Evaluation of two commercially-available Salmonella vaccines on Salmonella in the peripheral lymph nodes of experimentally-infected cattle

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Abstract

Background: Salmonella is a common inhabitant of the ruminant gastrointestinal tract, where it often resides asymptomatically and may be shed into the feces. More recently it was discovered that Salmonella may be contained within the peripheral, non-mesenteric lymph nodes, where it is impervious to in-plant pathogen control interventions and may serve as a source of Salmonella-contamination of ground beef. Over the past 10 years considerable research effort has been expended at understanding how this pathogen gets to these lymph nodes, the duration of infection, and, most importantly, screening and developing potential intervention strategies that may be employed on farm prior to the animal being presented for slaughter.

Methods: Utilizing an experimental model of Salmonella inoculation of bovine peripheral lymph nodes (PLNs), two pilot vaccine experiments were conducted to evaluate two Salmonella vaccines: Salmonella Newport Bacterial Extract (Experiment I) and Endovac-Bovi® (Experiment II) on preventing Salmonella acquisition by these nodes. In Experiment I, 4 months following the booster vaccination, 30 steers were inoculated with three Salmonella serotypes intradermally: Newport, Montevideo, and Anatum administered to the right legs, left legs, and to the caudal thorax and abdomen, respectively. Cattle were inoculated every other day over the course of five days (three total inoculation events) and 6 and 12 days following the final Salmonella inoculation, 16 and 14 head in each treatment were euthanized, respectively. In Experiment II, 12 head of Holstein steers were utilized. Seven days following the booster and weekly thereafter for 3 weeks (four total inoculation events), cattle were inoculated as above and euthanized 7 days following final inoculation. Right and left sub-iliac, popliteal and pre-scapular lymph nodes were collected in each experiment, weighed and cultured for Salmonella.

Results: In Experiment I, no treatment differences were observed in Salmonella prevalence 6 days post-inoculation (necropsy 1). However, in vaccinated cattle at the second necropsy, a reduction ($p = 0.05$) in Salmonella prevalence was observed in the sub-iliac and pre-scapular lymph nodes as well as when all nodes were evaluated collectively ($p = 0.04$). In Experiment II, the vaccine reduced ($p = 0.03$) Salmonella prevalence in the right popliteal and tended ($p = 0.09$) to decrease prevalence in both popliteal lymph nodes.

Conclusion: Under these experimental conditions, the data generated provide evidence of a partial vaccine effect on Salmonella within PLNs and indicate that further research may be warranted.

Keywords: cattle, lymph node, Salmonella, vaccine

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**Introduction**

Recent research indicates that the lymphatic system, and peripheral lymph nodes (PLNs) in particular, may be a significant contributor to the contamination of ground beef with *Salmonella*.\(^1\) *Salmonella* prevalence in these nodes varies significantly and is influenced by cattle type [feedlot fattened versus those removed (or culled) from herds for productivity reasons], region, and season.\(^2,3\) Others\(^4\) reported that lymph nodes collected from cattle slaughter plants had an overall *Salmonella* prevalence of 1.6% while research examining *Salmonella* prevalence in lymph nodes of cattle originating from different feedlots reported a wide range in prevalence (0–88%) among the different operations.\(^5\) In a study in which 3300 sub-iliac lymph nodes were collected across the United States from feedlot-fattened and culled cattle, the authors reported a median *Salmonella* prevalence of 11.8% and 0.65%, respectively.\(^2\) Further, for *Salmonella*-positive lymph nodes, concentration of *Salmonella* varied from 0.1 to greater than 3.8 CFU (log\(_{10}\)) g\(^{-1}\) of lymph node.\(^2\) Conventional wisdom suggests that *Salmonella* within the PLN originates in the gastrointestinal tract, likely escaping into systemic circulation, where it is captured in the lymph and transported to the regional lymph node. Researchers, utilizing wild type isogenic tag-strains of *Salmonella*, noted that transmission of *Salmonella* from the gastrointestinal system to the lymphatic system was frequently observed.\(^6\) However, in other experimental-challenge studies, researchers had very little success in producing *Salmonella*-positive PLNs following oral dosing of *Salmonella*.\(^7\)

As PLNs are below the surface of the carcass, and frequently encased in adipose tissue, they are protected from currently used in-plant pathogen interventions that focus on preventing or removing surface contamination. Based on a preliminary risk assessment, *Salmonella*-harboring PLNs are likely the primary contributor to *Salmonella* in ground beef,\(^1\) and in lieu of removal during slaughter, solutions will need to be implemented on the pre-slaughter side of production. Currently, few pre-harvest interventions are available for controlling *Salmonella* in cattle and are limited to vaccines and the feeding of direct fed microbials. In previous research, whole-herd vaccination with a vaccine containing siderophore receptors and porin proteins (SRPs) from *Salmonella* Newport was associated with a reduced prevalence of fecal *Salmonella* (8.0% versus 36.8%) when compared with herds that did not vaccinate.\(^8\) Others reported there was no evidence of a reduction in the fecal shedding of *Salmonella* in sub-clinically infected dairy cows\(^9,10\) although an improvement in milk production was observed in one study.\(^10\) Similarly, no vaccine effect was observed on fecal prevalence of *Salmonella* in feedlot cattle.\(^11\) In prior work, we reported modest efficacy of a vaccine administered to control *Salmonella* in the PLN.\(^12\) Therefore the objective of the current research was to use an experimental model of *Salmonella* infection of the PLN\(^12,13\) to examine the efficacy of two commercially-available *Salmonella* vaccines, *Salmonella* Newport SRP vaccine and a *Salmonella* Typhimurium bacterin-toxoid vaccine, in pilot studies to reduce *Salmonella* in the PLN.

**Methods**

All animal care and experimental procedures were reviewed and approved by the Animal Care and Use Committee of the Food and Feed Safety Research Laboratory, USDA (ACUC No. 2013001). All research below was conducted at this same laboratory. Thirty and 12 Holstein and Holstein-cross steers (average body weight = 137 kg and 103 kg ± approximately 15 kg) for Experiments I and II, respectively) were purchased from a single supplier on two occasions and transported to our laboratory in College Station, TX, where they were maintained on pasture and supplemented with a commercial non-medicated calf starter. Upon arrival, steers were weighed, identified with an ear tag, and metaphylactically administered tulathromycin (Draxxin\(^6\), Zoetis Inc., Kalamazoo, MI, USA), and an anthelmentic (Cydectin, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA) per label directions.

**Experiment I**

Cattle were brought to the laboratory in the summer of 2012 and maintained on native pasture prior to acclimation to pen and diet. Animals were randomly assigned to treatment (control or vaccine; 15 head per treatment). For randomization to treatment, steers were assigned a random number generated from a random number table, those numbers ranked, and the first 15
Experiment II

Twelve head of Holstein steers were utilized and group housed as above in a large outdoor pen and fed a commercial beef feed and Bermuda grass hay (50:50). One-half of the steers were administered Endovac-Bovi® with Immune Plus® [commercially available Salmonella Typhimurium bacterin-toxoid vaccine (IMMVAC Inc., Columbia, MO, USA)] on day 0 (October 2012) followed by a booster vaccination 14 days later. Control steers received a sham injection of sterile saline (equal volume). Seven days following the booster and weekly thereafter for 3 weeks (four total inoculation events), all cattle were inoculated intradermally with the three strains of Salmonella [Newport (3.3 × 10⁶ CFU ml⁻¹); Montevideo (8.5 × 10⁶ CFU ml⁻¹); and Anatum (3.3 × 10⁶ CFU ml⁻¹)], euthanized and necropsied (7 days following final Salmonella inoculation; November 2012), as described above. One control steer died unexpectedly during the experimental period of an unknown cause. Six isolates from each Salmonella-positive samples were serogrouped as above.
Statistical analysis
Data were analyzed using the commercially available software (SAS version 9.4 software, SAS Institute Inc., Cary, NC, USA). Qualitative data (proportion of positives) by node type (popliteal, pre-scapular and sub-iliac) were subjected to chi-square analysis using the PROC Frequency procedure. The effect of vaccine on *Salmonella* prevalence in all the PLNs combined was examined using logistic regression techniques and the model adjusted with animal as the co-variate to account for potential clustering of the outcome within animal. Non-zeros, that is, those instances where *Salmonella* was recovered from the PLN, yet was below the limit of quantification, were assigned a concentration that varied from 0.1 to 0.5 log CFU g⁻¹ depending on size of the lymph node. Quantitative data (log transformed, base-10) were analyzed using analysis of variance techniques. Individual animal served as the experimental unit and differences were considered significant at a 5% level of significance.

Results and discussion
In the first experiment utilizing the SRP vaccine, no treatment differences were observed in *Salmonella* prevalence of the PLNs on the first necropsy, 6 days post-inoculation (Table 1). There was a trend (p = 0.08) for more *Salmonella*-positive pre-scapular nodes in the vaccinated steers compared with control animals. Overall, 52.2% and 55% of the PLNs were *Salmonella*-positive in control and vaccinated steers, respectively (Table 1). Four steers were culture negative for *Salmonella* in all of the lymph nodes examined. Increasing the time from inoculation to necropsy was associated with a detectable effect of the vaccine. A reduction (p = 0.05) in *Salmonella* prevalence was observed in the vaccinated cattle in the sub-iliac and pre-scapular lymph nodes as well as when all nodes were evaluated collectively (p = 0.04). Similar associations were observed for the popliteal lymph nodes (p = 0.06) and for the lymph nodes (sub-iliac, pre-scapular, and popliteal) from the right side of the body (p = 0.09; Table 1). *Salmonella* concentrations in the PLN were insufficient [0.1–1.5 CFU (log₁₀) g⁻¹ lymph node], meaning very few lymph nodes across both necropsies contained quantifiable concentrations of the challenge strains of *Salmonella*, for statistical analysis. This has been encountered previously by the authors even with multiple applications of *Salmonella* and

### Table 1. The percentage of *Salmonella* positive lymph nodes (popliteal, pre-scapular, and sub-iliac; by node and combined) in cattle vaccinated with *Salmonella* Newport Bacterial Extract vaccine (SRP) prior to experimental inoculation with *Salmonella*. Cattle necropsied 6 or 12 days post-inoculation (Experiment I).

| Node       | Necropsy 1 (day 6) | Necropsy 2 (day 12) |
|------------|--------------------|---------------------|
|            | Control | Vaccine | p-value | Control | Vaccine | p-value |
| Popliteal  |         |         |         |         |         |         |
| Right      | 62.5    | 37.5    | 0.32    | 57.1    | 14.3    | 0.09    |
| Left       | 50      | 50      | 1       | 71.4    | 42.9    | 0.28    |
| Both       | 56.3    | 43.8    | 0.48    | 64.3    | 28.6    | 0.06    |
| Pre-scapular |       |         |         |         |         |         |
| Right      | 62.5    | 87.5    | 0.25    | 57.1    | 14.3    | 0.09    |
| Left       | 12.5    | 50      | 0.11    | 57.1    | 28.6    | 0.28    |
| Both       | 37.5    | 68.8    | 0.08    | 57.1    | 21.4    | 0.05    |
| Sub-iliac  |         |         |         |         |         |         |
| Right      | 75      | 50      | 0.3     | 57.1    | 14.3    | 0.09    |
| Left       | 50      | 50      | 1       | 57.1    | 28.6    | 0.28    |
| Both       | 62.5    | 50      | 0.48    | 57.1    | 21.4    | 0.05    |
| All nodes  | 52.2    | 55      | 0.89    | 62.3    | 19.9    | 0.04    |

SRP, siderophore receptor and porin protein
suggests differences in experimental *Salmonella* strains *versus* those acquired naturally and/or a different response by the host animal upon exposure.

Multiple *Salmonella* strains were inoculated into these experimental animals for two reasons. First, as cattle frequently harbor multiple serogroups within their gastrointestinal tract, it was deemed important to evaluate the vaccines against multiple *Salmonella* strains. Second, as this research was some of the early research following the discovery of *Salmonella* in the PLNs, it was unknown whether different serotypes varied in their likelihood of finding their way into a PLN. Serogroup distribution (data not shown) among those isolates examined from Experiment I was similar among treatments. All isolates matched one of the three challenge strain serogroups. The majority of recovered isolates were identified as serogroup E1 (76% and 60% in control and vaccinated cattle, respectively). Serogroup C1 accounted for 18% and 30%, and C2 for 6% and 11% of the recovered isolates in control and vaccinated cattle, respectively. As the *Salmonella* strains were applied using similar concentrations and an equal number of applications, the over-representation of the serogroup E1 (Anatum) compared with C1 (Montevideo) and C2 (Newport) is something of a surprise. Further, E1 isolates were recovered from all lymph nodes, not just the sub-iliac lymph nodes as might be anticipated given the site of Anatum administration (abdomen and caudal thorax). That said, four applications of the device were made to both the right and left sides of the abdomen and caudal thorax, both locations that are served by the sub-iliac lymph nodes, which may explain in part the abundance of recovered E1 isolates observed in this study.

According to the manufacturer, the SRP vaccine induces an antibody response against SRPs produced by *Salmonella* to acquire iron and thereby limiting iron acquisition. The vaccine was developed using SRPs specifically from *Salmonella* Newport, and while SRPs are utilized by the vast majority of *Salmonella* serotypes, to date there is only anecdotal evidence that the vaccine is effective against other serotypes. This, however, does not explain the relative low frequency of recovery of C2 isolates compared with C1 and E1 in the current study, as there were few isolates (6% and 11%) recovered from cattle in both treatments. A more likely explanation of these differences likely lies in serotype differences and/or the location of administration. These data are somewhat consistent with field data in that the SRP vaccine has been associated with mixed results when fecal shedding of *Salmonella* was evaluated. To date, however, it is unknown to what extent gastrointestinal populations of *Salmonella* are associated with the prevalence of *Salmonella* in the PLNs. If they are associated, these field data may lend support of serotype-specific efficacy. For example, an apparent lack of effect of the SRP vaccine on serogroup E1 (Anatum) was reported11 when fecal shedding of *Salmonella* in feedlot cattle was examined. Previously, we reported a modest effect of this same vaccine when Newport and Montevideo were used to challenge cattle12 and therefore were optimistic that a similar response would be seen in this study with Montevideo and Anatum. Subsequent research, conducted in part as a result of the results herein, evaluated the same SRP vaccine on *Salmonella* in the PLNs of fed cattle in a commercial production setting.15 Those researchers reported no difference between vaccinated and control cattle and noted that the high *Salmonella* prevalence in this particular feedlot may have overwhelmed any beneficial effect exerted by the vaccine.

In the second experiment, the Endovac-Bovi® vaccine was associated with reduced (*p* = 0.03) *Salmonella* prevalence in the right popliteal lymph nodes and tended (*p* = 0.09) to decrease prevalence in both popliteal lymph nodes when evaluated together. While not statistically significant, some evidence for a lowered (*p* = 0.14) *Salmonella* prevalence in the right pre-scapular and left sub-iliac nodes was observed (60% versus 16.7% for control and vaccinates, respectively; Table 2). One animal was culture negative for *Salmonella* in all of the nodes examined. It is important to note that fewer animals were included in Experiment II than in Experiment I; therefore, we lacked statistical power to detect what appeared to be substantial differences between vaccinated and control animals. *Salmonella* concentrations above the limit of detection were observed in only four steers and eight total nodes, ranging from 1.9 to 2.4 CFU (log_{10}) g⁻¹ of lymph node, and did not appear to be associated with treatment status (data not shown). Forty-five percent of the examined isolates belonged to serogroup C1.
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(Montevideo), while 35% and 19% belonged to groups E₁ (Anatum) and C₂ (Newport), respectively (data not shown).

The Endovac-Bovi® vaccine used in this experiment is made utilizing a genetically engineered Salmonella Typhimurium that has temporarily or permanently lost its ability to produce part, or all, of the capsular O side-chain carbohydrate. This impairment reportedly exposes the inner aspects of the bacterial cell wall that may serve as antigens to the host immune system. If this core is indeed common to all Salmonella, cross-protective immunity might be possible across most serotypes. If so, this may explain why the distribution of recovered serotypes was more similar in Experiment II when compared with Experiment I, in which E₁ was the predominant serogroup recovered. As in Experiment I, it is possible that increasing the time from inoculation to necropsy, and thus allowing more time for the vaccine to work, may have produced more significant results.

While these pilot vaccine studies observed significant vaccine effects, the failure of the SRP vaccine to work under commercial conditions in subsequent research and the challenges of incorporating a vaccine program into the cattle feeding industry such that it does not require additional processing of the cattle to administer a booster vaccine suggest that significant improvements need to be made in this technology prior to further evaluation and ultimate adoption by the cattle industry. That said, vaccines are viewed by many as viable pre-harvest intervention strategies and research is on-going to develop effective vaccines for Salmonella within the PLNs of cattle.

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Conflict of interest statement
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References
1. Li M, Malladi S, Hurd H, et al. Salmonella spp. in lymph nodes of fed and cull cattle: relative assessment of risk to ground beef. Food Control 2015; 50: 423–434.
2. Gragg SE, Loneragan GH, Brashears MM, et al. Cross-sectional study examining Salmonella enterica carriage in subiliac lymph nodes of cull and feedlot cattle at harvest. Foodborne Path Dis 2013; 10: 368–374.
3. Webb HE, Brichta-Harhay DM, Brashears MM, et al. Salmonella in peripheral lymph nodes of cattle.

| Table 2. The percentage of Salmonella positive lymph nodes (popliteal, pre-scapular, and sub-iliac; by node and combined) in cattle vaccinated with Endovac-Bovi® vaccine prior to experimental inoculation with Salmonella. Cattle necropsied 7 days post-inoculation (Experiment II). |
| --- |
| **Node** | **Control** | **Vaccine** | **p-value** |
| Popliteal | | | |
| Right | 60 | 0 | 0.03 |
| Left | 60 | 50 | 0.74 |
| Both | 60 | 25 | 0.10 |
| Pre-scapular | | | |
| Right | 60 | 16.7 | 0.14 |
| Left | 80 | 83.3 | 0.89 |
| Both | 70 | 50 | 0.34 |
| Sub-iliac | | | |
| Right | 40 | 50 | 0.74 |
| Left | 60 | 16.7 | 0.14 |
| Both | 50 | 33.3 | 0.43 |
| All nodes | 60 | 36.1 | 0.30 |
healthy cattle at slaughter. Frontiers Microbiol 2017; 8: 1–10.

4. Arthur TM, Brichta-Harhay DM, Bosilevac JM, et al. Prevalence and characterization of Salmonella in bovine lymph nodes potentially destined for use in ground beef. J Food Prot 2008; 71: 1685–1688.

5. Haneklaus AN, Harris KB, Griffin DB, et al. Salmonella prevalence in bovine lymph nodes differs among feedyards. J Food Prot 2012; 75: 1131–1133.

6. Porwollik S, Genovese K, Chu W, et al. Neutral barcoding of genomes reveals the dynamics of Salmonella colonization in cattle and their peripheral lymph nodes. Vet Micro 2018; 97–106.

7. Brown TR, Edrington TS, Genovese KJ, et al. Oral Salmonella challenge and subsequent uptake by the peripheral lymph nodes in calves. J Food Prot 2015; 573–578.

8. Loneragan GH, Thompson DU, McCarthy RM, et al. Salmonella diversity and burden in cows on and culled from dairy farms in the Texas high plains. Foodborne Path Dis 2012; 9: 549–555.

9. Heider LC, Meiring RW, Hoet AE, et al. Evaluation of vaccination with a commercial subunit vaccine on shedding of Salmonella enterica in subclinically infected dairy cows. J Am Vet Med Assoc 2008; 233: 466–469.

10. Hermesch DR, Thompson DU, Loneragan GH, et al. Effects of a commercially available vaccine against Salmonella enterica serotype Newport on milk production, somatic cell count, and shedding of Salmonella organisms in female dairy cattle with no clinical signs of salmonellosis. Am J Vet Res 2008; 69: 1229–1234.

11. Dodd CC, Renter DG, Thompson DU, et al. Evaluation of the effects of a commercially available Salmonella Newport siderophore receptor and porin protein vaccine on fecal shedding of Salmonella bacteria and health and performance of feedlot cattle. Am J Vet Res 2011; 72: 239–247.

12. Edrington TS, Loneragan GH, Hill J, et al. Development of challenge models to evaluate the efficacy of a vaccine to reduce carriage of Salmonella in peripheral lymph nodes of cattle. J Food Prot 2013; 76: 1259–1263.

13. Edrington TS, Loneragan GH, Hill J, et al. Development of a transdermal Salmonella challenge model in calves. J Food Prot 2013; 76: 1255–1258.

14. Brichta-Harhay DM, Arthur TM, Bosilevac JM, et al. Microbiological analysis of bovine lymph nodes for the detection of Salmonella enterica. J Food Prot 2012; 7: 854–858.

15. Cernicchiaro N, Ives SE, Edrington TS, et al. Efficacy of a Salmonella siderophore receptor protein vaccinSe on fecal shedding and lymph node carriage of Salmonella in commercial feedlot cattle. Foodborne Path Dis 2016; 13: 517–525.