Reduction of *Salmonella* in Cattle Feces and on Cattle Hides

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Reduction of *Salmonella* in Cattle Feces and on Cattle Hides: Project Summary

**Background**

*Salmonella* is a major food safety concern for the meat industry. Past research has demonstrated that cattle hides are the major source of *Salmonella*, as well as *E. coli* O157:H7 contamination of carcasses in processing plants. *Salmonella* poses a risk to human health due to foodborne illness, and it can also have adverse effects on animal health.

Multi-drug resistant (MDR) *Salmonella enterica* Newport and Typhimurium continue to emerge as public and livestock health and food safety challenges. A *Salmonella enteric* Newport bacterial extract vaccine (Epitopix LLC, Willmar, MN) was approved and conditionally licensed by the U.S. Department of Agriculture (USDA) Animal Plant Health and Inspection Service (APHIS) Center for Veterinary Biologics for use in live cattle in 2004. The vaccine interrupts iron acquisition by the pathogenic bacteria, which affects the bacteria’s ability to survive.

If proven effective in cattle production settings, this anti-*Salmonella* vaccine could be a valuable tool to reduce asymptomatic fecal shedding in cattle (for food safety and biosecurity benefits) and to minimize adverse *Salmonella* infection-associated morbidity and mortality (animal health and animal production benefits).

The researchers utilized a gut physiology apparatus (Ussing chamber) that allowed them to evaluate the effectiveness of the vaccine at much less expense than a typical vaccine challenge study, and without sacrificing the experimental animals.

The initial objective of this study was to determine if intestinal mucosa derived from cattle vaccinated with the *Salmonella* Newport vaccine were more resistant to adherence and invasion following ex vivo challenge with multi-drug resistant *Salmonella* Newport and Typhimurium strains compared to non-vaccinated cattle.

A secondary objective of the study, which has not been achieved, was to determine the length of time *Salmonella* survive on the hides of live cattle. Over 1,300 animals were sampled, but the hide prevalence of *Salmonella* was not sufficient to proceed with this phase of the experiment. The researchers have made plans to complete this aspect of the project at a later time.

**Methodology**

Thirty-two yearling steers weighing between 800 to 1,000 pounds were sourced from the U.S. Meat Animal Research Center (USMARC) feedlot. Half of the steers were randomly selected and vaccinated with the Epitopix *Salmonella* Newport Bacterial Extract. The cattle were vaccinated with a two-dose regimen two weeks apart according to the manufacturer directions. The remaining 16 steers served as unvaccinated controls.

Terminal cecal tissue (in the proximal large intestine) was surgically harvested from the animals and placed on eight separate Ussing chambers. The tissues were challenged with four diverse *Salmonella* strains in duplicate. The two *Salmonella* Newport strains were selected because the Epitopix vaccine is generated from a *Salmonella* Newport strain. *Salmonella* Dublin is a cattle
host-adapted strain. MDR *Salmonella* Typhimurium DT104 and MDR *Salmonella* Newport are the two most important *Salmonella* serotypes in dairy and beef cattle from a public health and clinical veterinary perspective. Serum samples were also collected from all of the cattle.

Western blot assays still need to be run to confirm that *Salmonella* vaccinated cattle developed an immune response specific to the antigens in vaccine. This work is in progress.

**Findings**

The researchers completed Ussing chamber experiments on 120 control tissues derived from 15 non-vaccinated control steers and on 80 *Salmonella* vaccinated tissues derived from ten *Salmonella* Newport vaccinated steers, after which it was determined that decontamination procedures for the Ussing chambers were complicating results. After recognizing this, 16 control tissues and 70 vaccinate tissues were run on the Ussing chambers and a different decontamination method (hot water) was used.

<table>
<thead>
<tr>
<th>Group</th>
<th>N Tissues</th>
<th>Mean Inoculum</th>
<th>Mean Adherence</th>
<th>Mean Invasion</th>
<th>Adhered per Inoculum&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Invaded per Adherence&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Invaded per Invasion&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>1,467,917 (379,696)</td>
<td>5,640 (7,864)</td>
<td>286 (213)</td>
<td>0.41 (0.56)</td>
<td>13.04 (17.83)</td>
<td>0.021 (0.015)</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>4</td>
<td>1,616,667 (223,242)</td>
<td>9,010 (14,987)</td>
<td>310 (308)</td>
<td>0.62 (1.06)</td>
<td>12.46 (13.11)</td>
<td>0.019 (0.017)</td>
</tr>
<tr>
<td>DT104</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dublin</strong></td>
<td>4</td>
<td>1,300,000 (119,319)</td>
<td>6,628 (6,602)</td>
<td>441 (222)</td>
<td>0.50 (0.47)</td>
<td>22.39 (33.73)</td>
<td>0.034 (0.019)</td>
</tr>
<tr>
<td>MDR Newport</td>
<td>4</td>
<td>1,061,667 (75,056)</td>
<td>3,588 (1,899)</td>
<td>216 (95)</td>
<td>0.33 (0.16)</td>
<td>6.19 (0.62)</td>
<td>0.020 (0.008)</td>
</tr>
<tr>
<td><strong>Newport</strong></td>
<td>4</td>
<td>1,893,333 (350,259)</td>
<td>3,335 (2,873)</td>
<td>175 (132)</td>
<td>0.18 (0.18)</td>
<td>11.10 (9.76)</td>
<td>0.009 (0.005)</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>70</td>
<td>1,400,476 (528,791)</td>
<td>18,190 (48,645)</td>
<td>522 (813)</td>
<td>1.40 (3.62)</td>
<td>11.40 (15.99)</td>
<td>0.043 (0.070)</td>
</tr>
<tr>
<td><strong>Typhimurium</strong></td>
<td>18</td>
<td>1,595,185 (650,862)</td>
<td>7,387 (12,668)</td>
<td>314 (350)</td>
<td>0.51 (0.93)</td>
<td>14.81 (16.98)</td>
<td>0.023 (0.027)</td>
</tr>
<tr>
<td><strong>DT104</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Dublin</strong></td>
<td>18</td>
<td>1,260,370 (646,758)</td>
<td>7,529 (5,433)</td>
<td>934 (1,244)</td>
<td>0.72 (0.59)</td>
<td>14.39 (13.05)</td>
<td>0.082 (0.111)</td>
</tr>
<tr>
<td>MDR Newport</td>
<td>16</td>
<td>1,189,167 (373,356)</td>
<td>36,525 (77,293)</td>
<td>380 (477)</td>
<td>2.98 (5.95)</td>
<td>8.27 (20.74)</td>
<td>0.035 (0.047)</td>
</tr>
<tr>
<td><strong>Newport</strong></td>
<td>18</td>
<td>1,533,704 (219,705)</td>
<td>23,357 (59,620)</td>
<td>445 (747)</td>
<td>1.55 (4.11)</td>
<td>7.82 (12.44)</td>
<td>0.029 (0.049)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Bacteria adhered (cfu)/bacteria inoculated into luminal side of Ussing chamber (cfu), percentage
<sup>2</sup> Bacteria invaded (cfu)/bacteria adhered (cfu), percentage
<sup>3</sup> Bacteria invaded (cfu)/bacteria inoculated into luminal side of Ussing chamber (cfu), percentage

Statistically, there was no difference in *Salmonella* attachment and invasion to cecal tissues from vaccinated and non-vaccinated cattle. However, mean *Salmonella* adherence per inoculum was numerically higher for cecal tissues from vaccinated (1.4%) versus non-vaccinated (0.4%) cattle.
Similarly, *Salmonella* invasion per inoculum was numerically greater in the vaccinated than control tissue (0.04% versus 0.02%). This phenomenon of greater adherence and invasion in cecal tissue from vaccinated cattle was true for all four *Salmonella* strains tested. Although in all instances the four *Salmonella* strains were able to adhere and invade the cecal tissue, attachment and invasion rates were lower than expected. One explanation may be that cecal tissue lacks Peyer’s Patches, a common site of invasion for *Salmonella* in the gastrointestinal tract. In addition, lymphocytes (which produce specific antibodies) occur at relatively low density in the cecal mucosa and cecal submucosa compared to other large intestinal tissues. Although cecal biopsies are relatively easy to surgically harvest, tissues from other gastrointestinal sites may be more likely to reveal differences, if any exist, in adhesion or invasion of *Salmonella* in vaccinated versus non-vaccinated cattle.

This project suggests that there is no difference in either *Salmonella* cecal mucosa adherence or invasion between *Salmonella* vaccinated and control (non-vaccinated) steers. The researchers had two potential explanations for the observed findings. The first being, the vaccine was ineffective or the vaccinated steers did not respond immunologically in the gut to the Epitopix *Salmonella* Newport vaccine components. The second explanation involves the experimental protocol and that the Ussing chamber approach using cecal tissue mucosa was insensitive to vaccine-induced immunological effects. Unfortunately, cecal tissue was the only large intestinal tissue that the researchers could safely and easily surgically harvest from live cattle without high risk to the health of the steers.

**Implications**
The preliminary data suggests that there is no difference in either *Salmonella* adherence or invasion between *Salmonella* vaccinated and non-vaccinated steers. The researchers felt, however, that more data need to be collected to confirm this finding. Additional work is also being conducted to achieve the second objective of this experiment and determine the rate of survival of *Salmonella* on cattle hides.

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