RISK FACTORS ASSOCIATED WITH PRESENCE OF SALMONELLA IN PIGS IN CANADA

Sylvain Quessy1, Evelyne Guevremont2, G. Beauchamp1, S. D’Allaire1, S. Fournaise3, C. Poppe4, T. Sanderson5, Ann Letellier1

1University of Montreal, 3200 Sicotte, St-Hyacinthe, Quebec, J2S 7C6, Canada, Ph: 450-773-8521 ext 8398, Email: sylvain.quessy@umontreal.ca.; 2Canadian food inspection agency, St-Hyacinthe, Quebec; 3Olymel, St-Hyacinthe; 4Public Health Agency of Canada, Guelph, Ontario; 5Consultant Veterinarian, Maxwell, Ontario

Abstract Carriers of Salmonella in finishing pigs are believed to be a main source of carcass and pork contamination at the slaughterhouse and during meat processing. To better control the presence of Salmonella at the farm and slaughter, it is important to adequately identify on-farm risk factors associated with presence of these bacteria in animals. Thus a study was performed to identify the risk factors associated with the presence of Salmonella in live animals and on carcasses from 312 herds in Canada. A questionnaire was designed to gather information on several factors present on the farm and was completed by the producers. Results from the multivariate logistic regression analyses of all the factors indicated a significant effect of the type of feed grind (p = 0.0001), pen size (p = 0.006), truck load capacity (p = 0.008), clinical signs of salmonellosis (p = 0.03) and a marginal effect of number of sources of finishing pigs (p = 0.098). Carcasses from highly contaminated herds were more likely of being Salmonella positive. Limiting the presence of Salmonella in the meat should therefore begin by reducing the number of herds highly contaminated by Salmonella.

Introduction Many HACCP-based good production practices to be used on farm have been developed in order to manage biological hazards, such as Salmonella and chemical hazards. The purpose of these practices is, ensuring a better use of antimicrobial agents and decrease risk of drug residues in meat products. So far, in pigs, these models have been built based on principles of risk analysis to manage well known risk factors or to avoid various sources that contaminate the herd. Very limited information was available in Canada on risk factors associated with the presence of Salmonella in pig operations. In most cases, we have had to rely on published literature to build on farm control programs.

Use of antimicrobial agents in intensive production units has been associated with the emergence of bacterial strains resistant to several antibiotics. While the majority of authors agree that the use of antibiotics in animals can be linked to only a small percentage of the resistances observed among humans, almost all stakeholders agree that antibiotics must be used more judiciously in livestock production in order to stem the emergence of resistant strains, even if only for the sake of animal health.

Resistance to multiple antibiotics is regularly observed in Salmonella strains isolated in Canada (Rheault et al., 2001). Furthermore, these multiresistant strains are associated with acute health problems on a growing number of hog farms in Canada (Desrosiers 1999). It has been reported in Europe that use of broad-spectrum antimicrobial agents can foster the presence of Salmonella in livestock (Evans and Wegener, 2003). Since resistant Salmonella isolates have genes encoding resistance to many of the antibiotics commonly used in livestock production, thus ensuring their selection and proliferation, it is important to determine if the antibiotics most commonly used on farms in Canada can be associated with the presence of multiresistant strains.

The objectives of this study were to identify the risk factors associated with the presence of Salmonella in swine herds and determine if the presence of multiresistant strains of Salmonella can be associated with the use of some antimicrobial agents on farm.

Materials and Methods

Questionnaire: A questionnaire was designed to collect information on potential risk factors and was completed by the producers. It addressed risk factors at the farm and to a lesser extent those associated with the transportation of animals. There were three phases in the development of the questionnaire. The first involved reviewing of the literature in order to collate all the risk factors associated with husbandry and transportation. In the second phase, a group of experts in the epidemiology and control of Salmonella in pigs were consulted and asked to validate the documented risk factors listed in the first draft of the questionnaire. They also suggested other poten-
tial risk factors. Finally, twenty hog farmers were asked to validate the questionnaire for understanding clarity. In addition, all the participating producers were contacted and given an explanation of the scope and goals of the study, along with instructions on how to complete the questionnaire properly. Most producers were again contacted after they had received the questionnaire to ensure validity of some of their answers.

**Collection of samples at the slaughterhouse:** Blood samples, one-gram sample 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 slaughter-houses 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). A total of over 22,000 samples were obtained.

**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 of the University of Montreal. The samples were incubated according to the official procedures described in the 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. A total of 432 Salmonella-positive isolates were characterized for their resistance to antibiotics by placing antibiotic discs on the agar media using the NCCLS-approved method.

**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 (Côté et al., 2004).

**Statistical Analyses:** a univariate logistic regression was done for each type of analysis in order to identify significant discrete-type risk factors (presence/absence) in relation with a discrete dependent variables, namely serology and lymph node scores. The serology score was 0: 0% seropositive; 1: > 0% and <= 20% seropositive; and 2: > 20% seropositive, where 20% represented the 75th percentile of distribution. The codes for lymph nodes were: 0: 0% positive lymph nodes; 1: > 0% and <= 74% positive lymph nodes; and 2: > 74% positive lymph nodes, where 74% represented the 75th percentile of distribution. A multivariate logistic regression was used to establish which of the variables identified in the univariate analysis were the most significant. All these analyses were done using version 8.1 of the SAS program (SAS Institute, Cary, NC). Other analyses were done using Student’s t-tests for comparing prevalence in small populations, unless otherwise indicated.

**Results** The prevalence of seropositive animals, which indicates the status that had the animal several days prior to slaughter was 13.6%. The prevalence observed in lymph nodes, which determines the status of the animal at its entry into the slaughter chain, was 32.3%.

The multivariate analysis of risk factors for the seroprevalence of Salmonella revealed a significant effect of the type of feed grind (p = 0.013), pen size (p=0.006), truck load capacity (p=0.008), signs of salmonellosis (p=0.03) and a marginal effect of number of sources of finishing pigs (p = 0.098). The odds of having a high score in serology were nearly 8 times lower (odds ratio = 0.13) with feed in mash form than with feed in pelleted form. The odds of having a high score in serology were lower when the truck load capacity was under 200 pigs (OR=0.32) and when pen size was smaller (under 170 square feet, OR=0.31). The odds were lower without signs of salmonellosis in the herd (OR=0.089). Finally, the odds were lower with one animal source than with two or more sources (OR=0.31).

We studied the relationship between the status of the lymph nodes and the serology. In many cases (69/84), the serology was negative whereas the lymph nodes were positive, suggesting that several animals become contaminated at the end of the finishing period or during transportation or the pre-slaughtering period. When the serology was positive, the lymph nodes were often positive as well (86/95), indicating that contamination occurred most likely in the herd at least 3 weeks earlier, which is the length of time usually required to develop a serological response to the infection.

At the batch level, results from the logistic regression model, with the sampled slaughterhouse as the random factor, indicated a positive and significant relationship between the % of
seropositive and the % of positive lymph nodes. The risk that a batch had a high score of positive lymph nodes increased by a factor of 3.8 for batches with a serology score of 2 compared with 0 (p < 0.0001). The risk increased by a factor of 2.9 (p = 0.005) for serology score 2 compared with 1. The increase of the risk was not different from 1 (odds ratio = 1.29, p = 0.42) when the serology score was 1, compared with 0.

The association between the prevalence in serology and the bacteriological status of carcasses at the batch level was determined. Results from the logistic regression model, with the sampled slaughterhouse as the random factor indicates a positive and significant relationship between the percentage of seropositive and the percentage of positive carcasses. The risk that a batch had a high score of positive carcasses increased by a factor of 5 when it had a serology score of 2 compared to 0 (p < 0.0001).

It was not possible to establish a relation with the use of any antimicrobial agent, whether in the meal as growth promoter or as therapeutic agent, and resistance profiles. Among the 432 *Salmonella* isolates, 57% were resistant to at least one antimicrobial agent. Resistance to 4 or more antimicrobial agents was observed in 11% of the isolates, while 19% of the isolates showed resistance to at least 3 antimicrobial agents. As expected, no difference was observed between the proportions of multi-resistant strains from carcasses and those from mesenteric lymph nodes.

**Discussion** Some important risk factors identified in this study might be difficult to control at the farm level or to justify economically although they contribute to increase the prevalence of *Salmonella*. For instance, on some farms, it might be difficult to reduce the numbers of source of incoming animals when all-in/all-out procedures are required. Also, the use of pelleted feed might be justified for productivity or practical reasons.

In addition, 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 of less than 20%. This clearly demonstrates that intervention at the farm is important in order to reduce the prevalence of *Salmonella* on carcasses.

**Conclusions** Herds with a high seroprevalence are much more at risk of being contaminated in the mesenteric lymph nodes. The carcasses from these highly contaminated herds are also much more at risk of being *Salmonella* positive. Limiting the presence of *Salmonella* in the finished product must therefore begin by reducing the number of herds highly contaminated by *Salmonella*.

This study does not allow the demonstration of an effect of antimicrobial agents on the emergence of multi-resistant strains of *Salmonella* in studied herds.

**References**

