Lesion severity at processing as a predictor of Salmonella contamination of swine carcasses

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Objective—To measure the relationship between gross lesions in swine carcasses observed at a processing plant and Salmonella contamination and to determine whether nonexpert assessments of lesion status would correspond with swine pathologists’ judgments.

Animals—Carcasses of 202 conventionally raised and 156 antimicrobial-free pigs in a Midwestern US processing plant examined from December 2005 to January 2006.

Procedures—4 replicates were conducted. For each, freshly eviscerated carcasses were identified as having or lacking visceral adhesions by a nonexpert evaluator and digital carcass photographs were obtained. Swab specimens were obtained from carcasses before the final rinse stage of processing, and bacterial culture for Salmonella spp and Enterococcus spp was performed. Subsequently, carcass photographs were numerically scored for lesion severity by 3 veterinary pathologists. Results were used to test the ability of lesion detection to predict bacterial contamination of carcasses and the agreement between judgments of the inexperienced and experienced assessors.

Results—The probability of Salmonella contamination in carcasses with lesions identified at the abattoir was 90% higher than that in carcasses lacking lesions, after controlling for replicate identity and antimicrobial use. The receiver operating characteristic curve and Cohen κ indicated close agreement between lesion detection at the abattoir and by the 3 pathologists.

Conclusions and Clinical Relevance—Findings indicated the presence of lesions could be used to predict Salmonella contamination of swine carcasses and that a nonexpert processing-line assessment of lesions could be used to discriminate between healthy and chronically ill swine before their entry into the human food supply. (Am J Vet Res 2012;73:91–97)
force may be required during the process, leading to leakage or spilling of intestinal contents. When leakage occurs, there is an approximately 40% probability of *Salmonella* contamination, given the reported percentage of swine with *salmonellae* in the gastrointestinal tract at the time of harvest. 

Few studies have quantitatively demonstrated the relationship between veterinary and human health related to animal-derived food products. A model has been developed that predicts the increase in the days of human illness (campylobacteriosis) per year according to the number of subclinically ill poultry slaughtered. 

By that model, even minor perturbations in *Campylobacter* loads in poultry products could have substantial impacts on public health.

A study involving pooled samples found that swine carcasses with tissue damage or lesions indicative of chronic, systemic infections are 2 to 3 times as likely to be contaminated with *Enterococcus* spp and *Campylobacter* spp at the end of processing, compared with nonlesioned carcasses. However, investigators failed to detect a meaningful correlation between peel-outs (ie, removal of the pleural and peritoneal linings after evisceration due to adhered visceral tissue) and *Salmonella* contamination, possibly because of low numbers of *Salmonella*-positive samples. The purpose of the study reported here was to quantify the relationship between lesions suggestive of subclinical illness (visceral adhesions) in swine and *Salmonella* carcass contamination through examination of individually identified and swabbed carcasses. A second objective was to determine whether in-plant, nonexpert processing-line assessment of pathological lesions would correspond with the findings of experienced swine pathologists for the discrimination of subclinically ill pigs from healthy pigs, with ultimate aim of improved food safety.

**Materials and Methods**

**Animals**—Swine included in the study were processed from December 2005 through January 2006 at a large Midwestern US abattoir. Only the first group of pigs to arrive at the abattoir each day was included to reduce the likelihood of environmental contamination from previously processed swine.

**Study design**—The study was conducted in 4 replicates of 2 days (Tuesday and Wednesday) each, separated by 2 weeks. On each occasion, swine from different farms were used. Pigs were intentionally selected to represent 2 types of farming practices: antimicrobial-free and conventional. Antimicrobial-free pigs (ie, those raised without use of antimicrobials for treatment, disease prevention, or performance purposes) were processed only on Tuesday morning each week. On the farms from which they originated, any ill pigs were removed from the barn and treated; those that recovered were harvested at another time. Conventionally raised pigs (ie, those raised on farms in which antimicrobials were used for any veterinarian-directed purpose) were selected for evaluation on the following morning. To avoid the potential confounding effect of barn of origin, pigs from 1 barn only were evaluated on each day. The 2 groups of pigs were from the same integrated pig production company, so they were managed in a similar manner (eg, similar veterinary care, housing, environment, and feed) and differed only with respect to antimicrobial exposure.

A third-year veterinary student performed the data collection, identifying freshly eviscerated carcasses as having lesions or no lesions on the basis of the presence or absence of lung tissue attached to the carcasses. Prior to the data collection, the student was trained by a veterinary pathologist (KJS) in identification of common pathological lesions associated with certain disease conditions in pigs. As part of this training, the student viewed pictures and specimens of lung tissue with the pathologist and was trained in sterile sample collection technique.

Collection began with a carcass that bore lesions, which was identified by the presence of visceral tissue adhered to the thoracic wall. After another 10 to 15 carcasses had been processed, a carcass without lesions was chosen. This spacing was determined on the basis of findings in a previous study, in which it was estimated that the probability of carcasses containing visceral adhesions requiring peel-out was 7% (or approx 1 in 13). We chose not to reduce the spacing to < 10 carcasses because it has been reported that 28% of carcasses have positive results of *Salmonella* culture when a *Salmonella*-positive carcass is processed beforehand and we wanted to minimize cross-contamination among carcasses.

A photograph was obtained of the interior of each split carcass, which was marked for swabbing. All photographs were obtained in the same manner with the same camera to minimize variability. Swab samples of the carcass interior were collected at the end of processing, just after the final USDA inspection point, before the final rinse procedure. A preliminary trial of this collection and identification procedure was performed before the study began, with the obtained photographs reviewed by one of the pathologists to ensure adequacy.

Gloves were worn during collection of swab samples from the pelvic, peritoneal, and pleural cavities of marked carcasses. One side of each carcass (the same side for each) was swabbed first with 1 side of a sterile sponge. The sponge was flipped over and used to swab the other side of the carcass, then placed in a plastic bag containing 10 mL of sterile buffered peptone water. Gloves were changed between carcasses. The swab sample collector obtained samples near the end of the line, after evisceration, and was therefore blinded to lesion status. Photography and sample collection ceased each day after 25 carcasses with lesions and 25 carcasses without lesions had been identified or until pigs from selected barns were no longer available.

Swab samples were put on ice and refrigerated immediately after collection. However, the sample collectors could not return to the microbiological laboratory until both Tuesday and Wednesday collections were completed. All samples in each replicate were processed after 48 hours of collection to ensure a consistent duration of refrigeration.

**Bacteriologic processing**—To test swab samples for the presence of salmonellae and enterococci, each
plastic bag containing the sample and sterile broth was placed in a laboratory paddle blender for 60 seconds. Next, 100 µL of broth was removed aseptically and placed on agar plates selective for enterococcal growth. Following incubation for 48 hours at 35°C, the plates were examined for suspect Enterococcus colonies. When colonies were found, 2 colonies/pig were subcultured onto blood agar plates, which were then incubated for 48 hours at 35°C. Colonies were Gram stained, tested for catalase production, and evaluated for growth in tubes containing NaCl. When a suspect colony was gram positive, catalase negative, and grew in tubes containing NaCl, it was considered to contain Enterococcus spp.

After a broth sample was removed for Enterococcus testing, 10 mL of enrichment broth was added to enrich the sample. The samples were then incubated for 24 hours at 42°C. Next, 100 µL of broth was plated onto 2 types of Salmonella-selective plates, which were incubated for 48 hours at 35°C. Suspect Salmonella colonies were subcultured in 2 types of biochemical media. To increase the sensitivity of Salmonella detection, the bags were allowed to sit at room temperature (25°C) for 6 to 8 days for additional delayed enrichment, after which 1 mL of the contents was removed and added to the tubes containing 9 mL of enrichment broth. These tubes were incubated at 42°C for 24 hours. Next, 100 µL of tube contents were plated onto Salmonella-selective plates and incubated for 48 hours at 35°C. Suspect colonies were confirmed with biochemical media as before.

Pathologist scoring—To evaluate the student’s ability to identify carcasses with lesions, the carcasses in the digital photographs were scored by 3 veterinary pathologists with extensive swine experience. One of these pathologists was board-certified in veterinary pathology, one was a pathology veterinarian with 30 years of experience in swine diagnostic testing, and the other was a veterinarian with a pathology doctoral degree and 5 years of experience in swine diagnostic testing. Pathologists were unaware of the student-assigned carcass classification and the microbiological status of the carcass. Each half of the carcass was scored from 0 to 3, with 0 indicating no adhered visceral tissue and 3 representing severe adhesions. The individual pathologist lesion score was obtained for each pathologist by adding the scores from each half, such that a range of 0 to 6 was possible for each carcass. The combined pathologist lesion score was obtained by summing all 3 pathologist scores, such that a range of 0 to 18 was possible for each carcass.

Statistical analysis—Agreement among the 3 pathologists’ lesion scores was measured by calculation of the Fleiss κ statistic, which was used to assess the reliability of agreement between raters. The interpretation of κ was 0 to 0.2 as slight agreement, 0.21 to 0.4 as fair agreement, 0.41 to 0.6 as moderate agreement, 0.61 to 0.8 as substantial agreement, and 0.81 to 1 as almost perfect agreement.

An ROC curve and LR plot were used to compare the diagnostic accuracy of the nonexpert with the expert pathologists at various lesion score cutpoints (eg, > 2, 3, or 4) with the aid of statistical software. For the LR plot, likelihood for agreement that lesions were present was defined as LR+ and likelihood of disagreement was defined as LR−, and the cutpoint in combined pathologist scores that yielded the largest LR+ and smallest LR− values was determined. Once the optimal cutpoint was established for the combined pathologist score, carcasses were reassessed as having lesions or lesion-free on the basis of the combined pathologist lesion scores and the cutpoint. The Cohen κ was calculated to evaluate the agreement between nonexpert and expert findings.

Each value of the combined lesion score (3 pathologists) was evaluated for an association with the probability of a carcass with a Salmonella-positive culture result. To evaluate the association between bacterial contamination and lesion detection by the student, multivariable logistic regression models were built by use of commercial software. Salmonella and Enterococcus contamination were treated as binary outcomes (present or absent), and the probabilities of carcass contamination with each were modeled. The main effect of interest was carcass lesion status as defined at the abattoir, with adjustments to account for the 4 replicates and for antimicrobial use on the originating swine farms. The goodness of fit of the final model was assessed by calculation of the deviance statistic by use of commercial statistical software. A value of P > 0.05 indicated no evidence of lack of fit. Regression diagnostic statistics were determined by use of deviance residuals, with absolute values of deviance residuals > 2 indicating outlying values. The relationship between bacterial contamination and lesion status was measured by calculation of PORs, with strength of association indicated by distance of PORs from 1. The significance of difference between proportions was evaluated with the 2-tailed Z test. Values of P < 0.05 were considered significant for all association tests.

Results

Animals—Three hundred fifty-eight swine carcasses were selected at the abattoir for inclusion in the study. Of these, 156 originated from swine raised without antimicrobial use and 202 originated from swine raised conventionally.

Microbial cultures—Salmonella spp were recovered from 36 of 338 (10.1%) carcasses, and Enterococcus spp were recovered from 39 (10.9%). The prevalence of carcasses contaminated with Salmonella (1% to 10%) differed significantly across the 4 study replicates, with the prevalence in the first replicate (1%) significantly lower than that in the other replicates. Carcasses of swine raised without antimicrobials were significantly more likely to be contaminated with Salmonella spp (27/156 [17.3%]) than carcasses of conventionally raised swine (9/202 [4.5%]), although carcasses of conventionally raised swine had a slightly, albeit nonsignificantly (P > 0.05), higher probability of Enterococcus contamination.

Agreement among pathologists—Photographs were obtained for all included carcasses (Figure 1). The distribution of pathologist scores revealed a wide range in lesion severity (Figure 2). The dis-
tribution of individual pathologist lesion scores approximately resembled a U shape, with higher frequency of scores in the lower and upper ends of the scoring range. Agreement was close among pathologists on lesion scores (ie, those assigned for a whole carcass by each pathologist). The scores of 2 particular pathologists shared more similarity (Fleiss κ, 0.74) than did the scores of the third pathologist with each of the others (Fleiss κ, 0.47 and 0.43). All 3 pathologists had moderate agreement on lesion scores (Fleiss κ, 0.54).

Agreement between nonexpert and experts—Two methods were used to evaluate agreement between carcass lesion status defined by the nonexpert student assessor (yes or no; abattoir positive or abattoir negative) at the abattoir and by the 3 pathologists during photograph evaluation. From the first method (ROC analysis), it was evident that a combined pathologist score > 2 had the strongest agreement with the nonexpert judgment (Figure 3). For the second method, LRs for the nonexpert assessment agreeing with the combined pathologist assessment were plotted in a graph (not shown). This plot also revealed that a combined pathologist score > 2 most strongly agreed with the nonexpert judgment. The Cohen κ value for agreement between a combined pathologist score of > 2 and the nonexpert judgment of lesion present was 0.95, indicating very close agreement.14

Association between bacterial contamination and nonexpert assessment—On the basis of the nonexpert judgment, 182 carcasses were defined as having lesions. Of these, 22 (12%) had positive results of Salmonella culture and 22 (12%) had positive results of Enterococcus culture. On the other hand, 8% and 10% of the carcasses judged as not having lesions had Salmonella and Enterococcus contamination, respectively (Table 2). For each value of the combined pathologist score, the prevalence of Salmonella contamination ranged from 0% to 100%, with a median of 10%. However, there was no evidence that probability of Salmonella contamination increased with increasing combined pathologist score, as determined on the basis of the extended Mantel-Haenszel χ² test (P = 0.82).

Multivariate logistic regression analysis of the association between bacterial contamination and lesion status defined by nonexpert assessment revealed that carcasses judged as having lesions were 90% more likely to have Salmonella contamination than carcasses in which no lesions were identified (POR, 1.9; 95% CI, 0.9 to 4.0), adjusting for replicate identity and on-farm antimicrobial practices. The probability of Enterococcus contamination in carcasses bearing lesions was not significantly different from that for carcasses without lesions. Carcasses from swine raised without antimicrobials also had a significantly higher probability of Salmonella contamination (POR, 6.7; 95% CI, 2.7 to 16.9) than did carcasses from conventionally raised swine. The prevalence of Salmonella contamination in replicates 2, 3, and 4 (POR, 12.6, 12.8, and 26.6, respectively) was considerably higher than in replicate 1.

Figure 1—Representative photographs of swine carcasses with (A) and without (B) visceral lesions. The carcass with lesions assigned a combined score of 18 (most severe score possible) by 3 pathologists was later found to be contaminated with Salmonella spp. There is large amount of organ tissue adhered to the pleural and peritoneal lining. The carcass without lesions, which received a combined pathologist score of 0 (no lesion evident), had negative results of bacteriologic culture for Salmonella spp.

Figure 2—Distribution of lesion scores for 358 swine carcasses processed at an abattoir. Three veterinary pathologists used photographs of splayed carcasses to assign a lesion score to each half, with 0 indicating no adhered visceral tissue and 3 representing severe adhesions. Thus, a total lesion score of 6/carcass was possible for each pathologist.
higher than that in replicate 1. A logistic regression model in which antimicrobial use was excluded as a variable revealed the POR between lesion status and Salmonella contamination was 1.7, which was 10% smaller than the POR from the full model. The POR decreased to a similar extent when replicate identity was excluded from the full model, suggesting both variables were confounders and should be left in the final models. Goodness of fit was sufficient for the Salmonella multivariate regression model ($P = 0.14$) and very good for the Enterococcus model ($P = 0.68$).

**Discussion**

In the study reported here, Salmonella contamination of carcasses was associated with the health status of the originating swine as judged by the presence of subclinical lesions (ie, visceral tissue adhered to the pleural and peritoneal lining). The use of carcass lesions as a health indicator is an established practice and has been commonly used to monitor the health of pigs.$^{18-21}$ Several studies$^{4,22,23}$ have been conducted to evaluate the association between gross lung lesions detected at abattoirs and various measures of on-farm health and performance during the growing period in swine. However, research into the possible association between carcass lesions and foodborne pathogen contamination at processing is lacking. Testing for pathogen contamination of carcasses is a standard public health practice in the United States$^{24}$ and the European Union.$^{25}$

The design of the present study limited our ability to determine the reason for the observed increased likelihood of pathogen contamination of carcasses that have lesions, compared with the likelihood in carcasses without lesions. Reasons could include the additional handling at evisceration required for carcasses with adhesions or the possibility of chronically diseased pigs being immune compromised and infected with Salmonella spp. However, our findings suggest the evisceration process may not be the only reason for the increase in carcass contamination because there was not a significant association between lesions and Enterococcus recovery, which could be used as an indicator of fecal contamination rather than infection (POR, 1.3; 95% CI, 0.7 to 2.5).

A potential limitation of the present study is the low sensitivity of bacterial detection by use of culture, which was possibly attributable to the swab sample...
method failing to collect all bacteria present. However, one can assume the low sensitivity of bacterial detection was applicable to all carcasses, regardless of lesion status, thereby resulting in nondifferential misclassification, which would have biased findings in favor of the null hypothesis, making it more difficult to detect a significant difference.\textsuperscript{26} Indeed, the actual association between swine carcass \textit{Salmonella} contamination and lesion status would be stronger than measured in the present study (ie, \textit{POR} > 1.9). The culture method used in the present study also lacked a confirmation step, such as serotype detection, which increased the probability of false-positive results and therefore decreased test specificity. However, this would have resulted in nondifferential misclassification bias as well, with the same potential impact on our findings.

The present study showed that trained pathologists are not required to differentiate carcasses with lesions from those without lesions in the abattoir, providing a lower-cost method for continuing this type of research. The dichotomous (lesion vs no lesion) nonexpert assessment appeared adequate to predict \textit{Salmonella} contamination of swine carcasses. No significant (extend-\textit{ed Mantel-Haenszel} $\chi^2$ test, $P = 0.82$) trend of higher \textit{Salmonella} contamination was evident with increasing severity of lesions.\textsuperscript{27} The nonexpert detection protocol described here was easy to standardize; such that nonexperts with minimum training could produce consistent assessments that are consistent with judgments of experienced pathologists.

On-farm antimicrobial use and replicate identity were necessary covariates in the logistic regression models we developed. However, the objective of the present study was not to compare antimicrobial-free and conventionally raised pigs with regard to bacterial contamination of carcasses. Each of these groups represented a different population. The antimicrobial-free group included only faster-growing swine that never required antimicrobial treatment for illness, yet conventionally raised swine may have received antimicrobials for prevention, control, or treatment of clinical illness. Consequently, comparison of lesion and contamination status between antimicrobial-free and conventionally raised pigs should be avoided, particularly without detailed historical data such as morbidity and mortality rates, rate of gain, and feed efficiency for the live pigs. Because of the dramatically varied \textit{Salmo-}
nella prevalence among replicates, replicate was included in the models. This emphasized the importance of including large numbers of pig groups or production lots in studies designed to understand the dynamics of \textit{Salmonella} prevalence on swine farms. In a previous study,\textsuperscript{1} a pooled sample method was used because it was an efficient means to test a high number of carcasses.\textsuperscript{26,28} In the present study, we chose to collect swab samples from individual carcasses so findings could be matched with the carcass of origin.

The \textit{POR} of 1.9 might be perceived as a small increase in the probability of \textit{Salmonella} contamination associated with carcass lesions, compared with epidemiological standards.\textsuperscript{30} However, when applied to the 95 million pigs harvested/d in the United States,\textsuperscript{31} the implications for public health are nontrivial. For exam-

\begin{itemize}
\item a. Speci-Sponges, Nasco, Fort Atkinson, Wis.
\item b. Whirl-Pak, Nasco, Fort Atkinson, Wis.
\item c. Buffered peptone water, Becton-Dickinson, Franklin Lakes, NJ.
\item d. Seward Stomacher 3300, Worthing, West Sussex, England.
\item e. Enterococcosel agar plates, Difco, Becton-Dickinson, Franklin Lakes, NJ.
\item f. CNA agar plates, Difco, Becton-Dickinson, Franklin Lakes, NJ.
\item g. Difco, Becton-Dickinson, Franklin Lakes, NJ.
\item h. Tetrathionate broth, Becton-Dickinson, Franklin Lakes, NJ.
\item i. Xylose-lysine-tergitol (XLT4) agar plates, Becton-Dickinson, Franklin Lakes, NJ.
\item j. Brilliant green agar plates, Becton-Dickinson, Franklin Lakes, NJ.
\item k. Triple sugar iron agar, Becton-Dickinson, Franklin Lakes, NJ.
\item l. Motility indole lysine medium, Becton-Dickinson, Franklin Lakes, NJ.
\item m. XLSTAT, Addinsoft, New York, NY.
\item n. Microsoft Excel 2007, Microsoft Corp, Redmond, Wash.
\item o. SAS, version 9.2, SAS Institute Inc, Cary, NC.
\item p. PROC LOGISTIC, SAS, version 9.2, SAS Institute Inc, Cary, NC.
\item q. INFLUENCE option, SAS, version 9.2, SAS Institute Inc, Cary, NC.
\item r. IPILOTS option, SAS, version 9.2, SAS Institute Inc, Cary, NC.
\end{itemize}

\textbf{References}


9. Rostagno MH, Hurd HS, McKeen JD, et al. Prenatal exposure to air sacculitis load on poultry carcasses from birds with and without \textit{Salmo-}
nella-contaminated carcasses to the US food supply. A similar scenario\textsuperscript{13} for poultry has been described for the association between \textit{Campylobacter} and \textit{Salmo-}
nella-contaminated carcasses to the US food supply. A similar scenario\textsuperscript{13} for poultry has been described for the association between \textit{Campylobacter} and human foodborne risk. \textit{Foodborne Pathog Dis} 2008;5:649–657.


