RESEARCH REPORT

pork checkoff

PORK SAFETY

Title: Antibody to *Salmonella spp.*, culture status, and associated risk factors among slaughtered pigs. **NPB #98-146.**

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I. Abstract:

Antibodies to Salmonella in pigs can be detected, indicating that pigs were exposed to one or more serotypes of Salmonella. We measured antibodies to *Salmonella* spp. in 3,354 pigs from 145 commercial pig herds in the upper Midwest. A mixed-antigen ELISA test was applied to meat-juice samples, detecting antibody response to many Salmonella species. We classified pigs into relatively lower (category I) and higher (category II) antibody response, with the threshold set by a cluster analysis. Overall, 45.6% of pigs were in category II (95% CI 43.9-47.3%). The variation in within-herd prevalence was large (SD 30.6%), suggesting a diversity of Salmonella cycling patterns among farms. Prevalence of category II antibody response was a poor predictor of *Salmonella* culture prevalence at slaughter. Herd or barn factors associated with an increased risk of high antibody category II prevalence were partial slotted or solid flooring (OR 3.75, p=0.09), poor growth performance (OR 6.8, p = 0.08) and bird access to the barn (OR 4.48, p=0.05). Batch pig flow (OR .35, p=0.09) and access to rodents (OR 0.2, p = 0.07) were associated with a decreased risk of antibody category II status. These findings suggest that improved hygiene may reduce Salmonella cycling and/or exposure on farms. The apparent protective association with rodent exposure is unexpected and not biologically plausible. It is possible that farms with rodent exposure tended to have other, unmeasured characteristics that could account for the apparent protective association. Further study of these factors is needed to test their effectiveness at reducing Salmonella exposure and cycling.

II. Introduction:

The United States Dept. of Agriculture, Food Safety Inpection Service for the first time has implemented regulations for the reduction of *Salmonella* contamination on

carcasses. Although these regulations do not specifically address on farm levels of Salmonella, they may induce packers to purchase preferentially from producers with low *Salmonella spp.* prevalence. Producers currently do not have access to an adequate scientific base of knowledge to control Salmonella on farms reliably.

As exports gain increasing importance for the U.S. industry, control of Salmonella becomes an important issue both to increase the safety and reliability of pork, but also to avoid potential non-tariff trade barriers. One major exporter, Denmark, implemented a control program starting in 1994. Development of a science-based control program appropriate to the diverse U.S. industry is thus an urgent challenge.

Detection of antibodies to *Salmonella spp.* has been used as the focus of a major Salmonella reduction program on Danish farms. Currently approximately 800,000 meat samples are assayed for Salmonella antibody using an ELISA technique.[1] The program claims to have reduced Salmonella shedding of marketed pigs by nearly 50%.[2] However, the serotypes of Salmonella in the U.S. differ from those in Denmark. In particular, *S. cholerasuis*, the most common clinical isolate in the U.S., is not reported in Denmark. Thus, the application of the Danish test is not assured under U.S. conditions.

III. Objectives:

A. Describe the prevalence of exposure to *Salmonella* in commercial production based on the presence of antibodies in meat juice.
B. Determine the relationship of antibodies to *Salmonella* (detected from meat samples) to culture results from lymph nodes
C. Describe the characteristics of farms with low exposure to *Salmonella* and with culture negative status vs. those with both high exposure and positive culture status at slaughter.

IV. Procedures:

Lymph nodes and meat samples were collected among slaughtered pigs in studies. In study I,[3] a previously completed NPPC-funded study, lymph nodes, serum samples and meat samples from 15 pigs for each of 30 farms were collected. In study II,[4] a complete study funded by the Illinois Council for Food and Agricultural Research, meat samples and caudal mesenteric lymph nodes were collected from 30 pigs from each of 141 farms over a 12-month period. The lymph node samples are cultured for *Salmonella* following a standardized laboratory protocol. The resultant meat juice is collected after freezing and thawing.

Meat juice samples were analyzed for the presence of antibodies using a slight modification of an existing ELISA method.[5] [6] Briefly, the antigens used in ELISA were prepared using heat extraction, purification, and concentration techniques. The ELISA antigens were extracted from four strains, each representing a separate serotype: *S. typhimurium, S. enteritis, S. anatum, and S. choleraesuis.* These four serotypes were diluted in PBS and 100 □l was then added to 96 microtiter plate. Plates were incubated overnight at 4 ^{o}C,. then were washed and blocked with 1% bovine serum albumin, followed by washes. Meat juice was diluted 1:10 in diluent buffer. One-hundred □l of diluted meat juice was then added to each microtiter plate well and incubated at 37 ^{o}C for 1 hrs. After washing, 50 □l alkaline phosphatase conjugated rabbit anti-swine IgG diluted in diluent buffer was added to each well and the plate was incubated 37 ^{o}C for 1

hour. After washing, nitrophenyl phosphate was added as a substrate and incubated at room temperature. The reaction was stopped and optical density (OD) was measured using a dual wavelength system (test filter: 410, reference filter: 450nm). A single serum positive control was run in duplicate and two negative serum controls were added in every plate to evaluate the variability of an ELISA and to allow for the calculation of a serum/positive (SP) ratio. The SP ratio was calculated as the sample OD divided by the average of the positive control OD values for a given plate.

No published cut-off values were available at the time of writing to categorize samples as negative or positive for *Salmonella* antibodies. We classified meat juice antibody samples based on a cluster analysis completed as part of a separate project in our laboratory.[7] Briefly, SP ratios from 300 randomly selected samples were used in an unweighted pair group means cluster analysis. Groups were selected based on the relative distances identified by the cluster analysis. The mean value and standard deviation was calculated for the lowest group. The cutoff was set at this mean value plus two standard deviations, equal to a SP ratio of 0.363. Pigs exceeding this threshold were classified as antibody category II, and pigs with results below this threshold were classified as category I. This procedure has been reported using serum samples from our laboratory.[8] Although this approach does not provide solid evidence to categorize pigs as *Salmonella* exposed or unexposed, it can still be useful in risk factor assessment, which depends more so on relative than absolute differences in the outcome. The approach also allows the description of the variability in antibody response.

Herds were categorized as high responders if the prevalence of category II antibody responders exceeded the mean for all herds in the study. The relationship of antibodies to culture status was evaluated by correlation analysis. Using a standardized and pretested survey, herd and barn-level characteristics were collected for each farm. Potential factors affecting within herd high responder status were assessed by logistic regression. Candidate factors for inclusion were those that were biologically plausible and had at least five herds both with and without the risk factor in our survey. Only those factors with p-values less than 0.1 will be discussed in this report.

Results:

Objective 1:

Preliminary investigation of the serum assay suggested that the most appropriate dilution for serum was 1:160, and for antibody 1:10. Using this dilution resulted in most samples on the flat portion of the ELISA OD titration curve. These dilutions were used for all subsequent work.

A total of 3,354 samples was assayed from 145 herds for antibodies to *Salmonella* spp. From study one, 43 herds were assayed by ELISA for antibody to Salmonella spp. in both meat-juice and serum. From study two, 2,742 meat juice samples from 102 herds were run using the same assay. Culture results were available for mesenteric lymph nodes from the same groups of pigs from both prior studies.

The prevalence of culture positive samples was 0.123 overall (95% CI 0.435-0.584), and was 0.144 and 0.103 for studies I and II respectively. The distribution of culture prevalence was skewed, with a few farms having a relatively high prevalence (Figure 1).

The prevalence of antibody category II was 0.456, with the 95% confidence interval of 0.439-0.473. The distribution of within herd antibody category prevalence was bimodal, with most herds either less than 20% or greater than 50% prevalence (Figure 2). The standard deviation of within-herd prevalence was 0.306, suggesting a high degree of variation between farms. This may imply that farms have differing

patterns of exposure to *Salmonella* spp.

Future work is needed to set an objective cutoff value to aid in the interpretation of this test. Because of variation in serotypes, including both experimental and field-based evidence in the setting of the breakpoints will be important and assessing the performance of the test.

Objective 2:

The association between culture results and antibody prevalence was not significant ($r = 0.075$, $p = 0.46$). Since the distribution of culture prevalence was highly skewed (Figure 1), the data were transformed with an arcsin conversion. However, the arcsin data transformation, often appropriate for skewed prevalence data, did not provide more evidence for a relationship ($r= 0.06$, $p = 0.56$).

The low correlation between the two outcomes suggests either that they describe two different aspects of *Salmonella* infection, and provide little overlapping information, or that the tests inadequately describe Salmonella occurrence. For example, *Salmonella* shedding and culture positive status can be sporadic, groups may become culture positive near the time of slaughter, whether or not they had previous exposure. In these cases, we would not expect good correlation between the tests simply because the pigs have not had adequate time to develop measurable antibody Alternately, each test has limitations in accuracy describing the *Salmonella* status of herds. For example, the limited sensitivity of culture-based method results in an underestimate of *Salmonella* occurrence. Sensitivity and specificity of the SalAD test are not currently quantified in commercial ("natural") infection; however, low to moderate sensitivity or specificity could contribute to the lack of association between the tests found here. Finally, and most likely, the lack of association may be attributable to a combination of factors.

Objective 3:

Because of the low correlation between mesenteric lymph node culture prevalence and antibody prevalence, we elected to analyze antibody category prevalence separately from culture prevalence. The assessment of culture prevalence alone is part of both studies I and II, and so will not be reported here. The logistic regression analysis detected significant relationships ($p<0.1$) for the following factors: batch pig flow (OR .35, $p=0.09$), <100% slotted flooring (OR 3.75, $p=0.09$), poor growth performance (OR 6.8, $p=0.08$), rodents access (OR =0.2, $p=0.07$), and bird access (OR 4.48, $p=0.05$).

Logically, antibody test results should be higher among herds that have increased frequency of exposure to *Salmonella* spp. generally, and especially in the last 2-6 weeks prior to sampling at slaughter. Other potential causes of higher test results might be reduced diversity in *Salmonella* serotypes, or shedding of *Salmonella* serotypes that have produce antibody responses that produce lower reactivity on this test. Although in the balance of this report references to high prevalence of antibody test responders will be referred to as high exposure herds, the reader should bear in mind that this is not the only possible explanation.

Batch pig flow appears protective to *Salmonella* exposure. Farms practicing batch flow are able to rear pigs in age segregated groups and enforce tighter internal biosecurity. In addition, improved hygiene is correlated with batch flow. Consequently, the reduced antibody response may be attributable to batch flow / age segregation, increased cleanliness, or both. Similarly, slotted flooring appears to make an additional contribute to hygiene, beyond that of batch flow alone.

Reduced growth performance, in this model, was indicated by being either

selected for poor growth, such as among culled, underweight pigs, or by being selected as the last group of pigs marketed from a batch. Logically, the increased risk of antibody positive status may have caused poor performance, or poor performance may have caused an increase in antibody response. These data would support either interpretation. In addition to likely slower growth performance, these pigs may have been in the barn either longer (last pigs selected) or for a shorter period (among culled pigs). Increase risk of antibody positive status may indicate increased susceptibility to infection, and increase in the duration of shedding, an increased antibody response to infection, or a delay in the timing of infection to the weeks shortly before slaughter.

The exposure to birds resulted in an increased risk of antibody positive status. Birds can shed *Salmonella* spp., and it is possible that transmission was taking place on these farms. However, the ability of birds to enter the building also suggests other issues about building design and maintenance. For this reason, it is possible that exposure to birds also implies access to other non-swine species.

The apparently contradictory findings between the increased risk associated with exposure to birds and the decreased risk associated with exposure to rodents cannot be adequately explained by the data collected here. It is unlikely that exposure to rodents causes reduced exposure to Salmonella. In fact, both rodents and birds can harbor and transmit *Salmonella* infection. One possible explanation may be that exposure to rodents is confounded by exposure another unmeasured, strongly protective factor or factors.

Finally, all associations detected in this analysis are subject to error, especially since the p-values for association are in the moderate range, between a p-value of 0.05 and 0.10. The real value of these detected associations may be to spur additional, prospective work, and to be considered in light of the findings of other exploratory studies.

References

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