

Effects of some methodologic factors on detection of *Salmonella* in swine feces

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Abstract

Bacteriologic culture of feces for *Salmonella* continues to be a central component of epidemiologic studies. We conducted a series of experiments on fecal samples collected from commercial swine farms to evaluate the effects of several methodologic factors on detection of *Salmonella*. Factors examined included fecal sample storage (no storage, 4C, -15C) and fecal sample weight. In addition we compared the standard method (Method 1) used in our laboratory [10g feces/buffered peptone water pre-enrichment/selective enrichment in Rappaport Vassiliadis (RV) broth] with another method (Method 2) used by ourselves and others in the USA (~1g sample/primary enrichment in tetrathionate and Hajna GN broths/secondary enrichment in RV broth). Immediate processing of samples yielded the best recovery of *Salmonella*, although storage at 4C for 6 days did not significantly reduce detection. Freezing of fecal samples resulted in significant reduction of detection. The weight of feces sampled had a marked linear effect on the detection of *Salmonella* using method 1. Direct comparison of Method 1 and Method 2 indicated comparable results, with Method 1 tending to yield higher detection of *Salmonella*. However, when conducted on samples of equal weight, Method 2 had significantly better detection than Method 1. The choice of methods can markedly affect the results of fecal sample culture. The preferred methodology for epidemiologic studies will be determined by many factors including logistics and cost. Our data highlight the imperfect sensitivity of culture methods, and the need for researchers to consider the sensitivity of their bacteriologic methods in the design and interpretation of field studies based on fecal culture.

Introduction

In response to the increasing emphasis on food safety in domestic and international markets for pork, several groups in the USA have recently embarked on epidemiologic studies of *Salmonella* in swine, many of which have been funded by national and state pork producer groups. Despite

advancement in newer diagnostic methods, conventional bacteriologic culture remains the foundation of epidemiologic studies of *Salmonella* spp.¹ A plethora of studies have compared microbiologic techniques for isolating *Salmonella* from diverse materials, and conflicting findings among studies abound. The scope of this methodologic dilemma is illustrated by the finding of a survey of 74 US laboratories culturing poultry tissue and environmental samples for *Salmonella*. No 2 laboratories used identical protocols, and the authors reported the use of 17 different selective enrichment media or combinations of enrichment media, considerable variation in the duration and temperature of selective enrichment, and the use of 14 different plating media.² The authors argued there was a critical need for some standardization of laboratory protocols, and the need to standardize procedures, or quantify differences among procedures was a common conclusion from break-out sessions at the 1st International Meeting on the Ecology of *Salmonella* in Pork Production in 1996.³

Disparate results of *Salmonella* prevalence in finishing pigs reported in studies by our respective groups.^{4,5} prompted the methodologic studies reported here. Factors that may have contributed to these varied findings include delay in sample processing and the use of different laboratory protocols. Differences in laboratory methods include 1) weight of fecal sample (25g or 10g vs. approximately 1g); 2) Preenrichment in BPW in method 1 vs. direct enrichment into TTB and Hajna broths; and 3) Incubation of RV broth at 42C vs. 37C. The objective in this paper was to compare the effect of some of these factors on detection of *Salmonella* in fecal samples from commercial swine farms.

Materials and Methods

The effects of storage of fecal samples on the recovery of *Salmonella*

Fecal samples weighing approximately 40 g each were collected from 84 gilts recently introduced into a commercial farrow-to-wean farm in eastern North Carolina. Fecal

samples were placed in sterile plastic bags, and transported for approximately 2 hours at ambient temperature to our laboratory. At the laboratory, the fecal sample from each gilt was divided into 3 sub-samples of approximately equal weight for 3 treatments: same day—samples processed on the day of collection; 6-day—samples stored at 4C for 6 days; or frozen—samples stored at -15C for 14 days. After the assigned storage treatments, all samples were processed similarly. Each fecal sample was weighed and reduced to approximately 10g (range, 8.5 to 11.5). To detect *Salmonella* organisms, fecal samples were diluted 1:9 by weight with BPW and incubated overnight at 37C. A 0.1 ml aliquot of the fecal-BPW solution was transferred to 9.9 ml Rappaport-Vassiliadis broth (RV) and incubated in a water bath at 42C for 24 ± 2 hours. A loopful of RV was plated on xylose-lysine-tergitol agar (XLT4) and incubated at 37C overnight. One colony per XLT4 plate with morphology consistent with *Salmonella* spp. was transferred to triple sugar iron and Christensen's urea agar slants. Isolates presumptively identified as *Salmonella* spp. were submitted for serotyping at the USDA National Veterinary Services Laboratory, Ames, IA..

The effect of fecal sample weight on the recovery of *Salmonella*

Rectal swabs and fecal samples were obtained from individual pigs housed at commercial farms in North Carolina. Rectal swabs were placed in 9 ml 2% buffered peptone water solution^a (BPW) for transport to the laboratory. Fecal samples were placed in sterile bags^b and stored at ambient temperature for transport to the laboratory. Samples were divided into weight categories at the laboratory. All samples were processed the day of collection. A total of 228 animals were sampled, comprising 178 sows and 50 finishing pigs. For all animals 1g, 10g and 25g samples were obtained. Data from all trials with like sample weight categories were pooled for analysis. Bacteriologic culture was performed as described above.

Comparison of isolation methods

We conducted a series of 5 experiments: 4 experiments comparing enrichment protocols in our laboratory, and 1 collaborative study with Dr. Cray's laboratory. In total, fecal samples were collected from 554 pigs on 5 commercial farms in North Carolina (96 to 121 pigs per farm). For the 4 'in house' trials, one loopful (using small animal fecal loops) of feces from the plastic bags was placed into each of 3 culture tubes, containing GN Hajna broth, sodium tetrathionate broth (TTB) or 2% buffered peptone water. A fourth treatment involved diluting approximately 10g of feces with 2% BPW solution in a 1:9 ratio by weight in plastic bags (method 1 – standard method in our laboratory). All samples (excluding those in TTB) were incubated overnight at 37C. For the TTB samples, the incubation period was 48hr. A 0.1ml aliquot was transferred to 9.9ml of RV broth and incubated at 37C (for the Hajna and TTB

samples) or at 42C (for the loopful and 10g BPW samples) for 24 hr. Each RV broth culture was streaked on xylosine-lysine-tergitol-4 agar and brilliant green sulfur agar plates which were incubated overnight at 37C. In the collaborative study with Dr Cray's laboratory, paired sets of samples were processed in each laboratory using both isolation methods (method 1: 10g feces/BPW/RV/XLT4); method 2: fecal loop/TTB and Hajna/RV/XLT4 and BGS) described previously.^{6,7} Broths from method 1 were streaked on XLT4 plates only, while broths from method 2 were streaked on both XLT4 and BGS plates (in accordance with current standard procedures at the 2 laboratories). Serotyping results from this study are pending.

Results

The effects of storage of fecal samples on the recovery of *Salmonella*

The results of this study have been reported elsewhere in full.⁸ Briefly, recovery tended to be highest for same-day processing, but the difference in the proportion of positive samples was not significant between same-day processing and storage at 4 C for 6 days. Storage at -15 C for 14 days resulted in a lower proportion of culture-positive samples, compared with same-day processing ($P = 0.02$, McNemar's chi-square test) or storage at 4 C for 6 days ($P = 0.08$).

The effect of fecal sample weight on the recovery of *Salmonella*

In all 3 comparison groups, there was a statistically significant difference among the fecal weight groups in the proportion of animals detected as positive for shedding *Salmonella* ($p < 0.001$, Cochran's Q test). The results of the study are being published in detail elsewhere,⁹ but summary data from one comparison (Table 1) are included to illustrate the marked effect of fecal sample weight on *Salmonella* detection. Trials including fecal swabs showed the detection was markedly poorer with swabs than fecal samples.

Table 1. Number of pigs positive and relative sensitivity for *Salmonella* detection by fecal sample weight (n=228)

Fecal Sample Weight	1 g	10g	25g
Number of Positive Fecal Samples	26	51	56
Relative Sensitivity	0.32	0.64	0.70

Comparison of isolation methods

Overall, pooling the results of the 4 experiments, method 1 (94 positive samples) yielded slightly more positive samples than did method 2 (91 positive, combining results of TTB and Hajna), but the difference was not statistically

significant. Confirmation of the results of the inter-laboratory study are pending and will be presented in full at the meeting. Interim results again indicate slightly more samples were positive using method 1, but again the difference was not statistically significant ($P = 0.25$, McNemar's chi-square). Using the kappa statistic as a measure of agreement, the results indicated closer agreement between laboratories within methods (kappa = 0.57, 0.62) than between methods within laboratories (kappa = 0.45, 0.55).

Discussion

To identify control measures for *Salmonella* on swine farms, an understanding of the epidemiological patterns of *Salmonella* infection and shedding on farms is necessary. Conventional bacteriologic culture remains the foundation of epidemiologic studies of *Salmonella* spp. and numerous studies have compared microbiologic techniques for isolating *Salmonellae* from a range of sources. Sampling and bacteriologic methods have varied greatly among previous epidemiologic studies of *Salmonella* in swine populations, greatly limiting the ability to compare results. At a time when considerable investment is being made in on-farm *Salmonella* research in the USA, we felt it was important to conduct some methodologic studies that would assist in the design and interpretation of such studies. These studies were based on 2 methods that have been used in the majority of recent published research in the US.

The most important implications from these studies are:

- Methodologic factors can markedly affect results
- The 2 standard methods involved yielded similar results, and results of the 2 laboratories did not differ significantly. This means that differences in results of previous studies from our laboratories are likely not attributable to methodologic differences
- Field samples can be stored for several days at 4C with minimal reduction in detection.
- Fecal sample weight markedly affects results with method 1, and may also with other methods.
- Delayed secondary enrichment increased detection by approximately 25% (data not shown), but requires considerably more resources.
- All methods have imperfect sensitivity, and researchers should consider compensating for this with increased sample size (e.g. for detection of positive herds), and also address this issue when interpreting results

An inescapable conclusion from reviewing the literature on *Salmonella* detection is that increased diagnostic endeavor, be it through more intensive sampling or the use of multiple enrichment broths or plating media, leads to

increased detection. Investigators need to weigh the costs of increased diagnostic effort against the expected gain in sensitivity of *Salmonella* detection in relation to the objectives of their studies. It would be imprudent to recommend standardized bacteriologic procedures for studies that are likely to have different aims and constraints. However, haphazard adoption by different investigators of the almost countless methodologic options does present a barrier to reconciliation of findings from different studies. Our fortuitous result that the different methods used in our respective laboratories yielded similar results provides a potential benchmark for other swine researchers in the USA. We suggest that adoption of methods for isolating *Salmonella* from swine feces that differ from those evaluated in this study should be accompanied by some assessment of their relative sensitivity for detecting *Salmonella* compared to one of the methods described here. Such a framework would provide flexibility for all researchers yet maintain some basis for comparison of results from different studies.

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