Project Title: Toward and Understanding of the Pre-harvest Ecology and Approaches to Control of *Salmonella*

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Background

Because peripheral lymph nodes (PLN) are internal, they escape the usual surface-focused antimicrobial interventions utilized during the slaughter process. Unless removed during trimming, they may constitute a significant source of *Salmonella* in ground beef, which might pose a food safety risk.

Given the challenge of PLN removal, solutions will need to be implemented on the pre-harvest side of production. If we are to do so, we should first explore the principle that prevalence is a simple function of both the incidence of infections (i.e., the rate of new PLN infections per unit time) *and* the duration of infection (DOI; i.e., time). Observing incidence is problematic or even impossible in field settings. However, if incidence is held as a constant, DOI can be estimated. Using an experimental model of infection developed in part by Beef Checkoff support, incidence can be controlled and the DOI observed. Given this knowledge, incidence can, therefore, be estimated using the observed prevalence and DOI. What remains unknown, however, is the variation in prevalence across a variety of PLN within a carcass.

The objectives of this study were to 1) evaluate within-carcass diversity of *Salmonella* among various PLN; and 2) characterize the duration of infection of *Salmonella* within PLN of cattle following intradermal inoculation.

Methodology

To accomplish objective 1, a fecal swab and six PLN were collected from 100 carcasses. Lymph nodes included the left and right subiliac, superficial cervical, and popliteal nodes. Samples were processed to recover *Salmonella* isolates. In addition, methods were used to quantify the concentrations of *Salmonella* within PLN.

To accomplish objective 2, a recently developed transdermal experimental challenge model was used to determine the duration of infection of *Salmonella* in the PLN of steers. Thirty-six Holstein steers (avg. BW=137 kg) were inoculated with *Salmonella* (d0), transdermally on each lower leg, and both sides of the back and paunch. Calves (n=4/time point) were euthanized beginning at 6 h and subsequently on each of days 1, 2, 4, 7, 9, 11, 14 and 21 post-inoculation. The following PLN (right and left) were collected and cultured (quantitatively and

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qualitatively) for the challenge strain of *Salmonella*: subiliac, popliteal, superficial cervical, and axillary.

Findings

In objective1, *Salmonella* was recovered from 32 and 80% of the PLN and fecal swabs respectively. The likelihood of recovery of Salmonella decreased over time in that across the sample collection days of 03OCT, 17OCT, 24OCT, and 31OCT, *Salmonella* was recovered from 58.9, 56.1, 15.8, and 9.1% of PLN, respectively. Further, *Salmonella* was recovered from 80, 100, 95 and 47.8% of fecal swabs, respectively. *Salmonella* was recovered from at least one PLN per carcass 100, 96.7, 50, and 37.1% of the time, respectively. Saliently, during the first 2collection days, *Salmonella* was recovered from all 6 PLN in excess of 20% of the carcasses.

In objective 2, lymph nodes were generally 100% positive following qualitative culture at 24 hours post-inoculation and remained so until day 14. By day 21, the percentage of *Salmonella* positive nodes decreased to approximately 50%, indicating the PLN were starting to eliminate the challenge strain. The exception were the axillary lymph nodes, the majority of which were *Salmonella* positive at the first necropsy, but sporadically positive throughout the remainder of the experimental period. The implication of which is unclear and warrants further evaluation.

Based on previous research with this experimental model, we expected that the DOI would last approximately 10 to 14 days. Due to our successful challenge that provided quantifiable concentrations, the DOI was much longer. This indicates that DOI is partly a function of the underlying concentration within the nodes. Under these experimental conditions, elimination from the nodes appears to begin somewhere between days 14 and 21 and it is yet unknown at what time post-inoculation it will be complete.

Implications

The data reported here in provide important new information on the pre-harvest ecology of *Salmonella* within groups of cattle and in particular, within their PLN. One of the key findings is that *Salmonella* can be widely dispersed across many PLN within a carcass. This highlights the limitations of in-plant controls in that it is not practical or may even be unachievable to remove all PLN given the number and distribution. The need for effective pre-harvest controls is clear.

To this end, we provided important new information on the duration of infection. Based on prior research, this appears to be somewhat concentration dependent but saliently, it is not overly long in that we expect quantifiable concentrations to be eliminated soon after day 21. The implication of which is that the observed prevalence of *Salmonella* within PLN in cattle presented for harvest can be reduced if an intervention–a vaccine or probiotic for example–effectively reduces the DOI. Another opportunity for control are interventions that reduce the rate of new infections.

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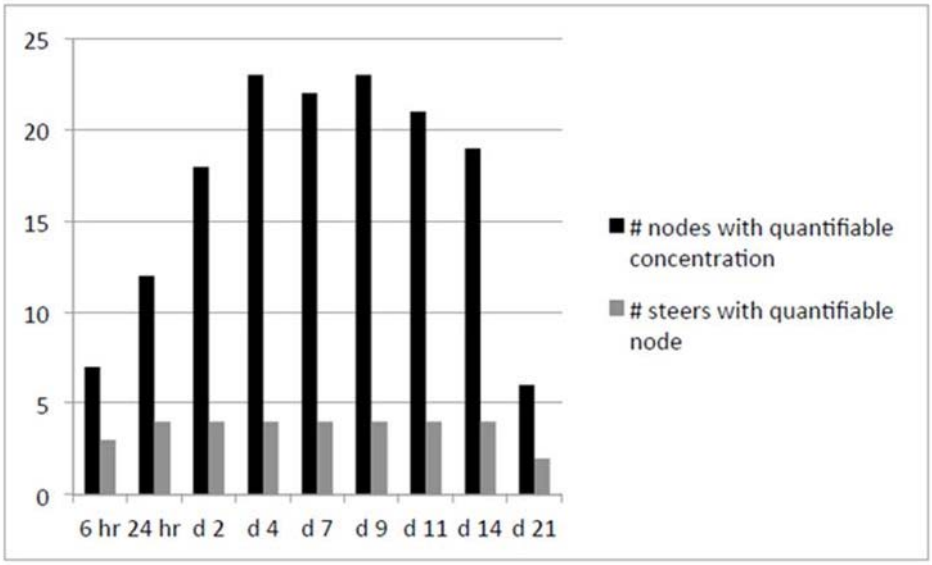

Figure 1. The number of steers with at least one peripheral lymph node *Salmonella* positive and the total number of peripheral lymph nodes *Salmonella* positive (following quantitative bacterial culture) resulting from intradermal administration of *Salmonella* followed by necropsy from 6 hours to 21 days post-inoculation. Four steers and 32 lymph nodes were examined at each time-point.

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