Solutions for the food safety threat posed by *Salmonella* in the lymph nodes of cattle presented for harvest

**Principal Investigators:** Guy H. Loneragan, Dayna Brichta-Harhay, and Tom S. Edrington

**Texas Tech University**

**Study Completed**
May 2012

*Funded by The Beef Checkoff*
Solutions for the food safety threat posed by *Salmonella* in the lymph nodes of cattle presented for harvest

**Background**

Informed regulatory oversight and improved process control through industry adoption of Pathogen Reduction/HACCP plans have dramatically reduced the human incidence of disease caused by *E. coli* O157. A testament to this success is that the proportion of USDA/FSIS ground beef testing positive for this pathogen has fallen by 90% in a decade. An important outcome of which is that the human incidence of *E. coli* O157 has reduced by almost half and beef is no longer the primary attributed vehicle for human exposure. Yet despite these impressive successes as well as improved sanitary slaughter processes, control of *Salmonella* in beef remains frustratingly elusive. In fact, the percentage of ground beef samples testing positive for *Salmonella* has not changed even during the same period of *E. coli* O157 success. Moreover, the human incidence of *Salmonella* has not decreased and if anything, it may have slightly increased.

These conflicting results ostensibly seem at loggerheads because our paradigm of carcass contamination, i.e., hide to carcass surface, is the same for both *Salmonella* and *E. coli* O157 and, furthermore, these two pathogens appear to be equally susceptible to validated in-plant interventions. Our preliminary data help explain this paradox in that *Salmonella* harborage in lymph nodes of cattle appears to be the likely avenue by which *Salmonella* evades carcass decontamination. Yet these phenomena also provide insights into opportunities to mitigate this food-safety threat.

To address to food safety threat posed by *Salmonella*, we developed a collaborative multidisciplinary, government-university team to undertake a series of research activities to fill critical knowledge gaps about sources of *Salmonella* in food as well as opportunities for its control. Our approach was consistent with the USDA/NIFA/NIFSI priority area 111.C. Consequently, the Beef Checkoff funds provided for this work served as co-support for a successful USDA/NIFA/NIFSI submission (USDA Contract # 2011-51110-31081).

The stated objectives for this work were to:

1. Objective 1. Characterize variation in regional, seasonal, and animal-type burden of *Salmonella* harborage in lymph nodes of cattle presented for harvest;
2. Objective 2. Identify and describe important biological and epidemiological factors associated with *Salmonella* in lymph nodes of cattle;
3. Objective 3. Develop and test practical solutions for control of *Salmonella* in the lymph nodes of cattle;

The pertinent objectives to which the funds from The Beef Checkoff applies are Objectives 1, 2 and 3.

**Methodology**

A lymph node surveillance program was conducted during the winter and spring of 2012. Lymph nodes were collected from 13 packing plants and assayed for the presence and concentration of *Salmonella*. In brief, surrounding fat and fascia were trimmed from lymph nodes, which were then
weighed, surface sterilized by submersion in a boiling water bath, placed into individual filtered
sample bags, pulverized using a rubber mallet and then enriched in 80 mL of tryptic soy broth.
Enrichments were subjected to immunomagnetic separation using anti-\textit{Salmonella} beads. Recovered
beads were transferred to 3 mL of Rappaport-Vassiliadis broth, incubated at 42°C for 18 to 20 h,
then streaked onto selective agar plates prior to incubation at 37°C for 18 to 20 h.

In a separate set of studies designed to establish a model of transdermal lymph node infection, initial
pilot studies were conducted in which an initial syringe application was trialed but found to be
ineffective. In a second study, an allergy skin testing device, each device consists of 10 testing probes
fitted at the end with a stainless steel lancet tip that protrudes from the probe enough to provide
intradermal, but not sub-cutaneous, administration of the \textit{Salmonella}. The device was dipped into
broth containing \textit{Salmonella} and then applied to the lower leg of the steer. Following \textit{Salmonella}
treatment, the steers were euthanized and necropsied to collect peripheral lymph nodes for \textit{Salmonella}
culture. Subsequent to these pilot studies, an experiment was conducted to evaluate whether a
commercially available vaccine might hold promise in the control of \textit{Salmonella} in the lymph nodes of
cattle. Sixteen Holstein crossbred steers were assigned to either a Control or Vaccine treatment and
inoculated in all legs with \textit{S}. Newport or \textit{S}. Montevideo using the skin test device described above.
Additionally, all steers were inoculated on the belly with \textit{S}. Senftenberg. Three and six days following
\textit{Salmonella} application, one half of the steers in each treatment were euthanized and necropsied at
which time lymph nodes were collected and cultured for \textit{Salmonella}.

**Findings**
Thirty four (2.9%) positive lymph nodes have been detected among 1,178 lymph nodes assayed to
date. The prevalence of positive lymph nodes harvested from feedlot animals (3.9%) was
approximately double that observed among cull cows (1.8%). Some regional variation was observed
in that fed plants in Texas (7.7%) appeared to have a greater prevalence of \textit{Salmonella} in the subiliac
lymph node than plants north of Texas (0.0%).

In terms of the transdermal challenge models, using the syringe, most of the lymph nodes examined
in the \textit{Salmonella}-treated steers were culture positive for the challenge strains. The serogroups of the
recovered lymph node isolates matched perfectly with the serogroups of the inoculated strains of
\textit{Salmonella} administered to each leg containing that particular lymph node. However, swelling around
the injection sites and mild to moderate lameness was observed in varying degrees in the three
\textit{Salmonella} treated steers and this method of application was deemed unsuitable. Inoculation of the
steer using the skin-allergy testing device resulted in \textit{Salmonella} positive lymph nodes. Isolates
cultured from the lymph nodes all belonged to serogroup B, the same serogroup as that
administered. No swelling or lameness was observed indicative of successful intradermal
administration. The third pilot study, confirmed the device selected to administer the \textit{Salmonella}
challenge is an effective means for inoculating \textit{Salmonella} transdermally and evaluating subsequent
uptake by the peripheral lymph nodes. All lymph node isolates belonged to the same serogroup
utilized to inoculate all legs. In the vaccine study, no treatment differences were observed in calves
inoculated with \textit{Salmonella} Montevideo except for a reduction in the percentage of right sub-iliac
lymph nodes that were culture positive in the vaccine treatment. The vaccine treatment decreased
the percentage of left superficial cervical lymph nodes that were positive compared to control steers
inoculated with \textit{Salmonella} Newport. Slight reductions in the percentage of \textit{Salmonella} positive sub-
iliac (right) and popliteal lymph nodes were also observed in the vaccine treatment, that when
combined and analyzed as percentage of all peripheral lymph nodes that were *Salmonella* positive, tended to be lower in the vaccine treatment.

**Implications**

Development of effective pre-harvest interventions to reduce the prevalence (and concentration) of *Salmonella* in the lymph nodes of cattle requires an understanding of the target population. Further, evaluation of potential interventions is enhanced when effective models are developed. Our initial lymph node surveillance data confirm the seasonal decrease in lymph node prevalence during the winter and spring time periods. Moreover, regional and animal-type variation is evident in the data in the feedlot prevalence is greater in the south and feedlot prevalence is greater than cull cow prevalence.

Prevalence is a function of both incidence (rate of new lymph node infections per unit time) and duration of infection. Hence interventions might be developed that target one or both of these determinants of prevalence. In our challenge models, we developed a repeatable challenge model that has substantial utility to evaluate both the duration of lymph node infection as well as opportunities to reduce it. Our preliminary vaccine work indicates that a commercially available vaccine may reduce the duration of infection of *Salmonella Newport* in lymph nodes.
Table 1. Prevalence (%) of *Salmonella* positive lymph nodes [peripheral and mesenteric (ileo-cecal)] following intradermal administration of *Salmonella* (Montevideo and Newport) to the lower legs and paunch region and effects of a SRP *Salmonella* Newport vaccine.

<table>
<thead>
<tr>
<th>Node</th>
<th>Montevideo</th>
<th></th>
<th></th>
<th>Newport</th>
<th></th>
<th></th>
<th>Combined strains</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Vaccine</td>
<td></td>
<td>Control</td>
<td>Vaccine</td>
<td></td>
<td>Control</td>
<td>Vaccine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P&lt;F</td>
<td></td>
<td></td>
<td>P&lt;F</td>
<td></td>
<td></td>
<td>P&lt;F</td>
<td></td>
</tr>
<tr>
<td>Sub-iliac right</td>
<td></td>
<td>75</td>
<td>0</td>
<td>0.03</td>
<td>25</td>
<td>25</td>
<td>1</td>
<td>50</td>
<td>12.5</td>
</tr>
<tr>
<td>left</td>
<td></td>
<td>0</td>
<td>25</td>
<td>0.28</td>
<td>75</td>
<td>25</td>
<td>0.16</td>
<td>38</td>
<td>25</td>
</tr>
<tr>
<td>Popliteal right</td>
<td></td>
<td>75</td>
<td>100</td>
<td>0.28</td>
<td>75</td>
<td>50</td>
<td>0.47</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>left</td>
<td></td>
<td>75</td>
<td>75</td>
<td>1</td>
<td>50</td>
<td>25</td>
<td>0.47</td>
<td>63</td>
<td>50</td>
</tr>
<tr>
<td>Superficial cervical right</td>
<td></td>
<td>75</td>
<td>100</td>
<td>0.28</td>
<td>75</td>
<td>75</td>
<td>1</td>
<td>75</td>
<td>88</td>
</tr>
<tr>
<td>left</td>
<td></td>
<td>100</td>
<td>100</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0.005</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Ileo-cecal</td>
<td></td>
<td>50</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>All peripheral nodes*</td>
<td>67</td>
<td>67</td>
<td>1</td>
<td>67</td>
<td>33</td>
<td>0.06</td>
<td>67</td>
<td>50</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*Excludes ileo-cecal

*For more information contact:*
National Cattlemen's Beef Association
A Contractor to the Beef Checkoff
9110 East Nichols Avenue
Centennial, Colorado 80112-3450
(303) 694-0305