Effect of buffered peptone water pre-enrichment on detected prevalence of Salmonella in swine feces

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Sensitive and specific detection methods are important to understand the epidemiology of Salmonella and to develop appropriate control strategies. The risk of Salmonella contamination of pork is associated with subclinical Salmonella infections in pigs. In most epidemiological investigations, microbiological culture has been used to deterimine Salmonella infection status for infections on swine farms or at slaughter plants. However, identification of Salmonella by culture among subclinically infected pigs may be highly influenced by the intermittent shedding status of pigs. The types and volume of samples used for culture as well as culture protocol used in microbiological examination can influence the sensitivity of the method (8).

Numerous laboratory culture techniques have been used for detecting Salmonella from variety of sources including foods and environmental samples (2). Application of non-selective pre-enrichment, such as buffered peptone water (BPW) broth has been widely recognized as a necessary preliminary step to restore damaged Salmonella cells in food samples. Pre-enrichment procedure using BPW has been used to enhance Salmonella isolation from swine feces as well (3). Alternatively, fecal culture results have been reported for a nationwide U.S. Salmonella survey using a two-step enrichment in which a pre-enrichment procedure was not used (10).

In general, it was suggested that culture of feces would give better detection rate for Salmonella than other materials including environmental samples and feed, in which Salmonella are likely to be damaged due to lack of moisture and potential effect of disinfectants (8). Thus, it is possible to apply enrichment media directly for detecting Salmonella from swine feces. Application of direct enrichment reduces the time required for identification of Salmonella compared to a protocol including BPW pre-enrichment. Further, higher sensitivity may be achieved by effectively suppressing the growth of other, non-*Salmonellae* bacteria that multiply following incubation in a pre-enrichment broth.

We designed this study to compare the effectiveness of adding a BPW pre-enrichment step to the reference procedure, a two-step selective enrichment protocol, and to assess the relative sensitivity of each of culture methods for detecting Salmonella in swine feces.

Materials And Methods

We collected samples from 300 individual fecal pats at a commercial Illinois slaughter plant. Two culture protocols were run in parallel on paired subsamples. We chose the sample size to provide >90% power of detecting differences between the two different culture methods at a of 0.05, assuming the population ratio of discordant pairs estimated in our preliminary work (4.3) (11).

Fecal samples were collected from the floor of lairage. The sampling unit was the abattoir pen, each representing one farm only. We used a convenient sampling scheme where we collected a maximum of 10 pigs from each available pen, resulting in 30 samples a day. Pens with fewer than 10 pigs were excluded from the study. Fresh, undisturbed fecal pats were sampled directly from the pen floor. Every attempt was made to collect feces as fresh as possible, presumably originating from a single pig to avoid mixing with other feces from other pigs or other contaminated materials. At least 25 g were collected for each sample using disposable spoons. Samples were placed on ice, transported to the laboratory via overnight delivery, and processed within 48 hours after collection. (10)

Each sample was divided into 2 portions, then cultured with and without a pre-enrichment step. For the pre-enrichment procedure, ten grams of feces were inoculated in 100 ml 2% BPW, then incubated for 24 hours at 37 C. Aliquots (0.1 ml) were transferred to 10 ml tetrathionate (TT) broth and incubated for 24 hours at 37 °C. One ml was transferred to 10 ml Rappaport – Vassiliadis (RV) enrichment broth, and incubated for 24 hours at 37 °C. A 0.1 ml aliquot was transferred to Xylose Lysine Tergitol – 4 (XLT-4) agar plate and struck for isolation. Suspect colonies were transferred to a brilliant green agar (BGA) plate to evaluate lactose production. All plates were incubated for 24 hours at 37 °C. Suspect colonies were confirmed with serum agglutination using polyvalent O antiserum to Salmonella. The reference procedure was the same as described in the pre-enrichment procedure, except that 10 grams of feces were transferred directly into 100 ml of tetrathionate broth, omitting the BPW step.

The association between the pre-enrichment and the enrichment procedure was evaluated using commercial software by McNemar's chi-square test for matched samples, with a critical p-value at 0.05. (EpiInfo 6 version 6.04a). A

sample was defined as an apparent true positive for Salmonella when Salmonella was identified in either cultural procedure. The apparent sensitivity of each procedure was measured based on the number of samples positive for Salmonella at each procedure over the total number of samples identified as positive in either procedure.

Results

Of 302 fecal samples collected, 56 samples (18.5%) were positive for Salmonella by at least one of the procedures (Table 1). Overall prevalence of the pre-enrichment procedure was 11.9% (36/302), while the reference procedure showed 14.6% (44/302), a difference of 2.7%. The apparent sensitivity of pre-enrichment procedure was 64.3% (36/56), compared to 78.6% (44/56) for the reference procedure using the direct enrichment. The McNemar's chi-square was 1.53 with p of 0.22, indicating no significant difference in detected prevalence between two procedures.

Table 1. A comparison of a direct enrichment (reference procedure) and pre-enrichment Salmonella culture procedures on paired fecal samples.

		Direct enrichment		Total
		+	–	
BPW	+	24	12	36
BPW	–	20	246	266
Total		44	258	302

Discussion

The results of this study suggest that the pre-enrichment with BPW provide no advantage over the direct enrichment in identifying Salmonella from swine feces. Further, the BPW pre-enrichment tended to be less sensitive, detecting a lower Salmonella prevalence compared to the direct enrichment procedure. This agrees with a report suggesting that a direct enrichment method increased detection of Salmonella in swine feces versus pre-enrichment methods (4).

This study differed from our approach in two ways that may contribute to difference in result. First, Edel and Kampelmacher reported a single step direct enrichment protocol, while we evaluated a two-stage enrichment protocol. Second, they cultured swine feces artificially contaminated, compared to field samples in our study.

Several factors may account for our findings. First, the type of samples we compared is less likely to have stressed the organism as compared with food and environmental samples. For stressed organisms, pre-enrichment may be more important, since the *Salmonellae* may have suffered sublethal injury (5). However, since fecal samples were collected shortly after defecation, the number of Salmonella in swine feces may have suffered little damage compared with food samples. Thus, pre-enrichment may be less helpful in fecal samples, when compared with food or environmental samples. Second, the tendency toward higher detection rate of the direct enrichment in this study may be due to overgrowth in BPW of competitive bacteria in feces, leading to compromise of the sensitivity and specificity of enrichment media followed (2).

The overall prevalence of Salmonella detected here (11.9%) was almost twice as high as found in the nationwide Salmonella control study in Denmark (6.2%) (1), while it was also less than half the amount (24.6%) detected in a study conducted in North Carolina (USA) (3). These discrepancies may be attributable to relatively small sample size of this study, differences in culture protocol, differences in the size of sample (7), differences in the types of sample (1), or regional differences in *Salmonella* prevalence. Davies et al. used 25 g of feces diluted 1:9 in 2% BPW, while Baggesen et al. cultured 5g caecal contents in 2% BPW, compared to 10 g feces used in this study.

The measured prevalence of Salmonella from this study also was different from the other work using exactly the same double-enrichment protocol with no pre-enrichment. The fecal prevalence of this study was 14.6%, which was higher than 9.7% shown in other study using the same culture procedure, but larger number of samples (1,072 individual feces) (8).

In summary, the addition of pre-enrichment step using 2% buffered peptone water to a double enrichment procedure generated no increased detection of Salmonella in feces of market weight pigs. Since both protocols detected similar levels of Salmonella, the simpler direct enrichment protocol is preferred for fecal culture.

References

1. Baggesen, D. L., H. C. Wegener, F. Bager, H. Stege, and J. Christensen. 1996. Herd prevalence of *Salmonella enterica* infections in Danish slaughter pigs determined by microbiological testing. Preventive Veterinary Medicine. 26:201-213.

2. D'Aoust J-Y. 1989. Salmonella, p.382-393. *In* M. P. Doyle(ed.), Foodborne Bacterial Pathogens. New York, Marcel Dekker.

3. Davies, P. R., W. E. M. Morrow, F. T. Jones, J. Deen, P. J. Fedorka-Cray, and I. T. Harris. 1997. Prevalence of salmonella in finishing swine raised in different production systems in North Carolina, USA. Epidemiology and Infection. 119:237-244

4. Edel, W., E. H. Kampelmacher. 1969. Salmonella isolation in nine European laboratories using a standardized technique. World Health Organization Bulletin. 41:297-306.

5. Edel, W., E. H. Kampelmacher. 1973. Comparative studies on the isolation of "sublethally injured" salmonellae in nine European laboratories. World Health Organization Bulletin. 48:167-174.

6. Fedorka-Cray, P.J., E. Bush, L. A. Thomas, and J.T. Gray. 1996. Results of the 1995 NAHMS Swine grower/finisher survey, p496-500. *In* proceeding of the 100th United States Animal Health Association meeting. Little Rock, Arkansas, USA.

7. Funk, J. A., P. R. Davies, and M. G. Nichols. 1997. Evaluation of sample weight for the isolation of Salmonella spp. from swine feces, p97-99. *In* Proceedings of 2nd International Symposium on the Epidemiology and Control of Salmonella in Pork. Copenhagen, Denmark.

8. Kim, J. Y., P. B. Bahnson, H. F. Troutt, and R.E. Isaacson. 1999. Salmonella prevalence in market weight pigs before and after shipment to slaughter. submitted. *In* Proceedings of 3rd International Symposium on the Epidemiology and Control of Salmonella in Pork. Washington D.C., U.S.A.

9. Nielsen, B., and D. L. Baggesen. 1997. Update on laboratory diagnosis of subclinical salmonella infections in pigs, p19-31. *In* Proceedings of 2nd International Symposium on the Epidemiology and Control of Salmonella in Pork. Copenhagen, Denmark.

10. O'caroll, JM., P. R. Davies, M. T. Correa, and B. D. Slenning. 1999. Effects of sample storage and delayed secondary enrichment on detection of *Salmonella* spp in swine feces. American Journal of Veterinary Research. 60:359-362.