Senftenberg. M1 resulted in 9 (14.5%) positive samples, which was superior (P<0.05) than M2 (6, 9.6%) and M3 (2, 3.2%), respectively.

PCR was superior (P<0.01) than conventional bacteriology only when RV was used as the DNA source. PCR using RV detected 27 out of 62 samples (43.5%), which was superior (P<0.01) than using SC or TT (3.2% and 8.0%, respectively).

**Discussion:** Considering the effect of sample weight on the detection of *Salmonella* bacteria (Davies et al., 2000), we cannot directly compare the three enrichment media used herein. However, we suggest the use of M1 whenever possible to obtain large amounts of fecal material (10g). Furthermore, the use of direct enrichment of swine feces in SC is discouraged.

The coupling of RV to PCR was significantly superior (P<0.01) than either TT or SC. Indeed, RV has been successfully used for PCR purposes in poultry-related samples (Oliveira et al., 2002). The best results of M1 (which used RV as enrichment broth) for isolation of *Salmonella* could indicate that this broth yielded higher numbers of *Salmonella* organisms, which could also explain the superior PCR results for RV. However, selective enrichment media may have different inhibitory properties to PCR. In fact, tetrathionate broth has been suggested to inhibit PCR (Stone et al., 1994). Our results indicate that RV broth may be successfully coupled to PCR after a simple boiling-centrifugation technique for DNA extraction. This is of great importance considering the cost-benefit ratio of this procedure.

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**References:**


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**COMPARISON OF ENRICHMENT SCHEMES FOR THE ISOLATION OF YERSINIA ENTEROCOLTICA**

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**Summary:** The food-borne pathogen *Yersinia enterocolitica* (YE) has been repeatedly linked to swine and is a world wide food safety risk. Microbiological culture methods for YE lack some functionality as the current gold standard requires a 21 day cold enrichment in phosphate buffered saline (PBS). In this study a shortened enrichment scheme using a higher incubation temperature and a more selective media (LB-BSI) was compared to PBS for the isolation of YE from swine feces. Both enrichments
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produced similar overall isolation rates but had a low level of agreement (kappa=0.11) for determining an individual pig's YE status. LB-BSI yielded a sensitivity and specificity of 22.7% and 87.2% respectively when compared to PBS. At the time of submission, further genotyping and serotyping assays are pending that may aid in discerning the source of disagreement between the methods.

Keywords: Sensitivity, specificity, feces, PBS, LB-BSI

Introduction: Yersinia enterocolitica (YE) is a food-borne pathogen causing an estimated 96,000 Americans to become ill each year (Mead, 1999). While the bacterium has been found in a variety of food and environmental samples, swine and pork products have been implicated as the primary reservoir of pathogenic YE (Davies, 1997). Fecal culture provides a relatively easy means to screen live pigs for the presence of YE. However, the standard methodology requires a three week wait for a negative result. Several enrichment schemes have been developed to shorten the wait time and aid in the isolation of YE from various sources (Fredriksson-Ahomaa, 2003). The aim of the study was to evaluate the effectiveness of Luria-Bertani-Bile Salts-Irgasan broth (LB-BSI) as a selective enrichment method for the isolation of YE from naturally infected swine feces as compared to the gold standard of cold enrichment in phosphate buffered saline (PBS). LB-BSI is advantageous compared to cold enrichment in PBS due to markedly shorter incubation time. Previous studies have not compared the methods in naturally infected feces.

Materials and Methods: During February to June 2003, 379 individual pig fecal samples representing 10 farms in Ohio and North Carolina were collected. Pigs included in this study ranged in age from 9 weeks to mature sows. Twenty gram fecal samples were collected by gloved hand from the rectum of each animal, placed in sterile Whirl-paks™ (Nasco, Fort Atkinson, WI, USA), and transported to the laboratory on ice. Samples were stored at 4°C for up to 24 hours until they were processed. Each 20 g sample was split into two 10g sub samples and placed in sterile specimen cups for enrichment in treatment A or B. Treatment A was the gold standard three week cold enrichment in PBS (Aleksic, 1999). Briefly, the 10 g sub samples were homogenized with 90 ml of 1X PBS (EMD Chemicals Inc., Gibbstown, NJ, USA) and incubated for 3 weeks at 4 °C before being plated. Treatment B was the Luria Bertani-Bile Salts-Irgasan (LB-BSI) selective enrichment based the methods of Bhaduri et al. (1997) and Hussein et al.(2001). Treatment B samples were diluted at a ratio of 1:10, homogenized, and incubated at 12 °C for 24 hours. After 24 hours, Irgasan (Ciba-Geigy Corp., Greensboro, NC, USA) was added at a final concentration of 5 µg/mL and re-incubated at 12 °C for an additional 48 hours before being plated. Following enrichment, 10 µL of each broth was streaked on separate Yersinia Selective Agar, CIN plates (Becton Dickinson and Company). Plates were incubated at room temperature for 48 hours. Colonies with morphology typical of YE were further biochemically identified by subculture onto Kligler Iron Agar (KIA, Becton Dickinson and Company) and urease broth (Becton Dickinson and Company). Colonies that fermented dextrose only and were urease positive were classified as presumptive YE. Presumptive isolates were stored at −80 °C for further characterization.

Results: At the time of the submission, all 379 samples have been processed through both treatments to the point of biochemical confirmation. The number of pigs found to be positive by the PBS and LB-BSI methods was 17.4% and 14.5% respectively. While the two methods detected a similar prevalence of YE there was lack of agreement between the two methods concerning YE status of individual pigs (kappa = 0.11, Table 1). Sensitivity was 22.7% and the specificity was 87.2% when comparing LB-BSI to the PBS cold enrichment.

Discussion: Given that the two enrichment schemes yielded similar prevalence rates for isolation of YE, yet had poor agreement at the pig level, the source of the disagreement needs further investigation. The stored isolates will be assayed for the presence of ail, a chromosomally encoded virulence gene associated with human clinical disease isolates, in order to assess the possibility of differential selection between the 2 methods for ail-harboring and non-ail harboring isolates. Additionally, the YE isolates...
will be serotyped to assess agreement between the methods for isolation of different serotypes. Serotyping and PCR results of the isolates may elucidate whether the disagreement between LB-BSI and PBS is a function of lesser sensitivity or greater inhibition of non-pathogenic YE.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>PBS</th>
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<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>LB-BSI</td>
<td>55</td>
<td>324</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>379</td>
<td>313</td>
<td></td>
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Table 1: Isolation of Y. enterocolitica from swine feces by PBS and LB-BSI

References:


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**Summary:** *Salmonella* (S.) Choleraesuis var. Kunzendorf is the major cause of swine salmonellosis in Poland. Eleven Xbal macrorestriction profiles and 3 ribotypes were noted amongst 36 isolates tested. Index of discrimination reached 0.88. Two clonal lineages were distinguished. One of the lines embraced the majority of 2000-2002 isolates showing over 80% genome similarity. The findings prove the clonal spread of the pathogen among swineherds in Poland.

**Keywords:** swine, salmonellosis, molecular typing, FIGE

**Materials and methods:** Thirty-six *S. Choleraesuis* var. Kunzendorf strains isolated from swine salmonellosis during 2000-2002 were genotyped by means of PFGE - Field Inversion Gel Electrophoresis (Hoeffer Scientific Instruments) and ribotyping. DNA samples were prepared as suggested by PulseNet