Comparison of Initial Enrichment on the Recovery of Salmonellae from Swine Lymph Nodes and Cecal Contents at Slaughter

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Abstract

We conducted an epidemiological survey to determine the prevalence of salmonellae in an integrated swine operation. Samples were collected at slaughter from 50 pigs per sampling period per farm and consisted of ileocolic lymph nodes and cecal contents. Culture techniques were as follows: samples (cecal and lymph node) were pre-enriched for 24 h with both tetrathionate and GN Hajna broth, were enriched with Rappaport Vassiliadis broth for 24 h, and were streaked onto brilliant green agar containing novobiocin and incubated for 24 h. Suspect colonies were inoculated into lysine iron and triple sugar iron agar, and H\textsubscript{2}S-positive cultures were confirmed by polyvalent-A Salmonella latex agglutination. We isolated salmonellae from 61% of pigs sampled. When comparing the frequency of isolations from sample sources, 58% were from lymph nodes and 42% were from cecal contents. There was no difference (P<0.05) in total isolations when tetrathionate was compared to GN Hajna (53% vs. 47%). There was a significant (P<0.05) advantage of utilizing tetrathionate for isolations from cecal contents when compared to GN Hajna (65% vs. 35%). On the basis of our results, we will modify future protocols so that we utilize tetrathionate for initial enrichment of cecal and fecal samples, and will employ GN Hajna for initial enrichment when culturing from swine tissues.

Introduction

Salmonella species are estimated to cause losses of>$100 million annually to the U.S. swine industry. Due to public health concerns, increased emphasis has been placed on determining the prevalence of on-farm salmonellae in swine (3). Pathogen control programs such as the Hazard Analysis of Critical Control Points (HACCP) program are designed to identify and evaluate the pathogen contamination of swine and pork. With the emphasis on testing for salmonellae and the time and labor intensity for traditional culture methods, any increase in the efficiency of culture techniques would be advantageous. When culturing swine and their environment for Salmonella Choleraesuis, GN Hajna broth has been considered by some as the initial enrichment of choice (1,2,4-6). However, when culturing for salmonellae other than S. Choleraesuis, tetrathionate broth has been routinely utilized for initial enrichment (7-9). We conducted a survey to determine the prevalence of salmonellae in pigs in an integrated swine operation. The purpose of the present study was to evaluate the recovery efficiencies for salmonellae from swine lymph nodes and cecal contents when GN Hajna and tetrathionate were compared as initial enrichments.

Materials and Methods

Culture procedures—Samples were collected at slaughter from 50 pigs per sampling period from 5 different farms for a total of 645 pigs sampled. Approximately 10 g of ileocolic lymph node and 25 g of cecal contents were collected from each pig, were individually placed into sterile plastic bags, and were transported on ice to our laboratory within 3 h of collection. One g of cecal content or 5 g of ileocolic lymph node was first enriched in either tetrathionate or GN Hajna broth, further enriched in Rappaport Vassiliadis broth, and plated onto brilliant green agar medium containing 25 \( \mu \)g/ml novobiocin. Enrichment and differentiation were accomplished at 37\(^\circ\)C during 24 h incubation. Salmonellae-like suspect colonies were inoculated into lysine iron and triple sugar iron agar and H\textsubscript{2}S-positive cultures were confirmed by agglutination with Salmonella O Antiserum Poly A-1 and Vi and Group C\textsubscript{1}, factors 5 and 6. Pure cultures of isolates were sent to the National Veterinary Services Laboratory to be serotyped.

Results and Discussion

We isolated at least one Salmonella organism from 61% of the pigs sampled. We had 415 samples serotyped (out of 558 positive samples) and they were represented by 33 serovars, but 85% of the isolates were represented by 10 serovars. Fifty-one pigs had multiple serovars isolated. When comparing the frequency of isolations from sample sources, 58% were from lymph nodes and 42% were from cecal contents (Table 1). There was no difference (P<0.05) in total isolations when tetrathionate was compared to GN Hajna (53% vs. 47%), although there was a numerical increase in lymph node isolations with GN Hajna compared to tetrathionate (55% vs. 45%). There was a significant
(P<0.05) advantage of utilizing tetrathionate for isolations from cecal contents when compared to GN Hajna (65% vs. 35%). Tetrathionate is considered more harsh than GN Hajna in that it restricts overgrowth of competing microorganisms from the gastrointestinal tract, and has been proposed as the primary enrichment of choice when culturing fecal samples (7-9). It has been suggested that GN Hajna is superior to other primary enrichments for isolation of S. Choleraesuis (1,2,4-6), hence the rationale for evaluating both enrichments in our study. However, none of our isolates were S. Choleraesuis. Of the 160 C group serovars isolated in our study, 81 were enriched with GN Hajna and 79 were enriched with tetrathionate (data not shown), thereby showing no advantage of GN Hajna for isolations of C group salmonellae. On the basis of our results, we will modify future protocols so that we utilize tetrathionate for initial enrichment of cecal and fecal samples, and will employ GN Hajna as the initial enrichment when culturing from swine tissues.

Table 1 Comparison of 2 enrichments for recovery of salmonellae from swine

<table>
<thead>
<tr>
<th>Sample</th>
<th>GN Hajna</th>
<th>Tetrathionate</th>
<th>Totals</th>
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<tbody>
<tr>
<td>Lymph node</td>
<td>176 (55%)</td>
<td>145 (45%)</td>
<td>321 (58%)</td>
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<tr>
<td>Cecal</td>
<td>84 (35%)</td>
<td>153 (65%)</td>
<td>237 (42%)</td>
</tr>
<tr>
<td>Totals</td>
<td>260 (47%)</td>
<td>298 (53%)</td>
<td>558 (100%)</td>
</tr>
</tbody>
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References


