

# Risk Factors at Slaughter Associated with Presence of *Salmonella* on Hog Carcasses in Canada

ANN LETELLIER,<sup>1\*</sup> GUY BEAUCHAMP,<sup>1</sup> EVELYNE GUÉVREMONT,<sup>2</sup> SYLVIE D'ALLAIRE,<sup>1</sup> DAN HURNIK,<sup>3</sup> AND SYLVAIN QUESSY<sup>1</sup>

<sup>1</sup>Faculté de Médecine Vétérinaire, Université de Montréal, 3200 Sicotte, Saint-Hyacinthe, Québec, Canada J2S 7C6; <sup>2</sup>Agriculture and Agri-Food Canada, Food Research and Development Centre (FRDC), 3600 Casavant West, Saint-Hyacinthe, Québec, Canada J2S 8E3; and <sup>3</sup>University of Prince Edward Island, Atlantic Veterinary College, 550 University Avenue, Charlottetown, Prince Edward Island, Canada C1A 4P3

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## ABSTRACT

Despite the application of hazard analysis and critical control point systems at slaughter and during processing, *Salmonella* contamination is still a significant biological hazard associated with pork products. A better understanding of risk factors in slaughterhouses and of contamination sources is therefore critical to improve control of this bacterium in the abattoirs. The objectives of this study were to identify the risk factors at slaughter that are associated with the presence of *Salmonella* on hog carcasses and to assess possible sources of contamination. A questionnaire on potential risk factors was developed. Over 7,400 hogs originating from 312 randomly selected production lots were tested. The lots were from 10 different abattoirs located in five different Canadian provinces. At slaughter, blood was collected for serological analysis, and mesenteric lymph nodes (MLN) and carcass swabs were collected for *Salmonella* analysis. Furthermore, pulsed-field gel electrophoresis was conducted to establish the genetic profiles of selected isolates from carcasses and MLN and to compare these profiles with those recovered from the slaughter environment. Multivariate regression analysis results indicated that the cleanliness of the hogs and the status of the scald water were factors significantly associated with the *Salmonella* status of the carcasses at the end of the slaughter process. Pulsed-field gel electrophoresis analysis showed that most isolates from carcasses were similar to those from animals (MLN) or the previsceration environment.

Salmonellosis and campylobacteriosis are the two most common foodborne diseases reported in Canada (19), resulting from microbial contamination of food, particularly of animal origin, and have a considerable impact on public health (16). Some hog slaughter processes, such as bleeding, dressing, and evisceration, expose sterile muscle to microbiological contaminants such as *Salmonella* that are present on the skin, in the digestive tract, and in the environment (5, 16). To reduce the risks of having food pathogens on carcasses, and the resulting impact on public health, government agencies such as the Canadian Food Inspection Agency and the Food Safety and Inspection Service of the U.S. Department of Agriculture (FSIS-USDA) have imposed regulations for hazard management on the meat industry, including the hazard analysis and critical control point (HACCP) system. These measures ensure intervention at critical control points in the slaughter process (20). While application of HACCP models have improved the situation and decreased the level of contamination by pathogenic bacteria, a significant percentage of meat products is still contaminated by *Salmonella* (14).

The farm-to-table approach was proposed by many authors as being the best method for efficiently controlling *Salmonella* in pork products (15, 21). There are strong indications that some on-farm interventions should reduce

the prevalence of *Salmonella* in the final products (2, 3, 4). However, their efficacy-to-cost ratio is subject to debate, because only some interventions are economically profitable at the farm level (10, 13). Control of this bacterium is essential at all production steps to decrease contamination levels in the final product, although authors disagree about the relative importance of various production steps. It is also important to have a better knowledge of the risk factors at slaughtering that may affect the occurrence of *Salmonella* on the carcasses to achieve better control of this pathogen. To our knowledge, no comprehensive study has yet been conducted in Canada to determine these factors. The overall objective of this study was to identify risk factors at slaughter associated with the presence of *Salmonella* in hog carcasses. A secondary objective was to genetically characterize the strains to assess the origin of the contamination.

## MATERIALS AND METHODS

**Questionnaire.** A questionnaire was developed to gather information on potential risk factors at slaughter and during transportation of animals. It was completed by slaughterhouse employees responsible for quality assurance. A group of experts, veterinarians and research scientists involved in the epidemiology and control of *Salmonella* in pigs, was consulted and participated in the development of the questionnaire. Personnel from two slaughterhouses were asked to validate the questionnaires for clarity. In addition, employees in charge of the quality assurance

\* Author for correspondence. Tel: 450-773-8521, Ext 18640; Fax: 450-778-8128; E-mail: ann.letellier@umontreal.ca.

programs in participating slaughterhouses were contacted and given an explanation of the scope and goals of the study, along with instructions on how to complete the questionnaire properly.

The questionnaire was divided into three sections: (i) slaughterhouse practices (cleaning and disinfection of pens, truck washing, frequency of knife disinfection, water treatment, etc.); (ii) information on the animal lots (time from farm to slaughter, cleanliness of the animals, tattoo number, and producer number); and (iii) any event during the slaughtering that may have affected the contamination of carcasses (mechanical problems, slaughter rate, stops, condemnation rate, contamination rate, gut ruptures, percentage of filled stomachs, and employee training).

**Evaluation of pig cleanliness.** For each lot in the study, pig skin cleanliness was evaluated on arrival at the slaughterhouse using the following criteria: clean pigs = no visible accumulation of fecal material on the body surface for more than 80% of pigs in the lot; dirty pigs = 25% or more of the body surface covered with fecal material for more than 50% of pigs in the lot; relatively clean pigs = lots not included in the above categories.

**Collection of samples at slaughterhouse.** One-gram samples of mesenteric lymph nodes (MLN) were collected using gloves and equipment disinfected between each sampling. In addition, samples for bacterial analysis were collected by swabbing the carcasses over the three USDA/Canadian Food Inspection Agency regulation anatomical sites (10). Blood samples were also collected from the same animals. A total of 7,441 hogs from 312 production lots were included in the study. The sampling was done in 10 slaughterhouses in Canada, namely, Quebec, Ontario, Manitoba, Saskatchewan, and British Columbia, within a 3-month period. For each lot, 20 to 25 pigs were sampled by selecting the first one randomly, and then by sampling every fourth animal. All three types of samples were taken from each of the 7,441 selected carcasses, for a total of over 22,000 samples.

**Environmental sampling.** At each slaughter visit (minimum 3 days), nine types of samples were collected by swabbing the immediate animal and carcass environments (pens, chutes, receiving areas, scalding water, evisceration floor, boots, gloves, aprons, and knives). For hog pen floors, a pool of five sites in each pen was sampled (10 by 10 cm per site, eight pens). For chutes and receiving areas, samples were collected from five sites. For scalding water, 50 ml were collected for analysis. For knives, composite samples were collected from the blade and handle of three knives. For the other types of sampling, samples were taken from three sites measuring 10 by 10 cm. The number of lots to be sampled per slaughterhouse was determined in advance, based on the daily slaughtering volume.

**Salmonella isolation and characterization.** Swabs were placed in sterile bags containing buffered peptone water, put in a refrigerator, and shipped in an icebox with ice packs to the laboratory at the Research Chair in Meat Safety of the University of Montreal. Samples were incubated using the official U.S. method, mandatory in Canada for slaughterhouses under the federal government jurisdiction as described in Mega-Reg, USDA (20). Briefly, samples were put in Rappaport-Vassiliadis (Difco, Detroit, MI) and tetrathionate brilliant green (BBL, Becton Dickinson, Cockeysville, MD) selective enrichment broths and then inoculated on selective agar media brilliant green sulfa agar (Difco) and double-modified lysine iron agar (Difco) supplemented with 20 µg/ml novobiocin (Sigma, St. Louis, MO). Lactose-negative colonies were tested for urease production (Difco) and for

typical reaction on triple sugar iron media (Difco). Colonies with typical biochemical patterns of *Salmonella* were tested using slide agglutination with a polyvalent O-antiserum (Poly A1-Vi, Difco). *Salmonella* isolates were serotyped at the laboratory of the Ministère de l'Agriculture, Pêcheries et Alimentation du Québec in St-Hyacinthe. In addition, for each slaughterhouse, a minimum of 10 selected isolates from carcasses, environment, and lymph nodes were genetically characterized by pulsed-field gel electrophoresis (PFGE) (6). Colonies of the pure overnight culture grown on blood agar were briefly suspended in a buffer solution of 75 mM NaCl–25 mM EDTA (pH 7.5) to an optical density of 1.5 at 625 nm. Then, 500 µl of this suspension was mixed with 500 µl of 1.5% low-gelling temperature agarose (Sigma) dissolved in sodium chloride–EDTA. The mixture was kept at 56°C until it was deposited in the molds. After 10 min at 4°C, the solidified plugs were transferred into the lysis buffer (3.6 ml of 1% [wt/vol] *N*-lauryl sarcosine–0.5 M EDTA, pH 9.5). To this mixture, 0.4 ml of a 10-mg/ml solution of proteinase K (Sigma) in 50 mM Tris–1 mM CaCl<sub>2</sub> (pH 8) was added. Cell lysis was carried out for 20 h at 56°C in a water bath. On the following day, the plugs were washed and stored in the appropriate buffer (10 mM Tris–10 mM EDTA, pH 7.5). Before digestion of bacterial DNA, plugs were preelectrophoresed to improve the clarity of restriction patterns. Agarose-embedded DNA was digested with 20 U of restriction endonuclease *Xba*I. Isolates showing no difference in their genetic profiles were submitted to another DNA digestion with 20 U of *Spe*I. Digestion was carried out at 37°C for 24 h. PFGE was performed with the Gene Navigator system (Amersham Pharmacia Biotech, CA) in a 1.2% high-gelling agarose (Sigma) gel in 0.5 × Tris-borate–EDTA buffer in accordance with the manufacturer's instructions. The gels were run for 20 h at 10°C at a constant voltage of 200 volts, using pulse times of 5 to 25 s with linear ramping and an electrical field angle of 120°. The gels were stained with ethidium bromide, destained in distilled water, and photographed on type 667 Polaroid instant sheet film apparatus under UV illumination. Two lambda ladder PFGE markers (Bio-Rad Laboratories, Hercules, CA) were used on each gel.

**Serological analyses.** Blood samples were analyzed by an ELISA (Maxivet, St-Hyacinthe, Quebec, Canada) to detect the presence of *Salmonella* antibodies, which indicate the status of the animals while on the farm (7). This serological assay was developed to allow the detection of serological response to more than 95% of the *Salmonella* serotypes commonly found in Canada (7).

**Statistical analyses.** Univariate logistic regression was used to identify discrete-type risk factors (presence or absence) in relation to the dependent variables, such as categories of prevalence of *Salmonella* on carcasses per lot. Logistic regression was used to analyze the possible links between risk factors (slaughterhouse practices) and the three types of *Salmonella* prevalence. Only variables for which the *P* value was 0.15 or less were retained for the final model. Then, multivariate logistic regression was used to determine which of the variables identified in the univariate analysis were statistically associated with the prevalence of *Salmonella*. For continuous variables, such as chlorine concentration and slaughter chain speed, the Spearman rank correlation was used. All these analyses were carried out using version 8.1 of the SAS program (SAS Institute, Cary, NC). Comparisons of prevalence were effected, unless otherwise indicated, using Student's *t* tests for small populations.

For the intraslaughterhouse analysis, prevalence of *Salmonella* was defined in three different ways and was used as the dependent variable. *Salmonella* on carcasses per lot (absence: 0%;

TABLE 1. Relationship between risk factors and *Salmonella* prevalence on carcasses at lot level

Risk factor	Spearman rank correlation	P value
Chain speed	$r = 0.53$	$0.10 > P > 0.05$
Chlorine concn	$r = -0.24$	$P > 0.20$
Quaternary ammonium concn	$r = 0.15$	$P > 0.50$
Frequency of washing the knife used for opening the abdominal cavity	$r = 0.58$	$0.10 > P > 0.05$

low:  $>0\%$  and  $\leq 12\%$ ; and high:  $>12\%$ ), seropositivity of lots (0:  $0\%$  prevalence; code 1:  $>0\%$  and  $\leq 20\%$ ; code 2:  $>20\%$ ,  $20\%$  corresponding to the 75th percentile of distribution), and prevalence of *Salmonella* in MLN per lot (0:  $0\%$  prevalence; code 1:  $>0\%$  and  $\leq 74\%$ ; code 2:  $>74\%$ , corresponding to the 75th percentile of distribution). However, several other independent variables could not be considered, because of lack of variation among lots.

For the interslaughterhouse analysis, the percentage of *Salmonella*-contaminated lots per slaughterhouse was used as the dependent variable. A specific slaughterhouse could appear twice in the analysis if it had changed cleaning product or chain speed. The independent variables were average speed of slaughter chain, average concentration of chlorine and quaternary ammonium compounds, use of one (single) or two products (combination) for disinfection, frequency of knife washing, and addition of chemical agents to the rinsing water. These factors were constant for a given slaughterhouse. The Wilcoxon test was used to examine whether the median prevalence differed among slaughterhouses depending on whether a combination of cleaning products was used and whether the carcasses were rinsed with chlorinated water.

## RESULTS

In this study, independent variables, when tested individually, indicated that *Salmonella* contamination of scalding tanks, knives, and boots, cleanliness of hogs, and the number of chain stops was associated with the prevalence of *Salmonella* in the lots. However in the final model, only two significant variables were retained: *Salmonella* contamination of scald water ( $P = 0.005$ ) and cleanliness of hogs prior to slaughtering ( $P = 0.008$ ). The odds of *Salmonella* presence on carcasses dropped by a factor of 0.39 when the scald water was *Salmonella*-free as opposed to not being *Salmonella*-free. Odds of *Salmonella* presence increased by a factor of 2.78 in lots with dirty pigs as opposed to clean ones. No difference was found between clean lots and relatively clean ones.

There was a positive, but not significant, correlation between the prevalence of *Salmonella* on carcasses and chain speed ( $r = 0.53$ ,  $P < 0.10$ ) and the frequency of knife

washing ( $r = 0.58$ ,  $P < 0.10$ ) (Table 1). There was no correlation between prevalence of *Salmonella* on carcasses and cleaning product concentration used: chlorine ( $r = -0.24$ ,  $P > 0.20$ ) or quaternary ammonium ( $r = 0.15$ ,  $P > 0.5$ ). *Salmonella* prevalence was similar for the two types of cleaning products ( $P = 0.11$ ) and for the two types of rinsing ( $P = 0.63$ ).

The relationship between the bacteriological status of the carcass and the serological status of the animal was determined (Table 2). In 43.4% of the cases (56 of 129), the serology was negative whereas the carcasses were positive, which suggested a recent contamination of the animal during transportation or cross-contamination of the carcasses at the slaughterhouse. When the serology was positive, the carcasses were positive in 67% of the cases (122 of 183), indicating that positive serological status strongly correlates with the positive status of a carcass. The logistic regression model, with the slaughterhouse as the random factor, indicated a positive relationship between the percentage of seropositivity and the percentage of bacteriologically positive carcasses. The odds that a lot would have a high score of positive carcasses increased by a factor of 5 when it had a serology score of 2 (lots with more than 20% of animals positive), compared with a score of 0 ( $P < 0.0001$ , Table 3).

The relationship between the bacteriological status of mesenteric lymph nodes (MLN) and carcasses was examined (Table 2). In many cases (80 of 93), the carcass was negative but the lymph nodes were positive, which in all likelihood, indicates that these animals were slaughtered in such a way that the carrier animal's infected tissues did not contaminate the carcass. In addition, when the carcass was positive, the lymph nodes were very often positive as well (75 of 86), which suggested contamination from the animal's infected tissues. The logistic regression model, used at the lot level, with the sampled slaughterhouse as the random factor, indicated a positive and significant relationship between the percentage of positive lymph nodes and positive carcasses for *Salmonella*. The odds that a lot would

TABLE 2. Relationship between bacteriological status of carcasses, serological and bacteriological status of MLN

Cases	No./total no. (%)	Interpretation
Serology negative, carcass positive	56/129 (43)	Suggests a recent contamination of the animal during transportation or cross-contamination of the carcass at the slaughterhouse
Serology positive, carcass positive	122/183 (67)	Indicates that positive serological status strongly correlates with a bacteriologically positive status of the carcass
Carcass negative, MLN positive	80/93 (86)	Indicates that these animals were slaughtered in such a way that the carrier animal's infected tissues did not contaminate the carcass
Carcass positive, MLN positive	75/86 (87)	Suggests contamination from the animal's infected tissues

TABLE 3. Relationship between the bacteriological status of the carcass and the serological status of the animal was demonstrated at the lot level (logistic regression model)

Relationship	Between	Odds
Positive and significant ( $P < 0.0001$ )	The percentage of seropositivity and the percentage of positive carcasses	The odds that a lot showed a high score of positive carcasses increased by a factor of 5 when it had a serology score of 2 compared with a score of 0
Positive and significant ( $P = 0.0006$ )	The percentage of bacteriologically positive MLN and the percentage of positive carcasses	The odds that a lot show a high score of positive carcasses increased by a factor of 5.4 when it had an MLN score of 2 compared with a score of 0

have a high score of positive carcasses increased by a factor of 5.4 when it had a lymph node score of 2, compared with a score of 0 ( $P = 0.0006$ , Table 3). As expected (7), most serologically positive animals showed positive lymph nodes (data not shown).

The PFGE genetic profiles of *Salmonella* strains isolated in each slaughterhouse from carcasses, preevisceration environment (entrance, pens, alleyways, scald tank), evisceration environment (floor, boots, knives, aprons), and lymph nodes (animal status) indicated that, in this study, most of the contamination of carcasses originated in the preevisceration environment. *Salmonella* strains isolated from the evisceration floor were, in approximately two-thirds of the cases, different from those isolated from the carcasses. Various serotypes were isolated in the study, and PFGE profiles were affected when the same serotype was isolated from carcasses, the MLN of the same animal, and from environment samples. As shown in Figure 1, *Salmonella* Typhimurium DT12 genetic profiles of isolates from four carcasses (lanes 4, 6, 8, and 10) were identical to those isolated in the pens (lane 2). The same profile was also observed in MLN from the same pigs (lanes 5, 7, 9, and 11), but isolates from boots and knives had different PFGE profiles. Same findings (data not shown) were observed for

Derby (eight pigs) and Schwartzengrund (eight pigs) serotypes.

## DISCUSSION

This study was designed to identify the risk factors associated with the presence of *Salmonella* on pig carcasses in Canada. It shows that, in Canada, the *Salmonella* serological status of on-farm livestock is closely linked to the presence of *Salmonella* on the carcasses, as reported in other countries (12, 18). Carcasses from herds where more than 20% of the animals were seropositive (category 2) were five times more likely to be positive than carcasses from negative herds, and three times more likely to be positive than those from herds with a prevalence of less than 20% (category 1). Although attention should be paid to controlling the risk factors identified in the current study at the slaughter level, it strongly suggests that on-farm intervention in decreasing the number of serologically positive animals would be of great value to decrease the percentage of *Salmonella*-positive carcasses. Therefore, targeting herds with higher prevalence of *Salmonella*-positive animals is important for control programs (12). Basic precautions taken at slaughter are likely to manage contamination and cross-contamination due to herds with low infection rates. However, when highly contaminated herds are slaughtered, these basic precautions are not sufficient to prevent contamination of a significant number of carcasses. Our findings suggest that, for Canadian herds, the intervention threshold should be for herds with a seroprevalence greater than 20%.

By genetically characterizing *Salmonella* strains, it was possible, in this study, to match the genetic profiles of strains isolated from pens or scald water with those from carcasses. It also suggests that the *Salmonella* strains from incoming animals are likely, within a limited period of time, to contaminate the slaughterhouse. The fact that the same genetic type was observed in the pens, the MLN, and many carcasses from the same sampled lot supports this hypothesis. This study was not designed, however, to determine the impact of a positive lot status on the bacterial status of carcasses from following lots. The random selection of pig lots did not allow for the sampling of a sufficient number of consecutive lots to draw any conclusion on this aspect.

Nevertheless, this study clearly shows that the status of on-farm livestock, established serologically, is closely linked to the presence of *Salmonella* on carcasses from the same lot. Since the genetic patterns of strains recovered

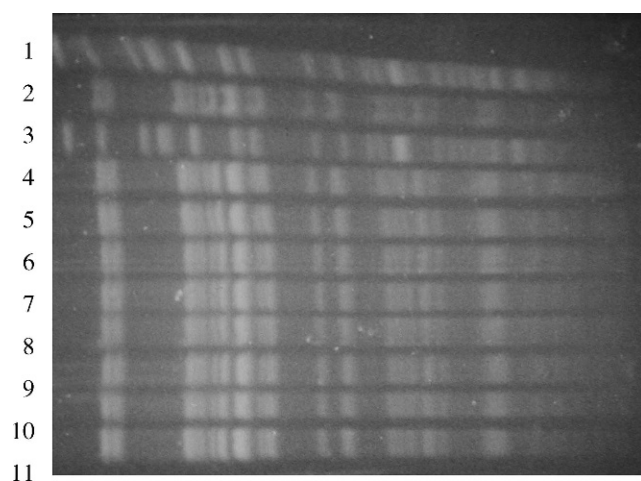


FIGURE 1. PFGE analysis of *Salmonella* isolates from a 1-day collect in a given slaughterhouse. Each lane indicates a DNA profile obtained with XbaI. Lanes 1 and 3 are *Salmonella* isolates from knives and boots, respectively, and in lane 2, *Salmonella* isolates from the holding pens. Isolates from carcass and MLN for pigs 1, 2, 3, and 4 are represented in lanes 4 to 5, 6 to 7, 8 to 9, 10 to 11, respectively.

from a given slaughterhouse changed from one sampling date to another (data not shown), it is highly unlikely that the contamination of the environment or pens occurred in the days preceding the sampling visit; the most likely explanation is that new strains were introduced by serologically positive animals that were shedding *Salmonella*. In a similar study in The Netherlands, Swanenburg et al. (17) reported that the PFGE genetic patterns found in carcasses were most often similar to the ones recovered from the slaughter environment. The difference in the design and slaughter procedures between European and Canadian abattoirs may explain in part these differences. In both studies, however, the genotypes recovered from lairage areas were strongly associated with those recovered from carcasses, indicating that attention should be paid to regular washing and disinfection of the lairage areas, particularly after the slaughtering of highly infected herds. In the same way, a longitudinal study of *Salmonella* dispersion and the role of environmental contamination in commercial swine production systems in Canada were investigated by Dorr et al. (8). They found that some genotypic clusters contained isolates originating in trucks and lairage swabs and also in cecal and/or mesenteric lymph nodes but not always from the farm environment. These findings underscore the significance of various environmental factors, including inadequate truck-washing systems, and highlight the role of lairage contamination by *Salmonella*. Avoiding direct or indirect contact between *Salmonella*-infected and *Salmonella*-free herds is also important. Wonderling et al. (22) in the United States demonstrated that, using PFGE profiles, 54% of genotypes found on hog carcasses were distinct from those in the feces. Our own results support their conclusion that each pig lot has the potential to introduce new contaminants into the plant previsceration environment and that feces from one pig can contaminate several subsequent carcasses, at least from the same lot. Another significant finding of the current study was the association between bacteriological status of the carcasses and water from the scald tank. These results coincide with those obtained by Hald et al. (11) in Denmark and clearly indicate that particular attention should be paid to the bacteriological quality of the scald water.

To our knowledge, this study is the first to establish a clear link between the cleanliness of the live pigs, as they enter the abattoir, and the final status of the carcasses. In beef, it has been shown that washing the animal is beneficial for the control of *Salmonella*, particularly when the degree of hide contamination is high (1, 9). It is generally assumed, for pigs, that washing before and after evisceration is sufficient to reduce the impact of the initial skin contamination. Our results would suggest that washing or spraying the animals before they are slaughtered could further reduce the level of skin contamination. One limitation to our study was that several risk factors could not be studied in detail because of the limited number of slaughterhouses involved and the fact that they often follow similar practices. In addition, high chain speed and a lower frequency of washing the knives used for opening the abdominal cavity tended to increase

contamination of carcasses. These risk factors deserve more thorough study in future research.

With regard to risk factors at the slaughterhouse associated with the presence of *Salmonella* in the final product, this study demonstrated the importance of the preslaughter and previsceration environment on the final status of carcasses. Namely, the cleanliness of the hogs and the status of the scald water proved to be significant factors associated with the final bacteriological status of the carcasses. Results obtained by genetic characterization and serology indicated that particular attention should be paid to the herd contamination levels of incoming animals and the previsceration environment to better control *Salmonella* in pigs at slaughter.

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