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

ANN LETELLIER,1* GUY BEAUCHAMP,1 EVELYNE GUÉVREMONT,2 SYLVIE D’ALLAIRE,3 DAN HURNIK,3 AND SYLVAIN QUESSY1

1Faculté de Médecine Vétérinaire, Université de Montréal, 3200 Sicotte, Saint-Hyacinthe, Québec, Canada J2S 7C6; 2Agriculture and Agri-Food Canada, Food Research and Development Centre (FRDC), 3600 Casavant West, Saint-Hyacinthe, Québec, Canada J2S 8E3; and 3University of Prince Edward Island, Atlantic Veterinary College, 550 University Avenue, Charlottetown, Prince Edward Island, Canada C1A 4P1

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 preevisceration 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...
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 samples of mesenteric lymph nodes (MLN) were collected 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.

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 receiving area was collected. For scalding water, 50 ml were collected for analysis. For knives, composite samples were collected from the blade and handle of each lot; dirty pigs

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 slaughtered 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 receiving area was collected. 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.

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low: >0% and ≤12%; and high: >12%), seropositivity of lots (0%; 0% prevalence; code 1: >0% and ≤20%; code 2: >20%, 20% corresponding to the 75th percentile of distribution), and prevalence of *Salmonella* in MLN per lot (0% prevalence; code 1: >0% and ≤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 1. Relationship between risk factors and *Salmonella* prevalence on carcasses at lot level**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Spearman rank correlation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chain speed</td>
<td><em>r</em> = 0.53</td>
<td>0.10 &gt; <em>P</em> &gt; 0.05</td>
</tr>
<tr>
<td>Chlorine concn</td>
<td><em>r</em> = −0.24</td>
<td><em>P</em> &gt; 0.20</td>
</tr>
<tr>
<td>Quaternary ammonium concn</td>
<td><em>r</em> = 0.15</td>
<td><em>P</em> &gt; 0.50</td>
</tr>
<tr>
<td>Frequency of washing the knife used for opening the abdominal cavity</td>
<td><em>r</em> = 0.58</td>
<td>0.10 &gt; <em>P</em> &gt; 0.05</td>
</tr>
</tbody>
</table>

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

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

Looking to 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
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 preevisceration 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 significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water significant finding of the current study was the association between bacteriological status of the carcasses and water.


