EVALUATION OF SAMPLE WEIGHT FOR THE ISOLATION OF

*Salmonella* SPP. FROM SWINE FECES.

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In many countries, contamination of meat and poultry products with bacteria potentially pathogenic to humans has become a major public health and trade concern. In 1996, there were significant legislative changes to the regulation of meat inspection in the U.S.A.. The new inspection procedures include mandatory microbiologic testing for 'generic' *Escherichia coli* and *Salmonella*, with specified standards for acceptable process control. As a result of these regulatory changes there has been an interest in identifying control measures at the farm level (pre-harvest) to reduce the risk of bacterial contamination of meat products.

To identify control measures for *Salmonella* on swine farms, an understanding of the epidemiological patterns of *Salmonella* infection and shedding on farms is necessary. Although there have been numerous cross sectional and some longitudinal studies of *Salmonella* prevalence or transmission in swine populations, there is great difficulty in comparing results due to different methods employed (including the sample taken, sample weight or volume, and isolation techniques).

In order to select methods to be used in longitudinal studies of the epidemiology of *Salmonella* on swine farms, we investigated the effect of sample weight on the relative sensitivity for detecting *Salmonella* in swine feces. Knowledge of the relative sensitivities of different weight samples would be useful for 1) comparing results of epidemiologic studies that have employed different methods, 2) comparing data within studies when sample weight may vary (samples from suckling pigs vs. finishing pigs), and 3) defining preferred methods when designing a study.

To our knowledge, there are no reports comparing the relative sensitivity of different sample weights for the detection of *Salmonella* in swine feces. McCall. et al demonstrated that fecal specimens (3-10g) were more sensitive for isolation of *Salmonella* from humans than rectal swabs, especially in sub-clinical, chronic carriers. Post-mortem comparisons of cecal swabs vs. cecal content samples have been conducted in swine. Newell et al. compared cecal swabs and cecal contents of 100 individual pigs at slaughter and isolated *Salmonella* from 6% of cecal swabs and 23% of cecal contents. Harvey et al. compared cecal swabs (0.5g) and 80g of cecal contents.

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from 814 swine at slaughter. Of 88 positive swine, 78 were identified with cecal contents as the sample, and 40 identified by cecal swab. Baggesen and Wegener reported that 5g cecal samples have a sensitivity of 0.5-0.6 for the isolation of *Salmonella* in naturally infected swine.

Comparisons of the relative sensitivity of individual rectal swabs and pooled fecal samples have been reported. Heard and Linton suggested the use of both rectal swabs (0.5g) and pooled fecal samples for identification of *Salmonella* carrier swine, as their results showed that of seven pens identified as containing swine shedding *Salmonella*, only two were identified by both individual rectal swabs and pooled pen fecal samples. Pooled feces identified 5 positive pens and rectal swabs identified 4 positive pens. Haddock compared rectal swabs to pooled pen samples in 16 market age pigs and found pooled samples identified additional *Salmonella* serotypes not isolated using rectal swabs. Ishiguro et al., noted that 3.5% of rectal swabs from market pigs were positive for *Salmonella*, while 30% of pen pooled samples (0.5-10g) were positive. Fedorka-Cray, et al. demonstrated that group pooled fecal samples were more sensitive than individual rectal swabs for isolation of *Salmonella typhimurium* from the feces of experimentally infected swine, and those infected by commingling with swine shedding *S. typhimurium*. Although pooled samples appear to be more sensitive for detection of *Salmonella* shedding in a group of animals, it may result in the loss of information important for longitudinal investigation of *Salmonella* transmission.

**Materials and Methods**

Four different sample weights of swine feces were compared for sensitivity of isolation of *Salmonella*. Swabs and fecal samples were collected per rectum from 226 individual sows or market pigs at two different farms. Farms were selected for inclusion in the study based on a history of high prevalence of pigs shedding *Salmonella* in the feces. Fecal samples (> 80g) were first divided into two sub-samples (>40g each) collected separately. One of the two sub-samples was stomached for 45 seconds. Each of the two sub-samples were then divided into 1g, 10g and 25g weights. The choice of fecal sample weights were based on current practices in our laboratory or by other investigators in the U.S.A.. All samples were then diluted 1:9 in 2% buffered peptone water (BPW) and incubated at 37°C for 16-20 hours. Swabs were incubated similarly in 10 ml of BPW. A 100μl aliquot was transferred to 9.9ml of Rappaport-Vassiliadis R10 broth and incubated at 42°C for 24 hours. Samples were then subcultured onto XLT4 agar and incubated overnight at 37°C. Colonies with morphology consistent with *Salmonella* were further identified using triple-sugar-iron agar and urea agar slants. Serotyping of isolates identified as *Salmonella* was conducted by National Veterinary Services Laboratory, Ames, IA.
Results and Discussion

Studies completed at the time of writing consistently indicate that sensitivity of detection increases with sample weight, and that the magnitude of effect across the range of weights (swab-25g) is sufficient enough to be of practical importance in the design and interpretation of epidemiologic studies. Because of costs and logistic problems inherent in handling large weight samples, the optimal approach may vary with the purpose of an individual study. Detailed results of this research will be presented at the time of the meeting.

References


