

**Title:** Determination of Salmonella Classification Levels for Selected Iowa Swine Herds and an Evaluation of Production Risk Factors for Classification Status - **NPB #02-187**

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**Abstract:** This study represents the first attempt to classify Midwestern production sites for *Salmonella* spp. sero-prevalence. The data suggest that the herds tested are similar in their distribution with respect to sero-prevalence of Salmonella as in Danish herds. Ignoring herd size, 90.2% of surveyed herds were negative or Level I, 8.2% were Level II herds, and 1.6% Level III. These results are similar to previous Danish studies (Alban et al., 2002; Mousing et al., 1997). Studies by Carstensen et al. (1998) suggested that herd size was statistically associated, albeit weakly, with *Salmonella* sero-prevalence, but the authors concluded the relationship was probably not biologically significant. The current data suggests that larger herds tend to have a higher sero-prevalence than smaller units; however, formal analysis has yet to be conducted to determine the direct association between herd size and salmonella sero-prevalence. Additional survey data is needed to evaluate this relationship.

The development and testing of survey documents for measurement of on-farm production practices is important to evaluation of on-farm risk factor analysis. The current survey vehicle demonstrated that producer interviews provided superior accuracy to producer self-assessment using the same questions. Common error sources were rooted in form and clarity of questions. Another important developmental hurdle was the limited agreement about on-farm risk factors by a pool of food safety and swine management experts. This limited expert agreement and the lack of "gold standards" to measure Salmonella exposure make evaluation of on-farm risk reduction strategies more difficult.

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**Introduction:** Salmonella infections are one of the three leading causes of human food-borne illness and deaths annually (Mead, 1999). Salmonellosis is recognized as an important zoonotic disease of worldwide importance, and is gaining status as a potential trade issue. The Centers for Disease Control and Prevention estimates 95% of human salmonellosis cases in the U.S. occur through food-borne transmission. Salmonella contamination reduction in meat products is a stated goal of USDA-FSIS under their HACCP implementation plan. They have imposed a pork industry performance standard of 8.5% isolation prevalence under the current plan. Recent statements by USDA-FSIS indicate potential for reduction of these national pork performance standards by up to 50% in the foreseeable future. Such a change would increase pressures on the pork chain to identify methods to further reduce pork contamination.

Our (ISU/NADC) research group has focused for the past four years on the farm to abattoir interface as an area requiring additional risk factors characterization and interventions development. We have demonstrated that the normal antemortem rest period in the abattoir is a major cause for Salmonella infections (5% vs. 39%) prior to harvest (McKean et al., 2001). Transport and lairage have been identified as risk potential factors in Salmonella contamination (Williams, L. P., and K. W. Newell, 1968; Hansen et al., 1964). A recent study demonstrated an increased isolation rate and diversity of serotypes when comparing on-farm and post-harvest Salmonella under two handling scenarios (Hurd et al., 2001). This increase was attributed to rapid infection as a result of exposure to the antemortem pen environment. These results are consistent with recent reports from the Netherlands. Abattoir lairage contributed 35% and 75% to tissue contamination cultures in sero-positive and sero-negative herds, respectively (Swanenburg et al., 2001), demonstrating that there may be a difference in Salmonella infections based on source herd status for market swine.

Since 1995, the Danish swine industry has conducted a Salmonella surveillance and control program to categorize production sites as to their risks for Salmonella contamination based on carcass meat juice antibody levels. This program has undergone several changes since initiation, but the concept of production-site risk categorization has remained a constant. Similar programs are being implemented in other European Union countries as Salmonella control strategies in pork. Currently, Danish herd classifications for Levels I, II, and III are approximately 95%, 3.3%, and 1.6%, respectively (Nielsen et al., 2001). This classification has been used to schedule transport and harvest of similar herd statuses to reduce antemortem cross contamination. It was reported that 94.4% of sero-negative Level I herds supplied animals with negative cecal cultures for Salmonella under this controlled harvest and transport production system. No similar systematic attempt to classify production sites in the USA has been attempted. This paucity of information hampers the ability to conduct farm to abattoir risk assessments.

In veterinary medicine, field-based questionnaires are frequently used in research to collect data for descriptive purposes or for risk factor determination. In production medicine, questionnaires may be used to determine if a farm has a high or low disease risk. These applications favor questionnaires for data collection because they are cost efficient. However, to be useful they must capture meaningful information accurately. Therefore, prior to use, questionnaires should be assessed for accuracy of information obtained. A questionnaire designed to capture information descriptive of facilities and routine management procedures associated with hygiene and biosecurity

protocols in swine production units, areas thought to be associated with exposure to and transmission of *Salmonella* spp., would be helpful to assist evaluation of Salmonella risk status of farms.

The four primary areas of questionnaire assessment are repeatability assessment, content, selected criteria applicability, and construct validation. Repeatability describes the capacity of a questionnaire to capture information consistently. Repeatability can be partitioned into internal consistency and external reliability. Asking the same question in a slightly different manner at different places in the survey and looking for the same answer tests for internal consistency, while re-administering the questionnaire to individuals and comparing responses tests the external reliability. To be useful a questionnaire must have a high degree of repeatability, and if repeatability cannot be demonstrated the questionnaire must be redesigned.

Content validation requires defining the questionnaire's domain, i.e. the topic area, and designing questions that will capture all the relevant information for that topic area. Obviously, if the questionnaire does not cover all the pertinent information required to capture the topic area, redesign is also required.

The remaining areas of questionnaire assessment are criteria or construct validity. Criteria validity compares questionnaire responses against the "true" answer. For questionnaires where the true answer is not available, such as quality of life issues, the extent to which the responses agree with the existing hypothesis can be compared; this is referred to as a construct validation. For our questionnaire, construct validity means responses would be associated with the introduction and transmission of *Salmonella* spp. Prior to examining construct validity, survey repeatability and content validity must be examined.

#### Objectives:

- 1) Determine the prevalence of serologic positive production sites in Midwest swine utilizing a commercial ELISA Salmonella test on selected meat juice samples collected for PRV case finding;
- 2) Categorize production sites using the new Danish classification scheme and compare with current Danish results; and
- 3) Develop and validate a production-based survey to use in future comparisons of selected sites classified as Levels I, II, and III under the Danish categorization scheme for potential production risk factors.

#### Materials & Methods:

*Classification survey:* An estimated 1,500 Midwestern production sites from seven Iowa-based abattoirs supplying samples for the PRV market swine surveillance program were surveyed to determine adequate sample size to complete the comparison with the Danish classification system. One selection criteria was designed to provide a substantial population most likely to utilize single site production practices. A separate subset of 30 producers readily identified as multi-site producers were selected for comparison purposes. The study population was finalized at ~1150 producers who submitted at least one lot (lot sizes – 20-180 head) per month – March to February 2002. During the test period the previously identified producers were flagged in the PRV database so that automatically, with each submission to an abattoir, their sample

identity could be marked utilizing the unique PRV laboratory number and appropriate samples taken. Four diaphragm samples were collected as a convenience sample from each lot submitted daily to the cooperating abattoirs as part of an Aujeszky's Disease surveillance program. A total of 1131 producers from this population met the one lot/week minimum sampling criteria at the end of the period. Each submitted, during a 12-week period April-June 2002, at least one lot/week, and samples were selected in the laboratory at the rate of up to 4 samples/producer/week. Where a producer submitted more than one lot per week only one lot (4 diaphragm samples) was selected, but the lot size for all submissions from these producers was recorded. These cumulative lot data were used to estimate the annual herd production for comparison with Danish populations.

*Production practices survey:* A survey to capture information on routine management procedures associated with hygiene and biosecurity and physical characteristics of the farm including flooring, housing type, ventilation type and type of animal raised was prepared. Information about factors associated with *Salmonella* spp. exposure which remain relatively static such as size of herd, feeding system, use of antibiotics and growth promotants, was collected, but not factors that vary rapidly over time, i.e. rodent pressure, number of visitors, etc.

The questionnaire was loosely based on a Danish instrument; however modifications were required to reflect USA production conditions. Additional questions were added based on a review of the literature and the opinion of five pre-harvest food safety or swine health management experts used for the content validation. The experts rated 37 categorical questions for relevance to *Salmonella* exposure. A question could be considered for inclusion in a questionnaire only if the all experts rated the it as relevant/highly relevant. The majority of questions required categorical responses (yes/no). Iowa State University Department of Statistics Survey Laboratory personnel evaluated the questionnaire with respect to reading level, absence of jargon and value-laden words, question length, and continuity of style.

The modified test-retest, conducted in June and July 2002, was administered to a convenience sample of 25 swine producers, who were clients of a cooperating Iowa veterinary practice. At each visit, a letter describing the aim of the questionnaire was provided to the producer with a request to complete the questionnaire alone (self-administered). Within 3 hours the questionnaire was administered (re-test) during a personal interview.

*Classification sample analysis:* Selected meat juice samples were tested using the IDEXX Swine Salmonella test kit<sup>®</sup>. Sample preparation, testing procedures, and the cut-off value for positive classification were designated by the manufacturer. Data was reported by lot identifier and the prevalence of positive samples/lot were recorded and held in a secure database.

*Statistical analysis - Classification data:* For the individual animal the prevalence and 95% CI were calculated using Proc Surveymeans (SAS 8.1<sup>®</sup>). For lots and farms the sampling probability was unknown, therefore Proc Means (SAS 8.1<sup>®</sup>) was used. Farms were classified based on their estimated annual production and monthly (4 week aggregated collections) sero-prevalence. Farms with <5000 annual production were classified: negative, Level I (0 % - <25 % positive), Level II (25-50%) and Level III

(>50%). Farms with >5000 annual production were classified: negative, Level I (0 % - <10 % positive), Level II (10-33%) and Level III (>33% positive).

*Statistical analysis - production survey data:* Derived from interviews, internal consistency was examined by determining the rank correlation between pre-determined pairs of questions related to biosecurity and hygiene protocols using Kendalls Tau-b. External reliability, examined using responses to the self-administered questionnaire and the interview matched by farm, was measured using a Kappa statistic which measures the degree of agreement beyond chance for categorical variables.

## **Results:**

*Classification:* From a total of 28,465 samples collected under the procurement algorithm, 26,325 were used in this analysis. The remaining samples were from herds that did not produce sufficient numbers within the sampling time period. Of those selected, 24,403 samples tested negative (92.7%) 1,915 (7.3%) positive and seven samples could not be tested because of insufficient sample volume. These 26,325 samples were collected from 6,521 harvest lots. In 1,229 (18.8%) lots at least one animal tested positive with an average lot prevalence of 38.8%. At least one positive animal was submitted from 506/1131 (44.7%) producers demonstrating that exposure to Salmonella during production is a common occurrence on a wide range of farms. These on-farm prevalence estimates do not demonstrate a link between significance for these exposures and Salmonella contamination of pork, but do provide a useful benchmark for future studies.

Farms with <500 head annual production were classified in the Danish system: negative - 232/319 (72.7%); Level I - 57/319 (17.8%); Level II - 25/319 (7.8%); and Level III - 5/319 (1.6%). Farms with between 500-5000 head were classified: negative - 371/784 (47.3%); Level I - 340/784 (43.4%); Level II - 61/784 (7.8%); and Level III - 12/784 (1.5%). For farms with >5000 head the classifications were: negative - 7/28 (25%); Level I - 14/28 (50%); and Level II - 7/28 (25%). In general there is a trend that larger annual production increased the likelihood that classification would be higher than in smaller herds.

*Production practices survey:* The questionnaire vehicle asked about facilities, hygiene protocols and biosecurity protocols. For the sets of general questions examined for internal consistency the correlation was <80%; however, there was a high correlation between responses to cleaning pens and rooms, i.e. >80% ( $p = 0.001$ ). There was also a 100% correlation between cleaning the site to a manure free stage and the use of disinfection ( $p = 0.001$ ). The responses between the self-administered and interview questions often agreed (Kappa point estimates from 0.52-1.00) relating to hygiene protocols and in-farm facility questions. Responses to questions about biosecurity protocols showed the greatest overall disagreement between the self-administered and interview questionnaire (Kappa statistic varied from 0.05 to 0.69). These significant variations indicated that few of the 37 questions would meet the general criteria for inclusion in a future questionnaire, i.e. 100% of the experts rated a question as three or four, and questions yielded repeatable and accurate estimations of the farm practices.

Overall the external consistency of the responses appeared reasonable, except for those questions relating to biosecurity practices. Questions about changing of clothes and footwear, hand washing, presence and use of shower-in facilities, and

quarantine facilities lacked repeatability. These response differences likely occurred due to the question form and lack of detail. Knowing that some responses are highly correlated with others allows the removal of duplicate questions which reduces the questionnaire length without reducing data collection.

The content validation study demonstrated that there was little agreement among the experts about the association between of the various risk factors and *Salmonella* exposure, and is consistent with another survey of a larger group of experts. This means that although the redesigned questionnaire can be used to collect data about the farms to investigate factors associated with *Salmonella*, it would be entirely inappropriate to use it to classify farms as high and low risk of *Salmonella* farms.

**Discussion:** This study represents the first attempt to classify typical Midwestern production sites for *Salmonella* spp. sero-prevalence using the Danish classification system. The data suggest that Midwestern herds are similar in their distribution with respect to sero-prevalence of salmonella as in Danish herds. Ignoring herd size, 89% of herds were negative or Level I, 9.5% Level II herds, and 1.5% as Level III. This is very similar to the results reported by Mousing et al., (1997): 93% of Danish herds were negative or level I, 3.9% were Level II, and 2.3% were Level III. These data do suggest that larger herds may tend to have a higher sero-prevalence. This interpretation however is limited by the fact that few herds are classified as large in this dataset – only 28. This observation follows a similar pattern reported in Danish studies in 1997 (Mousing et al., 1997) and by Carstensen et al. (1998) which suggested that herd size was statistically associated with *Salmonella* sero-prevalence in Danish herds but the authors concluded the relationship was probably not biologically significant. This analysis has yet to be conducted on USA herds to determine the ecology of salmonella, but should be completed to assist in refinement of risk analyses.

By formally assessing the questionnaire attempts can be made to improve validity of the data collected in a modified questionnaire for a larger risk factor study looking for associations between *Salmonella* spp. introduction and transmission to the type of facilities and hygiene and biosecurity protocols. The main modifications of our questionnaire should include (1) clarification of terminology by using interviews as a method of questionnaire delivery and (2) removal of highly correlated questions. The most serious limitation of any questionnaire, however, has not been addressed by this study, and that is the true measure of accuracy or criteria validation. Currently no gold standard exists to compare the responses. Therefore, one cannot be sure the responses from either the self-administered or interview questionnaire accurately reflects true characteristics for *Salmonella* risk. Given our experiences with the two questionnaire delivery methods, interviews lead to better data quality than self-administered documents. We will develop a questionnaire to be interview based for on-farm practices evaluation.

**Lay Summary:** This study represents the first attempt to classify typical Midwestern production sites for *Salmonella* spp. sero-prevalence using the Danish classification system. With 1,131 farms represented, this data provides a useful snapshot of Midwestern *Salmonella* prevalence and can be used as a benchmark for future efforts. The data suggest that Midwestern herds are similar in their distribution with respect to *Salmonella* sero-prevalence classifications as Danish herds. It also indicates that a

significant percentage of farms present a small number of market swine for harvest that have experienced prior *Salmonella* exposure

This study compared information obtained from production practice questionnaires generated by producer self-administration and by interview. Interview-based applications gave better repeatability and content validity. The main modifications of the current questionnaires should include: (1) clarification of terminology by using interviews as a method of questionnaire delivery and (2) removal of highly correlated questions. When modifying a production practices questionnaire, efforts should be made to clearly capture farm structure differences between on-site and off-site production systems, and how they affect biosecurity responses. Another area for improvement in future questionnaires is the use of common terminology.

The most serious limitation of any questionnaire has not been addressed by this study, and that is the true accuracy or criteria validation. No gold standard exists to compare the responses with on-farm *Salmonella* exposure, and therefore, the responses from either the self-administered or the interview questionnaire may not accurately reflect true on-farm *Salmonella* risks.

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