RISK FACTORS, AT SLAUGHTER, ASSOCIATED WITH PRESENCE OF SALMONELLA ON HOG CARCASSES IN CANADA

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Abstract Despite application of HACCP systems at slaughter and during processing, Salmonella contamination is still a significant biological hazard associated with pork products. A better understanding of slaughterhouse risk factors and contamination sources are therefore critical to improve control of this bacterium at slaughter level. The overall objective of this study was to identify the risk factors, at slaughter, associated with the presence of Salmonella in hog carcasses. We were also interested in the genetic characterization of the strains in order to assess the origin of contamination. A questionnaire on various potential risk factors was developed, over 7400 hogs were tested. These hogs originated from 312 randomly selected production lots. The lots came from 8 different provinces and were tested at time of slaughter in 10 different abattoirs. The tests included serology and bacteriology cultures from mesenteric lymph nodes (MLN) and carcasses. Furthermore, pulsed field gel electrophoresis (PFGE) was conducted to establish genetic profiles of selected isolates from carcasses and MLN to compare their profiles to those recovered from the slaughter environment. Multivariate regression analysis indicated that the cleanliness of the hogs and the status of the scald water were significant factors associated with the final bacteriological status of the carcasses. PFGE analysis showed that most isolates from carcasses were similar to those from animals (MLN) and/or pre-evisceration environment.

Introduction Salmonellosis and Campylobacteriosis are the two most reported foodborne diseases every year in Canada (Todd, 1997). The farm-to-table approach is now recognized as being the best approach for efficiently controlling Salmonella in swine herds and carcasses (Crump et al., 2002). In order for this approach to be efficient, all production steps must optimize control of this bacterium in order to decrease the contamination rates observed in the final product. There is still a need to better understand the sources of contamination and risk factors at slaughter associated with the presence of Salmonella on hog carcasses. The overall objective of this study was to identify risk factors, at slaughter, associated with the presence of Salmonella in hog carcasses. We were also interested in the genetic characterization of the strains in order to assess the origin of contamination.

Materials and Methods The questionnaire covered risk factors at the slaughterhouse and a portion of the transportation of animals. It was completed by slaughterhouse employees in charge of quality assurance. There were three phases in the development of the questionnaire. The first involved a review of the literature in industrialized countries with similar production schemes in order to identify all potential risk factors associated with the slaughter of hogs. In the second phase, a group of experts, veterinarians and research scientists involved in the epidemiology and control of Salmonella in hogs were consulted and asked to validate the first draft of the questionnaires on the documented risk factors. They also suggested other potential risk factors. Finally, personnel from two slaughterhouses were asked to validate the questionnaires for understanding and clarity. In addition, all the participating slaughterhouse employees were contacted and given an explanation of the scope and goals of the study, along with instructions on how to complete the questionnaire properly.

Collection of samples at the slaughterhouse: One-gram samples of mesenteric lymph nodes were collected. In addition, bacterial carcass samples were collected by swabbing the three predetermined anatomical sites were obtained for each animal. A total of 7441 carcasses were included. These pigs originated from 312 production batches and at 10 slaughterhouses in Canada receiving animals from Quebec, Ontario, Manitoba, Saskatchewan and British Columbia. Between 20 to 25 animals were randomly sampled per lot (first animal randomly selected, and every fourth one thereafter). Blood samples were also taken from the same animals selected for the lymph node sampling. A total of over 22,000 samples were obtained.
In addition, at each visit, nine samples were collected by swabbing the immediate animals’ and carcasses environment (pens, chutes, receiving, scald water, evisceration floor, boots, gloves, aprons and knives). For the samples taken from the hog pen floors, composite samples were collected from 5 sites per pen, at 8 pens for each day of collection. For chutes and receiving, samples were collected from 5 sites. For scald water, 50 ml was collected for analysis. For knives, composite samples were collected from the blade and handle of 3 knives. For the other types of sampling, samples were taken from 3 sites measuring 10 x 10 cm. Samples were collected over a minimum of 3 days at each of the participating slaughterhouses. The number of lots to be sampled per slaughterhouse was determined in advance, based on the daily volume of slaughtering at the slaughterhouse.

Bacteria isolation and Characterization: Swabs were placed in sterile bags containing a transportation medium, put in a cool place, and shipped to the laboratory of the Research Chair in Meat Safety at the University of Montreal. The samples were incubated in the official method set out in Mega-Reg (USDA, 1996), in selective enrichment broth (RV and TBG) and then inoculated on selective agar media (BGS and DMLIA supplemented with novobiocin 20µg/mL). Biochemical assays were conducted to confirm the identification, and serotyping was done at the MAPAQ laboratories in Saint-Hyacinthe, Quebec.

In addition, for each slaughterhouse, selected isolates from carcasses, the environment, and lymph nodes were genetically characterized by pulsed field gel electrophoresis (PFGE) using the method of Schwarz et al. (1994) in order to establish their genetic profile.

Serological Analysis: Blood samples were analyzed in order to detect the presence of Salmonella antibodies by the means of an ELISA to establish and determine the status of the animals when at farm. The serological assay used in this study has been developed to allow the detection of serological response to more than 95% of the Salmonella serotypes commonly found in Canada (Côté et al., 2004).

Statistical Analysis: A univariate logistic regression was done first in order to identify the significant discrete-type risk factors (presence/absence) in relation with some discrete dependent variables. For instance, association between each risk factor and categories of prevalence of Salmonella on carcasses per lot was analyzed to identify certain potentially significant risk factors. Next, we used multivariate logistic regression to establish which of the variables identified in the univariate analysis should be retained in the final model. For the analysis of the continuous variables, such as chlorine concentration and the slaughter chain speed, the Spearman rank correlation was used. All these analyses were done using version 8.1 of the SAS program (SAS Institute, Cary, NC). Other statistical analysis comparing two prevalences were done, unless otherwise indicated, by using Student’s t-tests for comparing prevalences in small populations.

Results Risk factors at the slaughterhouse associated with the presence of Salmonella on carcasses: For the intra-slaughterhouse analysis, the prevalence (%) of Salmonella on carcasses per lot per slaughterhouse has been used as an independent variable. This prevalence has been split into a three-level variable for analysis purposes (absence: 0%, weak presence: > 0% and <= 12%, and strong presence: > 12%).

However, it should be noted that several independent variables could not be considered, mainly because of the lack of variations among the lots. Logistic regression was used to analyze the possible links between the other practices and prevalence results. The first step involved testing each of the independent variables by itself. Significant variables with a threshold of 0.15 or less were considered in step two. These were: scalding tank, knife, boots, physical appearance of hogs, and the number of chain stoppages. Each of these variables is associated with the prevalence of Salmonella between lots at a slaughterhouse. The second step involved combining these variables so as to determine which of them would be retained in the final model.

The final model included two variables: scald water (p = 0.005) and appearance of hogs prior to slaughtering (p = 0.008). The odds of Salmonella presence dropped by a factor of 0.39 when the scald water was Salmonella free and increased by a factor of 2.78 in lots with moderately clean rather than clean hogs (25% or more of the body surface having accumulated fecal material on the skin). No differences were found between clean lots (no accumulation of fecal material on the body) and relatively clean ones (less than 25% of the body surface having accumulated fecal material).

For the inter-slaughterhouse portion of the analysis, we used the percentage of Salmonella-
contaminated lots per slaughterhouse as the dependent variable. The same slaughterhouse could appear in the analysis twice if it changed cleaning product or chain speed. The independent variables used were average speed of slaughter chain, average concentration of chlorine and quaternary ammonium, the use of one (single) or two products (combination) for disinfection, frequency of washing of the knife used for opening the abdominal cavity and the addition of chemical agents to the rinse water. These factors were constant within a given slaughterhouse. The Wilcoxon test was used to verify that the median prevalence in the slaughterhouses with combination cleaning products differed from the median prevalence in those that used a single cleaning product, and if the prevalence in the slaughterhouses that rinsed carcasses with chlorination differed from the prevalence in those that did not do so.

There was a positive but marginally non-significant correlation between a slaughterhouse’s chain speed and its prevalence ($r = 0.53$, $0.10 > p > 0.05$). There was no correlation between slaughterhouse prevalence and chlorine concentration ($r = -0.24$, $p > 0.20$) or the concentration of quaternary ammonium ($r = 0.15$, $p > 0.5$). There was a positive but marginally non-significant correlation between the prevalence and the frequency of washing the knife used for opening the abdominal cavity ($r = 0.58$, $0.10 > p > 0.05$). Slaughterhouse 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 status of the carcass and its serological status was demonstrated. In nearly half of the cases (56/129), the serology was negative but the carcasses proved to be positive, which suggests a recent contamination of the animal during transportation or cross-contamination of the carcass at the slaughterhouse. However, if the serology was positive, the carcasses were very often positive as well (122/183), which indicates that positive serological status strongly correlates with the positive status of a carcass. Testing at lot level, in the logistic regression model, with the sampled slaughterhouse as the random factor, indicates a positive and significant relationship between the seropositive percentage and the positive carcasses percentage. 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 ($p < 0.0001$).

We next examined the relationship between the bacteriological status of the mesenteric lymph nodes and the carcasses. In many cases (80/93), 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. However, if the carcass was positive, the lymph nodes were very often positive as well (75/86), which suggests contamination from the animal’s infected tissues. At lot level, we examined the association between the prevalence of *Salmonella* on carcasses and in lymph nodes. The logistic regression model, with the sampled slaughterhouse as the random factor, indicates a positive and significant relationship between the percentage of positive lymph nodes and the percentage of positive carcasses. The odds that a lot showed 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 ($p = 0.0006$).

Finally, we studied the genetic profiles of *Salmonella* strains isolated from carcasses, the environment and mesenteric lymph nodes. The study of the PFGE (pulsed field gel electrophoresis) genetic profiles of the bacteria isolated in each slaughterhouse from carcasses, the pre-evisceration environment (entrance, pens, alleyways, scald tank), post-evisceration environment (floor, boots, knives, aprons) and lymph nodes (animal status) indi-
cates that, in the majority of cases of contamination of carcasses by the environment, the strain isolated from the carcass comes from the pre-evisceration environment, whereas the strains isolated from the evisceration floor seem to differ from those isolated from the carcasses. The animals therefore appear to be contaminated by strains present in the pre-evisceration environment. Figure 3 shows an example of carcass contamination where the isolated strains (CP-269, CP-268, CP-266, CP-264) are identical to those isolated in the pens (CP-244). We also found the same profile on both the carcasses and the mesenteric lymph nodes.

Discussion This study, designed to identify the risk factors associated with the presence of *Salmonella* in carcasses, is the most comprehensive one undertaken to date on this topic in Canada. This study clearly shows that the status of livestock on the farm, established serologically, is closely linked to the presence of *Salmonella* on the carcasses. Carcasses from category 2 herds, i.e., herds where more than 20% of the animals are positive, were 5 times more likely to be positive than carcasses from negative herds, and 3 times more likely to be positive than those from herds with a prevalence less than 20%. Although attention should be paid to control the risk factors identified in the current study at slaughter level, it also suggests that on farm intervention to decrease the number of serologically positive animals should be of great value in order to decrease the percentage of positive carcasses.

By genetically characterizing strains isolated from carcasses, it was possible, in many cases, to match genetic profiles of strains isolated from the pens or the scald water to the strains isolated from the carcasses. In contrast, given the relatively small number of slaughterhouses and the fact that they follow similar practices, several significant risk factors could not be studied in detail. There are however statistical trends indicating that factors such as chain speed, the combination of cleaning products and the frequency of washing the knife used for opening the abdominal cavity deserve more thorough study in future studies.

Conclusions With regard to risk factors at the slaughterhouse, the study shows the degree of impact that controlling the pre-evisceration environment has 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 status of incoming animals and the pre-evisceration environment in order to better control *Salmonella* in pigs at the slaughter level.

References


