diluted 1:10 in sterile water. Each 20-µL reaction volume contained 12.4 µL of sterile water, 0.8 µL of each primer, 4 µL of EvaGreen Mastermix (Montréal Biotech, Montréal, Quebec, Canada), and 2 µL of *Salmonella* genomic DNA.

Primers and linear normalization regions were selected according to the protocol published by Bratchikov and Mauricas *(7)*. Real-time PCR and HRM curve analysis were performed using a LightCycler 96 real-time PCR thermocycler (Roche Diagnostics, Mannheim, Germany). Three genomic regions were amplified: *CR1, CR2,* and *YohM*.

For each HRM-typed strain, the combined analysis of the three curves generated by the three selected genes was attributed an HRM profile. HRM-typed strains were grouped according to their HRM profile and each profile was subsequently identified as a *Salmonella* serotype after submitting to serotyping at least one strain per HRM profile. Serotyping was performed by the Laboratoire d'épidémiosurveillance du Quebec (Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, Saint-Hyacinthe, Quebec, Canada).

Statistical analysis. Descriptive statistics was used to present the data. A *Salmonella*-positive carcass or environmental sample status was established when a positive culture was obtained on either one or both culture media (BGS and XLD).

Multivariable logistic regressions were used to model the carcass *Salmonella* status (positive or negative) according to the critical step and slaughtering order, conducted separately for each slaughter plant. The sampling day was included in the model as a fixed effect to adjust for potential clustering, and the model was adjusted for repeated measures within flocks. A full model including all variables was built; however, nonstatistically significant variables ($P > 0.05$, type 3 Wald test) were then removed one at a time from the model (backward selection) *(17)*. For statistically significant categorical explanatory variables, pairwise comparisons between categories were performed with *P* values adjusted using the Tukey-Kramer procedure for controlling the overall type 1 error rate at 5%. Odds ratios (ORs) were used to present the results *(17)*.

A logistic regression was used to model *Salmonella* environmental sampling status (positive or negative) according to the type of sampling (PS versus PO), performed separately for each slaughter plant. Because of the limited sample size, no comparison was done between CSLs.

Finally, a logistic regression model was used to predict the flock status (positive or negative) after chilling for specific HRM profiles, that is, detection of a specific profile on carcasses of a same flock sampled at either C4 or C5. One model was built for each profile and slaughter plant, and models were limited to HRM profiles detected after chilling in at least five flocks from a same slaughter plant. Evaluated predictors were the detection of this HRM profile on carcasses from the same flock at each of the previous steps, the detection of this profile in previous flock(s) slaughtered the same day in the same plant, and the detection of this same profile in environmental samples at PS. The model was adjusted for repeated measures within slaughter visit. Because of the limited sample size, only univariable analyses were performed. Odds ratios are used to present the results.

TABLE 2. *Number of carcass rinsate samples and proportion of* Salmonella*-positive carcass rinsate samples at each critical step of the slaughter process for each surveyed slaughter plant, Quebec, Canada, 2017*

			Logistic regression estimate				
Variable	No. of samples	% positive	β	SE (β)	*P* value[a]	OR[b]	95% CI (OR)[a]
Abattoir 1 (200 carcasses from 40 flocks)							
Constant			−3.66	1.01	<0.001		
Critical step[c]							
C1	40	92.5	6.18	1.15	<0.001	481	21–>999
C2	40	52.5	3.76	1.01	<0.01	43	2.7–685
C3	40	37.5	3.15	1.00	0.01	23	1.5–355
C4	40	45.0	3.46	1.01	0.03	32	2.1–496
C5	40	2.5	Ref.[d]			Ref.[d]	
Slaugthering order							
First lot	40	52.5	This variable was not kept in the final multivariable model.				
Second lot	40	42.5					
Third lot	40	42.5					
Fourth lot	40	42.5					
Fifth lot	40	50.0					
Abattoir 2 (179 carcasses from 39 flocks)							
Constant			−3.40	0.73	<0.001		
Critical step[c]							
C1	39	89.7	5.57	0.86	<0.001	263	29–>999
C2	39	43.6	3.14	0.79	<0.001	23	3.1–174
C3	39	38.5	2.93	0.78	<0.01	19	2.5–140
C4	39	5.1	Ref.[e]			Ref.[e]	
C5	23	0.0					
Slaugthering order							
First lot	37	48.7	This variable was not kept in the final multivariable model.				
Second lot	38	36.8					
Third lot	38	26.3					
Fourth lot	36	27.8					
Fifth lot	30	56.7					

[a] *P* values and 95% confidence interval (CI) odds ratios for the critical step variable were adjusted multiple comparisons using the Tukey-Kramer procedure.
[b] OR, odds ratio estimates.
[c] C1, after bleeding, with the feathers still attached to the carcass; C2, at time of transfer between the live-receiving and the evisceration departments, before evisceration; C3, before chilling; C4, after water-immersion chilling; and C5, after dry-air chilling.
[d] Reference category.
[e] Reference category. The C4 and C5 steps were merged for regression modeling.

All regression analyses were conducted in SAS 9.4 (SAS Institute Inc., Cary, NC) by using the Genmod procedure.

RESULTS

In total, 379 broiler chicken carcasses from 79 flocks were sampled between February and July 2017 in the two surveyed slaughter plants. On the eight sampling days in each abattoir, five flocks originating from a different farm were sampled, except for one sampling visit conducted in abattoir 2 for which only four different flocks were available at the time of plant visit. For the whole sampling period duration, 68 different broiler chicken farms in total were sampled, with some farms being sampled more than once. According to the selective medium used, 138 (36.4%) carcass samples were positive for *Salmonella* on both BGS and XLD agar plates, whereas 9 (2.4%) carcasses were positive only on BGS agar plates and 14 (3.7%) carcasses were positive only on XLD agar plates.

***Salmonella* carcass contamination varied among critical steps of the slaughter process.** *Salmonella* was detected on at least 1 carcass from all flocks sampled in abattoir 1 (40/40) and from 35 of the 39 flocks sampled in abattoir 2. The *Salmonella* carcass positivity at each critical step of the slaughter process and according to the slaughtering order for both surveyed slaughter plants is presented in Table 2. For both plants, C1 was the most frequently detected as positive for the presence of *Salmonella,* with >89% of the carcasses sampled at this critical step being *Salmonella* positive. *Salmonella* was detected at least once in every sampling point of the processing line identified as critical, except at C5 (air chilling) in abattoir 2. Similarly, only one positive sample was found at this step in abattoir 1 (Table 2).

For both slaughter plants, only the critical step variable was retained in the final multivariable logistic regression modeling the *Salmonella* status of carcasses, as the

TABLE 3. *Number of samples collected and proportion of* Salmonella*-positive samples at each critical environmental sampling location for both surveyed slaughter plants, Quebec, Canada, 2017*

Critical sampling location	Abattoir 1		Abattoir 2	
	No. of samples	No. (%) positive	No. of samples	No. (%) positive
Postsanitation (PS)				
Feather-plucking rubber fingers (E1)	8	6 (75)	8	5 (63)
Conveyor belt: live receiving to evisceration (E2)	8	1 (13)	8	0 (0)
Eviscerating machine (E3)	8	1 (13)	8	0 (0)
Floor surface evisceration (E4)	8	3 (38)	8	0 (0)
Conveyor belt before chilling (E5)	8	1 (13)	8	0 (0)
Conveyor belt after chilling (E6)	8	0 (0)	8	0 (0)
Stainless steel cut-up room (E7)	8	0 (0)	8	0 (0)
Total	56	12 (21)	56	5 (9)
Postoperation (PO)				
Feather-plucking rubber fingers (E1)	8	8 (100)	8	7 (88)
Conveyor belt: live receiving to evisceration (E2)	8	4 (50)	8	4 (50)
Eviscerating machine (E3)	8	1 (13)	8	6 (75)
Floor surface evisceration (E4)	8	6 (75)	8	7 (88)
Conveyor belt before chilling (E5)	8	3 (38)	8	5 (63)
Conveyor belt after chilling (E6)	8	3 (38)	8	0 (0)
Stainless steel cut-up room (E7)	8	3 (38)	8	0 (0)
Total	56	28 (50)	56	29 (52)

slaughtering order and date of slaughter were not significantly associated with carcass positivity during model building ($P > 0.05$). For abattoir 1, the proportion of *Salmonella*-positive carcasses was significantly associated with the critical step ($P < 0.001$, 3 df). According to pairwise comparisons, this proportion was significantly higher at all steps than for C5 (after dry-air chilling) (Table 2). It was also significantly higher at C1 (after bleeding) than at subsequent C2 (OR = 11, adjusted $P < 0.01$), C3 (OR = 20, adjusted $P < 0.01$), and C4 (OR = 15, $P < 0.01$) steps, respectively. For abattoir 2, the proportion of *Salmonella*-positive carcasses was also statistically significantly associated with the critical step ($P < 0.001$, 3 df). For this abattoir, the two chilling steps (C4 and C5) were combined to allow for model convergence, considering the very low number of *Salmonella*-positive carcasses from these two steps. Carcasses at both water-immersion and dry-air chilling steps were significantly less contaminated than at any other of the three previous steps (Table 2). The odds of positivity to *Salmonella* was significantly higher in carcasses sampled at C1 (bleeding) than at C2 (OR = 11, adjusted $P < 0.01$) and C3 (OR = 14, adjusted $P < 0.01$) steps, respectively.

Slaughter plant environmental contamination increases after operations. In total, 224 environmental samples were collected (112 in each abattoir) from the surveyed plants during the visits. Overall, 54 (24.1%) samples were found to be positive for *Salmonella* on both BGS and XLD agar plates, whereas 10 (4.5%) samples were positive only on XLD agar and 10 (4.5%) samples were positive only on BGS agar. The distribution of *Salmonella*-positive environmental samples is presented in Table 3. For both plants, the proportion of *Salmonella*-positive samples was significantly higher ($P < 0.001$) for samples collected after the slaughter activities (PO) than after the sanitation procedures (PS), before the slaughter activities. The odds of positivity at PS was 3.7 (1.3 to 10.4) times higher for abattoir 1 and 10.9 (95% confidence interval: 4.6 to 26.2) times higher for abattoir 2, respectively, than at PO.

Identification of *Salmonella* HRM profiles and serotypes. In total, 432 *Salmonella* isolates were recovered (302 isolates from carcasses and 130 isolates from the environmental sampling). Each isolate was attributed a three-digit number corresponding to the HRM profile obtained following analysis of the fusion curves for *CR1, CR2,* and *yohM* genomic regions (Supplemental Fig. S1). Among the 192 samples from which isolates were recovered from both BGS and XLD culture media, a same HRM profile was identified for the two isolates in 159 (82.8%) samples, whereas the analysis of the two isolates generated different HRM profiles corresponding either to a same *Salmonella* serotype for 7 (3.6%) samples or to a distinct serotype for 26 (13.5%) samples.

In total, 40 distinct HRM profiles corresponding to 15 different *Salmonella* serotypes were identified among all isolates. Up to eight different HRM profiles per serotype were identified (Table S1). Among these HRM profiles, 17 were identified only from the environmental samples, 17 profiles were found exclusively in carcass rinsates, and 6 profiles were found in both types of samples. Twenty-two HRM profiles were observed only in abattoir 1, 12 were unique to abattoir 2, and 6 were common to both plants.

The most commonly identified *Salmonella* serotypes in carcasses were Heidelberg, Schwarzengrund, and Kentucky in both abattoirs (Table 4). *Salmonella* serotypes Heidelberg, Schwarzengrund, Kentucky, Mbandaka, and Thomp-

TABLE 4. *Distribution of* Salmonella *seroytpes in carcasses, flocks, and environmental samples, for each slaughter plant, Quebec, Canada, 2017*[a]

	Abattoir 1				Abattoir 2			
Salmonella serotype	No. of positive carcasses ($n = 200$)	No. of positive flocks ($n = 40$)[b]	No. of positive environmental samples		No. of positive carcasses ($n = 179$)	No. of positive flocks ($n = 39$)	No. of positive environmental samples	
			PS ($n = 56$)	PO ($n = 56$)			PS ($n = 56$)	PO ($n = 56$)
Agona			—[c]	—			—	—
Enteritidis	4	3	—	2	7	6	—	3
Brandenburg	—	—	—	—	1	1	—	—
Fresno	—	—	—	1	1	1	—	—
Hadar	—	—	—	—	4	3	—	—
Heidelberg	44	25	6	9	26	21	4	10
Infantis	—	—	—	—	—	—	—	1
Kentucky	19	14	—	9	22	16	1	9
Mbandaka	10	8	1	2	4	4	—	1
O:4 H:i H:−	1	1	—	—	—	—	—	—
Schwarzengrund	19	15	5	6	13	9	—	7
Senftenberg	1	1	—	—	—	—	—	—
Thompson	3	3	1	—	—	—	—	—
Worthington	—	—	—	—	1	1	—	—
7:k−	—	—	—	2	—	—	—	—

[a] *n,* total number of samples; PS, postsanitation; PO, postoperation.
[b] A positive flock is defined as a flock with at least one positive carcass for the serotype.
[c] —, this serotype was not identified.

son were the only serotypes identified from the environmental samples collected at PS. *Salmonella* Heidelberg was isolated at least once from all environmental surfaces sampled, except from E6. For abattoir 1, the presence of a single serotype and of a single HRM profile was noted for 16 of the 40 flocks sampled, whereas analyses revealed the presence of two different serotypes and of two distinct HRM profiles in 17 and 16 flocks, respectively (Table 5). In this same abattoir, seven and eight flocks were found positive for the presence of three distinct serotypes and HRM profiles, respectively. Similarly, a single serotype and HRM profile was identified from 14 and 12 flocks of the 39 flocks slaughtered in abattoir 2, respectively. Fifteen and 17 flocks sampled from abattoir 2 revealed the presence of two distinct serotypes and genotypes, respectively, whereas three different serotypes and HRM profiles were found on the carcasses of 6 flocks sampled in this plant.

Predicting the final flock status. For abattoir 1, 18 flocks had at least one carcass positive for *Salmonella* after chilling (C4 and/or C5) (see Table S2). Among these, 17 flocks also showed a *Salmonella*-positive carcass in at least one of the previous steps of the slaughter process. The *Salmonella* status of carcasses sampled at previous steps (C1, C2, and/or C3) for these 17 flocks was distributed as follows: 3 flocks were exclusively positive to the same HRM profile in at least one of the previous steps, 10 flocks were positive to both the same and different HRM profiles, and 4 flocks were positive for a different profile only. When a same flock was found *Salmonella* positive at both C1 and C4/C5 in this same abattoir, *Salmonella* Heidelberg 1-1-1 and Kentucky 6-6-1 were the sole HRM profiles identified. For abattoir 2, only two flocks were positive after chilling and neither of these flocks showed similar HRM profiles between the carcasses sampled at C1 and those sampled at C4/C5.

Based on these results, the model predicting the flock status after chilling was only performed for abattoir 1 with *Salmonella* Heidelberg HRM profile 1-1-1 for which a sufficient number of positive samples was available to allow for model convergence. Overall, the flock status for *Salmonella* Heidelberg 1-1-1 at any of the previous steps (C1, C2, C3) of the slaughter process was predictive of the flock status for this same HRM profile after chilling (Table 6). Of note, for 10 of the 12 flocks found positive for *Salmonella* Heidelberg HRM profile 1-1-1 at C4/C5, this profile was also isolated from the carcass rinsates in at least one of the previous steps. The *Salmonella* Heidelberg 1-1-1 status of the previously slaughtered flock(s) in the same day was also predictive of a flock carcass status for this same HRM profile after chilling. No statistically significant association was found or could be tested with the status of environmental samples (Table 6). The distribution of positive samples by flock and sampling day is illustrated in Table S3.

DISCUSSION

Salmonella-positive broiler chicken carcasses were identified at each critical step of the slaughtering line, as reported in a similar study by Rivera-Perez et al. *(49)* conducted in Costa Rica. They found that the critical step showing the highest level of contamination was C1 (after bleeding), which is not surprising considering that the feathers, skin, crop, and cloaca of birds entering the

TABLE 5. *Distribution of flocks according to the number of* Salmonella*-positive carcasses, number of distinct serotypes detected, and number of distinct HRM profiles detected per slaughter plant, Quebec, Canada, 2017*

	No. of *Salmonella*-positive carcasses in the flock in abattoir 1 ($n = 40$ flocks)[a]						No. of *Salmonella*-positive carcasses in the flock in abattoir 2 ($n = 39$ flocks)					
	0	1	2	3	4	5	0	1	2	3	4	5
No. of different serotypes detected in the flock												
0	—	—	—	—	—	—	4	—	—	—	—	—
1	—	11	2	1	2	—	—	10	1	3	—	—
2	—	2	8	4	3	—	—	3	9	3	—	—
3	—	—	—	5	1	1	—	—	1	4	1	—
No. of different HRM profiles detected in the flock												
0	—	—	—	—	—	—	4	—	—	—	—	—
1	—	11	2	1	2	—	—	8	1	3	—	—
2	—	2	8	4	2	—	—	5	9	3	—	—
3	—	—	—	5	2	1	—	—	1	4	1	—

[a] *n*, total number of samples.

slaughter plant can all carry significant bacterial loads *(21)*. *Salmonella* contamination at this step could originate from the farm environment, from the transport coops, or even from an increased shedding of the pathogen from carrier birds during the stressful transport conditions *(27, 46)*. Some of the *Salmonella* contamination might also have originated from the first steps of the slaughter process, such as through the contamination carried on the hands of abattoir workers, the knife used for bleeding, or the shackles on which birds were hung *(32, 47)*. In contrast to other studies reporting the evisceration step as critical for magnifying poultry carcass contamination by *Salmonella,*

TABLE 6. *Results from univariable logistic regression modeling the flock status for* Salmonella *Heidelberg 1-1-1 HRM profile after chilling (C4 and C5) according to the flock status at previous steps (C1, C2, and C3), status of the previously slaughtered flocks in the same day, and the environmental samples collected at postoperation on the same day in abattoir 1, Quebec, Canada, 2017*

Salmonella Heidelberg 1-1-1 status of previous carcasses or environmental samples collected in the same day			% of positive flocks to *Salmonella* Heidelberg 1-1-1 at chilling	Univariable logistic regression estimates[a]				
Type of sample[b]	Status[c]	No. of flocks		β	SE (β)	*P* value	OR[d]	95% CI (OR)
Carcasses of the same flock at C1	Pos.	16	43.8	1.08	0.50	0.03	3.0	1.1–7.9
	Neg.	24	20.8	Ref.[e]			Ref.	
Carcasses of the same flock at C2	Pos.	8	87.5	3.63	1.16	<0.01	37.8	3.9–367
	Neg.	32	15.6	Ref.			Ref.	
Carcasses of the same flock at C3	Pos.	7	71.4	2.23	1.09	0.04	9.3	1.1–78.2
	Neg.	33	21.2	Ref.			Ref.	
Carcasses of previous flock(s)	Pos.	20	45.0	1.53	0.77	0.05	4.6	1.0–20.9
	Neg.	20	15.0	Ref.			Ref.	
Environment at PS in zone 1	Pos.	5	0.0	No convergence of the model.				
	Neg.	35	34.3					
Environment at PS in zone 2	Pos.	15	60.0	2.40	1.45	0.10	11.0	0.64–190
	Neg.	25	12.0	Ref.			Ref.	
Environment at PS in zone 3	Pos.	0	—	No convergence of the model.				
	Neg.	40	30.0					

[a] Constant (intercept) is not presented.
[b] C1, after bleeding, with the feathers still attached to the carcass; C2, at time of transfer between the live-receiving and the evisceration departments, before evisceration; C3, before chilling; C4, after water-immersion chilling; and C5, after dry-air chilling. PS, environmental sampling postsanitation (i.e., sampling performed after sanitation and before the start of the slaughtering day). Zone 1, feather-plucking rubber fingers (E1). Zone 2, conveyor belt between the live-receiving and the evisceration departments (E2), eviscerating machine (E3), floor surface in the evisceration department (E4), and conveyor belt before chilling (E5). Zone 3, Conveyor belt after chilling (E6).
[c] Pos., positive; neg., negative.
[d] OR, odds ratio estimates.
[e] Ref., reference category.

usually following a rupture of the intestinal tract, this critical step appeared to be under control in abattoirs in the this study, because a decrease in the prevalence of *Salmonella*-positive carcasses was observed for both plants at this step compared with C1 *(25, 49)*.

Water chilling was associated with a reduction in *Salmonella* carcass contamination only in abattoir 2. Chilling equipment and chemical antimicrobial agents used for carcass sanitation can vary considerably among processing establishments *(26)*. For the present study, the water renewal rate, the carcass residence time in the chiller, and the incorporation of a chemical processing aid into the cooling water are three major factors that could have contributed to the higher prevalence of *Salmonella*-positive carcasses in abattoir 1. Indeed, whereas abattoir 1 was using a non-countercurrent flow system with a water turnover of 35 L/min, carcasses sampled in abattoir 2 were chilled in a countercurrent flow system for which the water turnover was 110 L/min. It is well established that the water renewal rate contributes to reducing carcass bacterial loads and preventing bacterial multiplication as well as controlling the microbiological status of the cooling water *(28, 37)*. Although the carcass residence time in the chilling system was close to 2 h for abattoir 2, this time was limited to 1.5 h in abattoir 1, mainly due to the smaller size of the prechill reservoir for which the temperature control was more challenging owing to its limited capacity. Moreover, peracetic acid was added to the chilling water in abattoir 2, which was not the case for abattoir 1. Even if water chilling has been reported to reduce the mean aerobic bacterial load of poultry carcasses, immersing carcasses in a sanitizer-free chilling reservoir was also correlating with an increased frequency of *Salmonella* detection among these carcasses, probably reflecting the cross-contamination occurring at this step *(8, 32)*. Indeed, the 45% *Salmonella* prevalence observed for carcasses exiting the chiller tank in abattoir 1 does not support the hypothesis that the sole physical effect of washing during immersion chilling is the primary mode of action for chilling to remove *Salmonella* from broiler chicken carcasses at this step *(37)*. By contrast, the 5.1% prevalence obtained from the sampling of carcasses at C4 in abattoir 2 supports a significant contribution of chemical processing aids in managing the contamination of poultry meat products by *Salmonella*: the 37.5 and 38.5% *Salmonella* positivity identified just before entering the chilling reservoirs was highly similar between the two plants *(8, 26)*. Although most of these sanitizers show great efficacy for controlling foodborne pathogens, some evidence seems to indicate that the different *Salmonella* serotypes found in poultry would respond differently to the chemical action of the various sanitizers used during chilling, with chlorine-based products reported to be the most effective for *Salmonella* control in commercial conditions *(26, 33, 54)*.

Our results highlight dry-air chilling as critical in reducing the contamination with *Salmonella,* with the proportion of positive carcasses decreasing from 37.5 to 2.5% and from 38.5 to 0% in abattoir 1 and 2, respectively. It can be hypothesized that the absence of the mechanical washing effect (as provided by water-immersion chilling), the close contact between hung birds, the use of water sprays in the air chilling room, or even the carriage of cetylpyridinium chloride (a quaternary ammonium compound) resistance genes such as *qacEΔ1* by some of the *Salmonella* strains present on the air-chilled carcasses sampled might not have been significant contributors to a residual contamination by the pathogen *(13, 26)*. It can therefore be hypothesized that the use of cetylpyridinium chloride combined with a prolonged contact time and the desiccation effect of the forced-air cooling system can significantly reduce *Salmonella* contamination found on broiler chicken carcasses.

The great serotype diversity observed for carcasses at C1 suggests that multiple sources of the pathogen contribute to this contamination. *Salmonella* diversity was previously reported to be greatest at the broiler chicken farm level than at other stages of the production chain *(15)*. It is assumed that the diversity of *Salmonella* present at this step is representative of the farm and transport *(49)*. According to the Canadian Integrated Program for Antimicrobial Resistance Surveillance, the most frequently detected serotypes at the broiler farm level are *Salmonella* serotypes Heidelberg, Kentucky, and Enteritidis, with varying predominance over years *(10)*. Results from the current study are thus in line with what was observed at the national level. They also highlight the emergence of *Salmonella* Schwarzengrund, which has been identified as such in the United States, in addition to having been incriminated in the occurrence of human disease outbreaks attributed to the consumption of poultry meat products *(11, 14)*. *Salmonella* Heidelberg was the most frequently identified serotype in the present study. According to the FoodNet Canada annual report *(42), Salmonella* Heidelberg consistently ranks as one of the top three most prevalent causes of human salmonellosis in the country. Also, *Salmonella* Heidelberg was associated with a more invasive form of human disease and with a higher case fatality rate than other nontyphoidal *Salmonella* serotypes *(28)*. In 2017, a *Salmonella* Heidelberg outbreak was responsible for nine cases of foodborne illness in six Canadian provinces and territories, and frozen raw breaded chicken products were identified as the source of infection *(44, 45)*.

Even if the use of next-generation sequencing approaches such as whole genome sequencing would represent the most discriminant approach when it comes to tracking *Salmonella,* the use of an HRM typing approach on the isolates recovered during the present study was discriminant enough to define with more precision the role of critical steps of the slaughter process, of the slaughter plant environmental contamination, and of previous flocks slaughtered during a same sampling day, which contrasts with other studies reporting results on the dynamics of *Salmonella* carcass contamination within the slaughter process by using positivity and/or serotype data only *(6, 25, 39, 49)*.

Interestingly, the CSL for which a residual *Salmonella* contamination was detected at PS in both surveyed plants is closely related to the plucking step, which is recognized as one of the most critical steps contributing to the cross-contamination of carcasses moving along the kill line *(36,*

49). The evisceration step may represent an increased risk for the contamination of broiler chicken carcasses, as supported by the six of eight samples collected at this step (E3) in abattoir 2 being found positive for *Salmonella* at PO. The absence of any *Salmonella*-positive environmental samples collected after chilling at PO in abattoir 2 supports the hypothesis that the contaminated carcasses act as a primary source of cross-contamination *(25)*. The five of eight *Salmonella* positivity for E5 at PO and a 45% *Salmonella* positivity identified for carcasses sampled at C4 in abattoir 1 further support this hypothesis. These observations emphasize the importance of controlling *Salmonella* at the live production stage, before broilers reach the slaughter plant. The *Salmonella* Heidelberg 1-1-1 HRM profile distribution among critical steps of the slaughter process further supports the hypothesis that the incoming birds act as a primary source of *Salmonella* contamination for carcasses of a same flock and for subsequently slaughtered flocks through contamination of the processing equipment.

Notably, previous studies have shown that *Salmonella* can persist in broiler processing environments despite intensive cleaning procedures and disinfection of contaminated equipment and surfaces, an observation made for both plants investigated in the current study *(47)*. Indeed, HRM profiles of *Salmonella* serotypes Heidelberg, Kentucky, Schwarzengrund, and Thompson that were not found on sampled carcasses were recovered from both the plucker and the eviscerating machine (Table S1). Similarly, other HRM profiles were observed in the environment of processing plants at PO, whereas these profiles were not observed from the carcasses. These observations suggest that some *Salmonella* would enter the slaughter plant along with the incoming birds, but owing either to their lower numbers or to their differential ability to survive the slaughter process, would not contaminate the final meat product. Although the few HRM profiles that were found on carcasses sampled at C4/C5 were also present at C1, 14 other profiles persisted from C1 to C2, before not being detectable from the carcasses sampled at C3, C4, and C5 (Table S1). However, because only one carcass per flock was sampled for each of the critical steps selected, with a maximum of two distinct colonies kept for molecular typing, it is likely that the full diversity of *Salmonella* profiles present was not revealed *(13)*. The laboratory protocol used has allowed showing the presence of two distinct *Salmonella* HRM profiles on 22 and 28% of the *Salmonella*-positive carcasses sampled at C1 for abattoir 1 and 2, respectively. When adopting a similar isolation protocol combined with a serotyping and pulsed-field gel electrophoresis approach on one *Salmonella* strain per positive sample, Vinueza-Burgos et al. *(53)* showed that two or three distinct sero-genotypes could be recovered from a same flock with the cecal sampling of 25 chickens per flock at the slaughter plant level *(53)*. However, Cox et al. *(14)* recently showed that broiler chicken carcasses sampled at prechill can harbor an average of five distinct *Salmonella* serotypes and that a preenrichment step of the whole carcass rinsate volume yields even more diversity.

Salmonella strains can evolve through the acquisition of genetic elements to survive hostile environments created by the use of antibiotics, acid-based alternatives, carcass sanitizers, high and low temperatures, and the pH and osmolarity variations that are encountered in poultry facilities and processing plants *(1, 13, 35, 41)*. A repeated exposure of some *Salmonella* strains to various sublethal stresses seems to increase the tolerance and survival ability of these strains, without neglecting the ability of some serotypes of *Salmonella* to preferentially adhere to chicken skin *(20, 50)*. These observations highlight the importance for poultry processors to optimize the control of *Salmonella* cross-contamination along the slaughter line, but more importantly to the prevention of this contamination at the farm level.

In conclusion, our results reinforce the importance of the *Salmonella* status of the incoming birds on the contamination of the final product. They also illustrate the importance of both the water-immersion and dry-air chilling steps in the control of *Salmonella* on broiler carcasses and underline the importance of the design and features of the water-immersion chilling system in the final carcass *Salmonella* status. The HRM approach allowed for the description of the distribution of *Salmonella* in commercial poultry processing plants. This approach also allowed for the identification of some *Salmonella* types that persist through the slaughter process, an observation that would deserve particular attention. This study highlights the importance of better documenting the survival and dissemination ability of some specific *Salmonella* types at the slaughter plant level to optimize the microbiological quality of poultry meat products.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: https://doi.org/10.4315/JFP-20-250.s1

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