Evaluation of a ΔcyA Δ(crp-cdt) Salmonella choleraesuis Commercial Vaccine to Protect Against Clinical Signs Caused By And Reduce Shedding of Salmonella typhimurium in Pigs

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Introduction

The two most common causes of Salmonellosis in swine are Salmonella choleraesuis and S. typhimurium.(1) Salmonella typhimurium infections, most commonly seen in intensively reared weaned pigs, cause an enterocolitis of variable severity, followed by a carrier state that can last up to 28 weeks from onset.(1,2) Infected pigs are believed to be the primary source of infection for other pigs and pork carcasses.(1)

Efficacious modified live (ML) vaccines for swine are commercially available for S. choleraesuis but not for S. typhimurium.(3) However, cross protection between Salmonella serogroups has been demonstrated in poultry.(4) In cattle, ML vaccines administered by intramuscular (IM) injection have provided a degree of cross protection between S. typhimurium and S. dublin,(5,6) and between S. choleraesuis and S. dublin.(7) However, results of studies on the vaccination of cattle with orally administered avirulent S. dublin have been unable to demonstrate protection against S. typhimurium.(8)

We are unaware of any reports of studies of cross protection between S. choleraesuis and S. typhimurium in swine. The objective of these two studies was to evaluate whether protection is afforded against clinical Salmonellosis caused by Salmonella typhimurium and reduction in the shedding of Salmonella typhimurium by vaccinating three to four week old pigs with a commercial live modified ΔcyA Δ(crp-cdt) Salmonella choleraesuis vaccine (Argus SC™, Bayer Corporation, Agriculture Division, Shawnee Mission, Kansas).

Materials and Methods

Two studies were conducted to address the cited objectives. In Study A, forty-five, three to four weeks old, Salmonella negative crossbred pigs, were obtained from a commercial pig farm. The animals were randomly assigned to three groups, namely Group I (Vaccinated and challenged), Group II (Non-vaccinated and challenged) and Group III (Non-vaccinated and non-challenged). The pigs from Group

I were vaccinated orally with a single dose of the commercial S. choleraesuis vaccine (Argus SC™) delivered by means of a water proportioner. Three weeks, post-vaccination, pigs from Groups I and II were orally challenged with a virulent strain of S. typhimurium. During the fourteen days post-challenge period, the efficacy of the vaccine was assessed by evaluating physical condition, fecal scores, rectal temperature, body weight, and mortality. At the end of the observation period, the body weights were taken and the experiment was concluded.

The second study (Study B), was similar to the first one. However, the S. typhimurium challenge dose was adjusted to be lower in order to allow the detection of possible differences in shedding of the challenge organism, post-challenge. In addition, the presence of the challenge organism in tissues namely, liver, spleen, mesenteric lymph node, ileo-cecal junction and tonsils were evaluated.

Results

The post-challenge results from study A are shown in Table 1. In Group I, the percent days (5.0%) the pigs were "morbid" was significantly lower (P<0.0001) than compared to pigs in the non-vaccinated group (Group II) (23.8%). The vaccinated pigs mean body temperature had returned to normal (<103.4°F) within 48 hours after challenge, but the non-vaccinated pigs remained above normal until day 5. On days 2, 3 and 4 post-challenge, the mean body temperatures of the vaccinated pigs (103.4°F, 103.0°F, 103.2°F, respectively) were significantly lower than those of the non-vaccinated pigs (105.1°F, 103.9°F, 103.7°F, respectively) (Table 1). Eighteen of twenty (80%) non-vaccinated pigs (Group I), and 6 of the 20 (30%) vaccinated pigs (Group I) had diarrhea post-challenge (P < 0.01). The percent number of days, pigs in Group I had diarrhea (3.1%) were significantly (P < 0.01) lower when compared to the non-vaccinated pigs from Group II (23.8%). The average daily gain (1.20 lb/day) of the vaccinated pigs was significantly (P < 0.05) greater than that of the non-vaccinated pigs (Group II) (0.62 lb/day).

In Study B, due to the low challenge dose, pigs developed
only mild and transient clinical signs of disease. As a result, group differences in the ancillary data such as, average daily gain, rectal temperature, and clinical signs were not significant (Table 1). Post-challenge, the non-vaccinated pigs (Group II) shed *S. typhimurium* longer (36.2%) than the vaccinated pigs (10.7%) (P < 0.05). More non-vaccinated pigs (Group II) (79%) than vaccinated pigs (9.5%) were cultured positive for *S. typhimurium* in at least one tissue at necropsy (P < 0.01). *Salmonella typhimurium* was isolated from a significantly (P < 0.05) greater proportion of tissues from the non-vaccinated group (Group II) (34.7%) than from pigs in the vaccinated group (3.8%) (Table 1). The percent of pigs positive for the individual tissues namely liver, spleen, ileo-cecal junction, mesenteric lymph node and tonsil are shown in the Table 2. A significantly (P<0.05) higher percent of non-vaccinated pigs (Group II) were positive with the challenge organism in tonsils (52.6%), ileo-cecal junction (57.9%) and mesenteric lymph node (47.4%) tissues when compared with the vaccines (Group I) (4.8%, 4.8% and 4.8%, respectively).

### Table 1: Summary of Results from Pigs in Studies A and B, Post-Challenge

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment Groups</th>
<th>No. of Animals</th>
<th>Clinical Signs (Percent Days Positive)</th>
<th>Diarrhea (Percent Days Positive)</th>
<th>Percent Tissues Positive</th>
<th>Rectal Temperature (°F)</th>
<th>Average Daily Gain (lbs/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Day 2</td>
<td>Day 3</td>
</tr>
<tr>
<td>A</td>
<td>I</td>
<td>20*</td>
<td>5.0*</td>
<td>3.1*</td>
<td>ND</td>
<td>103.4*</td>
<td>103.0*</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>20*</td>
<td>23.8*</td>
<td>23.8*</td>
<td>ND</td>
<td>105.1*</td>
<td>103.9*</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
<td>ND</td>
<td>103.5</td>
<td>103.6</td>
</tr>
<tr>
<td>B</td>
<td>I</td>
<td>21</td>
<td>0.0*</td>
<td>0.1*</td>
<td>3.8*</td>
<td>103.8*</td>
<td>103.6*</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>19</td>
<td>1.1*</td>
<td>0.7*</td>
<td>34.7*</td>
<td>103.6*</td>
<td>104.0*</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>103.7</td>
<td>103.5</td>
</tr>
</tbody>
</table>

Group I = Vaccinated/Challenged; Group II = Non-vaccinated/Challenged; Group III = Non-vaccinated/Non-challenged.
Comparisons are done within each study between Groups I and II. Different superscripts within a column and study indicate significant (P < 0.5) difference. * One and three animals died post-challenge from Groups I and II, respectively.

### Table 2: Isolation of *Salmonella typhimurium* from Internal Organs of Pigs In Study B, Post Challenge

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Number of Animals</th>
<th>Liver</th>
<th>Spleen</th>
<th>Tonsils</th>
<th>Ileo-Cecal Junction</th>
<th>Mesenteric Lymph Node</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>21</td>
<td>0.0*</td>
<td>4.8*</td>
<td>4.8*</td>
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<tr>
<td>Group II</td>
<td>19</td>
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<td>5.3*</td>
<td>52.6*</td>
<td>57.9*</td>
<td>47.4*</td>
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<tr>
<td>Group III</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Group I = Vaccinated/Challenged; Group II = Non-vaccinated/Challenged; Group III = Non-vaccinated/Non-challenged.
Comparisons are done within each study between Groups I and II. Different superscripts within a column indicate significant (P < 0.5) difference.
Discussion

Oral vaccination of three to four week old pigs with the ΔcyA Δ(crp-cdt) live modified *Salmonella choleraesuis* vaccine (Argus SC™) significantly reduced clinical signs of disease and also reduced the fecal shedding of *Salmonella typhimurium*. Vaccinated pigs in Study A, gained significantly more weight after experimental challenge, had significantly lower morbidity and diarrhea scores, and shed *S. typhimurium* in feces significantly less often or at lower concentrations (study B) than non-vaccinated pigs. Even though isolation procedures for the recovery of *Salmonella typhimurium* from fecal samples and tissues was restrictive, more than 50% of the non-vaccinated animals in study B had the challenge organism in the ileo-cecal junction and tonsils. The clinical signs, body temperature, and culture results in the respective vaccinated pigs were similar to other reports of experimental *Salmonella typhimurium* infection of weaned pigs challenged with approximately the same dose of virulent bacteria.(2,8) In those studies, and as in Study A, the mean rectal temperatures peaked post-challenge in the first 2 days and returned to normal by Day 4.

The differences in the clinical signs of disease caused by *S. typhimurium* seen between vaccinates and non-vaccinates in study A were similar to those described in a report of an experimental ΔcyA Δcrp *S. typhimurium* vaccine.(8) The gene deletions in that vaccine are also among the deletions [ΔcyA Δ(crp-cdt)] present in the *S. choleraesuis* vaccine used in study A.

In study B, isolation of *Salmonella typhimurium* was primarily from the tonsils, ileo-cecal junction, and mesenteric lymph nodes of the vaccinated and non-vaccinated pigs. This is consistent with the previous studies in which deep tissues (liver and spleen) were rarely infected, but mucosal and lymphoid tissues were commonly infected.(2,8)

Although *Salmonella* may survive for long periods in the environment, it is widely believed that the carrier animal is the major source of infections for both animals and humans.(1) The significant reduction in shedding of *S. typhimurium*, and protection against clinical signs of the disease suggests that oral mass vaccination with the ΔcyA Δ(crp-cdt) live modified *S. choleraesuis* vaccine (Argus SC™) will be a useful tool in the prevention of *S. typhimurium* infection in swine. This may be of increasing importance as monitoring programs, which include monitoring for *Salmonellae*, are implemented in the food industry.

References


