Comparative evaluation of *Salmonella* detection assays in swine feces.

Marcos Rostagno\(^1,2\), Scott Hurd\(^2\), Jared Gailey\(^2\), James McKean\(^3\), Rômulo Leite\(^4\)

\(^1\)Federal University of Lavras, Department of Veterinary Medicine, Caixa Postal 37, CEP.37.200-000, Lavras, Minas Gerais, Brazil, Phone/Fax: 35-3829-1148, E-mail: rostagno@ufla.br
\(^2\)National Animal Disease Center: ARS: USDA, Ames, Iowa, USA.
\(^3\)Iowa State University, College of Veterinary Medicine, Ames, Iowa, USA.
\(^4\)Federal University of Minas Gerais, Veterinary College, Belo Horizonte, Minas Gerais, Brazil.

Summary: The performance of four commercially available *Salmonella* detection assays were comparatively evaluated for detection of *Salmonella* in swine fecal samples. The detection assays included two antigen capture enzyme-linked immunosorbassays (ELISA 1 and 2), a DNA hybridization assay, and an immunochromatographic assay. For the “gold-standard”, the combination of results from two isolation methods were used. Briefly, both methods included pre-enrichment (Tetrathionate or Rappaport-Vassiliadis broth), enrichment in Rappaport-Vassiliadis broth, and plating on XLT-4 agar. The sensitivity and agreement (Kappa statistics) with the “gold-standard” for each evaluated detection assay were: 87.36% and 0.64 for the ELISA 1; 98.85% and 0.96 for the ELISA 2; 97.70% and 0.92 for the DNA hybridization assay; and 80.46% and 0.48 for the immunochromatographic assay. From these results, it can be concluded that there are good *Salmonella* detection assays currently available, that could be useful in investigations using clinical samples, like swine feces.

Keywords: *Salmonella*, Swine, Detection assays.

Introduction: Due to the increasing emphasis on food safety in the pork market, several studies on the epidemiology of *Salmonella* in swine have been done during the last years. These investigations require accurate means of discriminating between infected and non-infected animals, which is dependant on detecting evidence of the agent in clinical samples, usually feces. This detection can be a major problem with *Salmonella*, as epidemiologic studies generally require testing large numbers of samples or pigs, and conventional bacteriological methods for its isolation and identification are labor intensive and time consuming. Several *Salmonella* detection assays, which use different principles for detection are commercially available. They were developed for use in the food industry. However, they may have application in preharvest food safety research. In this study, the performance of four commercially available *Salmonella* detection assays were comparatively evaluated for detection of *Salmonella* in swine fecal samples.
Material and Methods: *Salmonella* detection assays were comparatively evaluated for detection of *Salmonella* in swine feces, using 10g samples (n=100). For the “gold-standard”, the combination of results from two conventional bacteriological isolation methods were used. Briefly, both methods included pre-enrichment (Method A: 100mL of Tetrathionate broth; Method B: 100mL of Rappaport-Vassiliadis broth, 24h), followed by enrichment in 10mL of Rappaport-Vassiliadis broth (24h), and plating on Xylose-Lysine-Tergitol-4 (XLT-4) agar (24h). Suspect colonies (up to 3 colonies per plate) were biochemically identified as *Salmonella* or non-*Salmonella*, using the BBL Crystal Enteric/Nonfermenter identification system. The detection assays evaluated in this study included two antigen capture enzyme-linked immunosays (ELISA 1: TECRA *Salmonella* Visual Immunoassay; and ELISA 2: Assurance Gold *Salmonella* EIA), a DNA hybridization assay (Gene-Trak), and an immunochromatographic assay (Path-Stik). The cut-off values (O.D. values) applied for the enzyme-linked immunoassays were 0.3 for ELISA 1, and the average of 2 positive controls multiplied by 0.25 for ELISA 2, as recommended by the respective manufacturers. The enzyme-linked immunoassays and the DNA hybridization assay were applied after enrichment, directly on aliquots taken from the Rappaport-Vassiliadis broth. The immunochromatographic assay was applied after 6 hours of post-enrichment in buffered peptone water.

Results: From the fecal samples used in this study (n=100), 78 were positive for the isolation of *Salmonella* by method A, and 75 were positive by method B. The combination of the results from both methods (A + B) constituted 87 positive samples. The sensitivity and agreement (Kappa statistics) with the “gold-standard” for each evaluated detection assay were; 87.36% and 0.64 for the ELISA 1; 98.85% and 0.96 for the ELISA 2; 97.70% and 0.92 for the DNA hybridization assay; and 80.46% and 0.48 for the immunochromatographic assay. All detection assays, except by the immunochromatographic assay, detected no false positive samples. The immunochromatographic assay detected 1% of false positive samples. The false negative results for each evaluated assay were; 11% for the ELISA 1; 1% for the ELISA 2; 2% for the DNA hybridization assay, and 18% for the immunochromatographic assay.

Discussion: Results from this study and from others (Cherrington and Huis in’ Veld,1993a,b; Harvey et al.,1999; Wegener and Baggesen,1997), demonstrate that there are *Salmonella* detection assays currently available from the food industry with potential application on swine clinical samples. These assays could be useful in epidemiological investigations using clinical samples, like swine feces. These assays may allow the processing of large numbers of samples as usually required in many epidemiologic studies of *Salmonella* in swine populations. The ELISA 2 and the DNA hybridization assay had the best performance in the comparative
evaluation done in this study, with 98.85% and 97.7% of sensitivity, and 96% and 92% of agreement with the “gold-standard”, respectively. Both proved to be highly sensitive and specific (no false positive results) for the detection of *Salmonella* in swine fecal samples, after enrichment in Rappaport-Vassiliadis broth. The ELISA 2 was easier and faster to perform, when compared to the DNA hybridization assay, but both resulted in time and cost savings comparing to the culture methods applied. In both cases, reliable results were obtained after 48 hours of inoculation of the samples in the pre-enrichment broths. Time savings over the conventional isolation methods are possible, however the pre-enrichment and enrichment steps are still needed to obtain the necessary number of *Salmonella* in order to be detected by the assays. The ELISA 2 and the DNA hybridization assay were comparable and performed better than the other evaluated assays for detection of *Salmonella* in swine fecal samples. The use of these two detection assays is a reliable and useful tool, besides of being less laborious and less expensive than conventional bacteriological isolation methods.

References: