Herd-level risk for *Salmonella* culture positive status in slaughtered pigs.

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**Keywords:**

**Introduction:** Quantitative definition of risk factors is needed for farms to develop programs and procedures to reduce *Salmonella* shedding economically and reliably. It is essential that this information be available to producers before implementation of proposed *Salmonella* reduction regulations. Producers should then be able to retain market access and choose informed interventions while avoiding unnecessary costs.

*Salmonella* spp. have been isolated from 25.7% of market swine in the US (Nelson et al., 1982), and 18% of pork samples obtained from apparently healthy market pigs in Canada (Lammerding, et al., 1988). When pig carcasses are examined for the presence of salmonella, the prevalence of positive isolations depends in part on which tissues are examined. *Salmonellae* were isolated from 21% of the intestines and 21% of the swabs of pig carcasses in a Dutch study but from only 3% of the mesenteric lymph nodes (Oosterom et al., 1985). A Canadian survey revealed an 80% positive isolation rate from feces and a 7% rate from pork meat (Mafu et al., 1989).

*Salmonella* outbreaks have been traced back to pig farms (Maguire et al., 1993). *Salmonella* have been isolated from pigs both before and after shipment to slaughter, and both locations can be considered indicators of the risk of *Salmonella* contamination at the slaughter plant. While samples collected at the farm gate are an unequivocal reflection of the farm, samples collected at slaughter have been shown to be strongly correlated with farm samples (Kim et al., 1999). However, transmission during transport to and lairage in the slaughter plant is possible, and samples collected at slaughter may indicate *Salmonella* that originate either on the farm or at the slaughter plant, or from trucks or intermediate holding facilities.

We designed this study to describe the strength of association between potential herd-level risk factors and the detection of *Salmonella* in commercially produced, slaughter-weight pigs.

**Materials and Methods:** Farms participating in a health monitoring program were
eligible for this study if they were in the state of Minnesota and were within a 200 km radius of one slaughter plant. A maximum of two farms were chosen at random each week, based on those marketing during that week. Herds were eligible for inclusion in the study only once. The contact for the herd was established as the veterinarian conducting the slaughter health inspection. This list was randomized, and permission was requested from the farm.

A survey of 72 questions was administered by personal interview. The survey was designed to collect information in the areas of health, facility design, hygiene and biosecurity, pig-flow management, and transport and lairage. The field technicians (Fransen L, and Grass J) validated responses by direct visual inspection, and asked for clarification if the survey response did not match the physical evidence observed at the site. In cases where certain questions could not be answered at the time of the visit, respondents were provided a copy of the survey and asked to return it by post.

Herd managers were advised of the categories of information included on the survey one week in advance of the herd visit. One of the two technicians visited each farm within 48 hours of shipment to slaughter. With the farm manager, the technician individually identified the animals to be shipped with a numbered ear tag.

Feces and a serum samples were collected from each pig at the farm visit. Approximately 30 g of feces was collected from each of 20 pigs either by digital extraction with a gloved finger, or from the non-contaminated surface of a fecal pat after observed defecation. If a 30g sample could not be obtained, another pig was sampled in its place from among the marketing group.

At slaughter, pigs were individually marked on the exterior of the carcass to retain the ID number on the ear tag, which was removed before evisceration for hygienic reasons. Intestinal tracts were individually identified, then placed in tubs and set aside for later processing. Because tracts were handled without decontamination of gloves between contact with each tract, their surfaces were considered cross-contaminated. Samples were therefore collected by dissection and reflection of tissues in a way to prevent contamination of the underlying sample. Ten to twenty grams of cecal content was collected by gently scraping the surface of the cecal mucosa with a disposable plastic spoon. Approximately 30 grams of caudal mesenteric (cecal) lymph nodes were collected aseptically by dissection and reflection of the overlying mesentery. Lymph nodes exposed to potential contamination were not collected. Samples were packaged in individual sterile plastic bags, placed on ice within two hours of collection, and sent to the laboratory on ice for processing the next day. Samples were collected Monday through Thursday only. While 20 pigs were tagged at the farm, only the first 15 pigs slaughtered were collected at the plant. Non-matching farm samples were discarded.

All samples were qualitatively analyzed for the presence of *Salmonellae* by conventional bacteriological methods designed for the isolation of *Salmonella spp* (Fedorka-Cray et al., 1998), with the addition of XLT-4 plating for isolation.
Individual pigs were categorized positive if any sample collected was positive, i.e. fecal isolation at the farm or at the slaughter plant or by isolation from mesenteric lymph nodes or cecal content at the slaughter plant. The proportion of pigs positive per herd was then calculated.

Multiple linear regression analysis was performed, using a combination of best subset regression and stepwise model selection methods. Variables were considered for analysis if there was a biologically plausible relationship to *Salmonella* shedding, and, for categorical variables, if at least five farms were observed in each category. Because of limited number of farms studied, the total number of variables included in the initial best subsets approach was limited to 14. A subset was defined as all possible models within a fixed number of predictor variables.

Based on published literature, variables with experimental or observational data suggesting a linkage to *Salmonella* were given a higher priority, as were variables that contributed to the least number of missing cases. Incomplete or missing surveys were assumed to have values missing at random. Transformation of the outcome variable was considered when suggested by regression diagnostics.

The criteria applied to the evaluation of best subsets were the coefficient of determination (R^2), adjusted coefficient of determination (Adj.R^2) and the Mallow’s Cp statistic. Of all possible models, the 5,000 models with the highest R^2 were identified within each subset. Models including interactions without the corresponding main effects were then eliminated. Within a subset, models were ordered by decreasing values of R^2, with those models within 5% of the maximum R^2 per subset considered for further evaluation. To aid in the selection of the number of variables included, the statistics Mallow’s Cp and adjusted R^2 for each subset was plotted against number of predictors (Weisberg, 1985). The model with the highest R^2 in each subset was evaluated. In addition, backward stepwise selection was run starting from the saturated model, with a p-value to exit of 0.1 and a p-value to enter of 0.15.

To investigate the potential effects of missing values, and to estimate the effects of variables with missing values, eight variables were considered for forward stepwise model selection, starting with the model selected by the methods described above. The variables offered were pig density (area allocated per pig), estimated age of pigs at slaughter, use of animal origin feed ingredients, diagnosis of salmonellosis in the past 12 months, mixing pigs from different barns during transport to slaughter, truck hygiene prior to transport to slaughter, the use of growth promoting or therapeutic antibiotics, and the use of wooden gating.

**Results:** The mean inventory of growing pig barns was 379 pigs (s.d. 201), with a range of 110 to 1,200 pigs. Pigs were moved to different locations (barns or rooms) one to five times following weaning; 42.4% of farms moved pigs to two locations, and 40.7% moved pigs to three locations. The mean herd-level proportion of *Salmonella*
test positive pigs was 0.277 (s.d. 0.294). The distribution was positively skewed.

Factors for Salmonella isolation in finishing pigs were evaluated based on survey responses for 51 farms for which complete data were available. An arcsin transformation, 2*arcsine *(proportion)⁻⁰·⁵ (Cohen et al., 1983), was applied to the outcome variable to correct for nonconstant variance of residuals.

The highest Adj. R² was a model with 10 predictor variables (R² = 0.42). However, the increase in Adj. R² was relatively small in models with more than five variables. The increased R² from the four to five variable model was 0.039; stepwise increases for models with more than five variables were all less than 0.03. Among the five variable subset, 45 models had an R² within 5% of the maximum R² for the subset (30%). Among these, the variables appearing in 10 or more models were: presence of birds, non-batch pig movement, bowl waterers, wet/dry feeders, substandard performance, and prior diarrhea.

Regression analysis of the models in each subset with the highest R² revealed a model (R² = 0.22) (Table 1) with significance of all variables at an α level of ≤ 0.1. The same models were identified by the backwards stepwise elimination approach. The main effects model predicted Salmonella prevalence in the range 2% to 61% depending on the combinations of risk factors present.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Intercept</td>
<td>0.264</td>
<td>0.36</td>
</tr>
<tr>
<td>Birds</td>
<td>0.450</td>
<td>0.10</td>
</tr>
<tr>
<td>Wet/dry feeders</td>
<td>0.489</td>
<td>0.09</td>
</tr>
<tr>
<td>Bowl waterers</td>
<td>0.585</td>
<td>0.00</td>
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</tbody>
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Table 1. Linear regression model predicting the transformed prevalence of Salmonella in slaughtered pigs.

Discussion/Conclusions: The increased prevalence of Salmonella associated with birds may suggest a role in transmission. Birds are known to carry Salmonella, and bird feces and dead birds may be consumed by pigs. Alternately, farms with facilities that do not exclude birds may have other, unmeasured biosecurity risks or other characteristics that result in increased shedding at the time of slaughter. Wet/dry feeders commonly used in Minnesota allow pigs to consume feed either dry or mixed with water at the feeder. Wet feed can remain behind, allowing a environment conducive to bacterial growth. Further, some of these feeder designs have relatively
larger, open troughs that would be more easily contaminated than dry feeders commonly employed. Further investigation seems warranted, since details on feeder design were not included in the survey. Bowl waterers were of two basic designs, either with or without nipples. In either case, bowl waterers retain water between individuals and offer a fomite to transfer Salmonella.

No variables associated with transport and lairage, including mixing of pigs in trucks, assembly of pigs at multi-farm collection facilities, sanitation of trucks and time between shipment and slaughter, were associated with Salmonella prevalence. This is in contrast to evidence from other sources that ceca and associated lymph nodes can become culture positive within a short time (Fedorka-Cray et al., 1994 and Hurd et al., 2001). All pigs were held in lairage for variable periods of time, allowing the potential for transmission of bacteria. These findings suggest that the transport and lairage variables measured here played a relatively minor role in Salmonella transmission. Pigs in this study were handled in a manner similar to all slaughtered pigs at this plant, except that they were segregated at the slaughter plant on arrival, and placed in cleaned pens to facilitate movement to the slaughter line. It is possible that this level of segregation reduced cross contamination.

The fact that many alternate models had similar predictive power as measured by $R^2$, coupled with the Adj. $R^2$ and the curve of Mallow’s Cp statistic suggests that additional variables are predictive of Salmonella prevalence, but that the number of farms studied gave insufficient power to statistically establish the relationships. While we cannot conclude that commonly found variables with non-significant p-values are indeed risk factors, these variables can be considered for further investigation.

The predictive value of our model, with an $R^2$ of 22%, suggests that although the model predicts a substantial amount of variability, much unexplained variation remains. As expected in observational studies, variables that may be causative were not considered in the model because we lacked herds with or without the putative risk factor. Finally, the limited number of farms examined prevented us from examining interaction effects.

References: