Effect of competitive exclusion on transmission of *Salmonella* Choleraesuis between early weaned pigs

Anderson RC¹, Genovese KJ², Harvey RB¹, Stanker LH¹, Keith NK³, DeLoach JR⁴ and Nisbet DJ¹

¹USDA-ARS, Food Animal Protection Research Lab, College Station, TX 77845
²Texas A&M University, College Station, TX 77843
³Keith Associates, Springfield, MO 65804
⁴MS BioScience Inc., Madison, WI 53704

**Introduction**

*Salmonella* serovar Choleraesuis accounts for the vast majority of diagnosed cases of swine salmonellosis in the United States (24). Enterocolitis caused by *Salmonella* Typhimurium ranks second as a cause of salmonellosis (24) but this organism ranks high as an important food borne pathogen. Infection by *Salmonella* Typhimurium and other broad host range serotypes can occur through exposure to contaminated feedstuffs, infected rodents, birds or other swine. In contrast, transmission of the host-adapted serotype, *Salmonella* Choleraesuis, is thought to occur primarily via contact with infected carrier pigs (24) which may shed the pathogen from their gastrointestinal and possibly their respiratory tract (11, 13).

Competitive exclusion technology (CE), the exclusion of enteric pathogens from the alimentary tract by preferential colonization with mutualistic and commensal microbes, is an attractive strategy to reduce *Salmonella* infection of swine. Such technology has been proven effective in enhancing colonization resistance of avian species to *Salmonella* (3-8, 15, 17-18, 20-21, 25-26). More recent evidence suggests that CE reduces cecal concentration as well as fecal shedding of *Salmonella* Choleraesuis from experimentally infected swine (2, 10, 16, 22), although the innate invasiveness of the pathogen may be unmitigated (2). Feces likely are a major source of salmonellosa infection to previously non-infected animals (14) and continued exposure of pigs to salmonella-contaminated feces tends to prolong shedding of the pathogen (9). Thus, it is plausible that reduced concentration and incidence of shedding of salmonella may reduce the potential for horizontal transmission. The objective of the present study was to assess the potential of CE treatment to reduce horizontal transmission of *Salmonella* Choleraesuis between early weaned pigs.

**Materials and Methods**

Our experimental design consisted of experimentally infecting CE-treated and nontreated piglets with *Salmonella* Choleraesuis and then measuring the potential of these infected pigs (designated as seeders) to naturally transmit the pathogen to previously uninfected pigs (designated as contacts) upon co-mingling (1). Thus the potential for horizontal transmission of *Salmonella* Choleraesuis was tested via co-mingling of seeders and contacts as follows: 1) CE-treated seeders with CE-treated contacts, 2) untreated seeders with untreated contacts, 3) CE-treated seeders with untreated contacts and 4) untreated seeders with CE-treated contacts.

The porcine derived competitive exclusion culture, consisting of an undefined consortium of native gut microbes, was propagated from cecal contents of a healthy 6 week old pig and was maintained in continuous flow culture as described for avian cultures (17). Treatment with this CE culture was accomplished via oral gavage (5 ml) of each piglet within the litters designated. Treatment was given on the day of birth only. The viable cell count of the undefined consortium, determined by viable cell count on Brucella Blood Agar as previously described (2), was to 2 to 9 x 10⁶ anaerobic colony forming units (CFU).

Piglets were weaned at 14 days of age to concrete floored pens (6 m²). At 15 days of age each litter was split into two equal or nearly equal numbered groups of 3 to 5 piglets per group (depending on number of piglets/litter). The piglets of each group were then either challenged via oral gavage with 10⁶ to 10⁷ colony forming units (CFU) of a novobiocin (NOV) and nalidixic acid (NAL) resistant strain of *Salmonella* Choleraesuis (to serve as seeders) or removed to a separate remote pen (to serve as contacts). The NOV+NAL resistant mutant was propagated from a *Salmonella* Choleraesuis var. *kimzendorf* 3246pp isolate generously provided to us by Dr. P. Fedorka-Cray (Athens, GA). The challenge concentration was determined via viable cell count on Brilliant Green agar containing both 25 μg NOV/ml and 20 μg NAL/ml (BGA/NOV+NAL). Seeders and contacts originating from different litters were co-mingled 2 to 3 days post-challenge and at no time did seeders outnumber contacts within a pen following co-mingling.

Beginning at weaning, rectal swabs were collected daily from each piglet for qualitative cultivation for salmonellae. Wildtype *Salmonella* were not recovered from any of the piglets prior to challenge or co-mingling or from fecal samples collected from the maternal sows within 48 hrs prior to farrowing and upon the first defecation post-farrowing. Fecal samples from the sows were
simultaneously cultured for wildtype *Salmonella* via initial culture of approximately equal portions (1 to 2 g) in GN-Hajna and tetrathionate broth (20 ml each), further enrichment of these (100 µl each) in 5 ml Rappaport-Vassiliadis (RV) broth. Selective differentiation was accomplished on Brilliant Green Agar containing 25 µg NOV/ml (BGA/NOV). Rectal swabs collected from piglets prior to *Salmonella* Choleraesuis challenge were also cultured for wildtype salmonellae as above except in 5 ml rather than 20 ml GN-Hajna or tetrathionate broth. Rectal swabs collected from piglets post-challenge were preenriched in 5 ml GN-Hajna broth, further enriched in RV broth as above and plated to BGA/NOV+NAL. Approximately one week post-*Salmonella* challenge, piglets were euthanized by injection with sodium pentobarbital and necropsied for collection of ileocolic lymph nodes and cecal contents. These specimens (1 to 2 g) were cultured for the challenge strain as were the post-challenge rectal swabs except 20 rather than 5 ml GN-Hajna broth was used. Quantitative bacteriology of cecal contents was accomplished by plating serial ten-fold dilutions (10<sup>7</sup> to 10<sup>9</sup>) to BGA/NOV+NAL plates. All incubation steps were carried out at 37°C for 18 to 24 hr. Plates were examined for typical salmonellae-like colonies and suspect colonies were confirmed via serum agglutination using *Salmonella* Antiserum Poly A 1-IV and Group C<sub>1</sub>, Factors 5 and 6.

The basic experimental design as described above was replicated in three separate experiments with each replicate representing an experimental unit. Within each experiment, average proportions (*Salmonella* Choleraesuis-positive) and cecal concentrations of *Salmonella* Choleraesuis were determined for seeders and for contacts and each set of data were analyzed for differences using Analysis of Variance (23). All animals were cared for according to standard swine husbandry practices and were fed diets formulated to meet or exceed NRC requirements (19).

**Results**

Most of the seeder piglets, whether CE-treated or not, were infected by *Salmonella* Choleraesuis at 23 to 26 days of age as determined by recovery of *Salmonella* Choleraesuis from ileocolic lymph nodes and cecal contents collected at necropsy (Table 1). No significant differences in shedding or proportions of piglets yielding *Salmonella*-positive lymph nodes or cecal contents were observed between any of the seeder groups. Differences observed in concentrations of *Salmonella* recovered from cecal contents from the seeders are indicated (Table 1). As for the contacts, average proportion of pigs shedding *Salmonella* Choleraesuis at least once post-challenge was significantly lower (*P* < 0.09), as was the proportion of piglets yielding *Salmonella*-positive lymph nodes (*P* < 0.05), for only those CE-treated piglets commingled CE-treated seeders. Other differences were not significant (Table 1).

**Discussion**

Our oral challenge of greater than 10<sup>7</sup> CFU *Salmonella* Choleraesuis was sufficient to infect most (>88%) of the experimentally challenged piglets (seeders), as evidenced by qualitative recovery of the pathogen from ileocolic lymph nodes or cecal contents. We thus conclude that an adequate natural challenge was presented to those piglets not previously infected (contacts). In contrast to that observed earlier with pigs similarly challenged (1-2), we observed as high or higher recovery of *Salmonella* Choleraesuis from cecal contents as we did from ileocolic lymph nodes (Table 1). However, recovery was highest from the lymph nodes of most contact groups (Table 1) thus indicating, as observed elsewhere (1, 12-13), that the concentration of *Salmonella* Choleraesuis encountered by an animal and prior passage of the pathogen through a host likely influence the potential establishment of a carrier state in that animal.

Previously, we observed reductions in fecal shedding, as determined by daily culture of rectal swabs, and cecal colonization of *Salmonella* among experimentally challenged piglets that had been inoculated at birth and at weaning with CE cultures (2). In the present study, differences in fecal shedding were not significant (*P* > 0.10) for the CE- or untreated seeders. However, CE treatment was given at birth only in this study, so as to accommodate expectations of industry usage, and this may have influenced the effectiveness of CE treatment post-weaning. Moreover, the degrees of freedom available for our analysis may have limited our ability to detect differences.

With respect to contacts, we did obtain evidence for reduced horizontal transmission of *Salmonella* Choleraesuis between CE-treated seeders commingled with CE-treated contacts as the pathogen was not recovered from ileocolic lymph nodes of any of these CE-treated contacts nor were any of these contacts observed to shed the pathogen (Table 1). In contrast, *Salmonella* Choleraesuis was recovered from ileocolic lymph nodes of significantly more (*P* < 0.05) of the CE- or untreated contacts of the other commingled groups (Table 1). Proportions of piglets yielding *Salmonella*-positive cecal contents or either *Salmonella*-positive cecal contents or lymph nodes was highest for untreated contacts commingled with untreated seeders and was lowest for the CE-treated contacts commingled with CE-treated seeders (Table 1) which suggests that CE worked best if given to both seeders and contacts. These proportions; however, were not significantly different but again our ability to detect differences was likely limited by the degrees of freedom available from this study (Table 1).
Table 1. *Salmonella* Choleraesuis status of seeder (experimentally infected) and contact (naturally exposed) piglets following commingling.

<table>
<thead>
<tr>
<th>Pairings</th>
<th>Total number of piglets</th>
<th>Proportion of pigs shedding at least once post challenge</th>
<th>Incidence of shedding&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Proportion of culture-positive necropsy specimens&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean cecal conc'n (log&lt;sub&gt;10&lt;/sub&gt; CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated seeders</td>
<td>3</td>
<td>13</td>
<td>85</td>
<td>49</td>
<td>4.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Untreated contacts</td>
<td>3</td>
<td>13</td>
<td>53&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9</td>
<td>1.4</td>
</tr>
<tr>
<td>CE-treated contacts</td>
<td>3</td>
<td>14</td>
<td>43&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td>CE-treated seeders</td>
<td>3</td>
<td>11</td>
<td>100</td>
<td>46</td>
<td>2.1&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Untreated contacts</td>
<td>3</td>
<td>11</td>
<td>36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6</td>
<td>0.6</td>
</tr>
<tr>
<td>CE-treated seeders</td>
<td>3</td>
<td>13</td>
<td>92</td>
<td>43</td>
<td>3.4&lt;sup&gt;b,i&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Proportions of piglets yielding *Salmonella*-positive ileocolic lymph nodes (ILym), cecal contents (CC), or either are expressed as % and were calculated as the average of the three replicates.

<sup>b</sup>Number of experimental replications.

<sup>c</sup>Incidence represents the average cumulative incidence calculated the seven days immediately post *Salmonella*-challenge.

<sup>d</sup>For contacts, unlike superscripts indicate significant differences (*P* < 0.09).

<sup>e</sup>For contacts, unlike superscripts indicate significant differences (*P* < 0.05).

<sup>h</sup>For seeders, unlike superscripts indicate significant differences (*P* < 0.05).

<sup>i</sup>For seeders, unlike superscripts indicate significant differences (*P* < 0.05).
References


