Escherichia coli O157:H7 Shedding in Vaccinated Beef Calves
Born to Cows Vaccinated Prepartum with Escherichia coli O157:H7 SRP Vaccine

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ABSTRACT

Extensive research, intervention equipment, money, and media coverage have been directed at controlling Escherichia coli O157:H7 in beef cattle. However, much of the focus has been on controlling this pathogen postcolonization. This study was conducted to examine the performance, health, and shedding characteristics of beef calves that were vaccinated with an E. coli O157:H7 SRP bacterial extract. These calves had been born to cows vaccinated prepartum with the same vaccine. Cows and calves were assigned randomly to one of four treatments: (i) neither cows nor calves vaccinated with E. coli O157:H7 SRP (CON), (ii) cows vaccinated with E. coli O157:H7 SRP prepartum but calves not vaccinated (COWVAC), (iii) calves vaccinated with E. coli O157:H7 SRP but born to cows not vaccinated (CALFVAC), (iv) cows vaccinated with E. coli O157:H7 SRP prepartum and calves also vaccinated (BOTH). Calves born to vaccinated cows had significantly higher titers of anti–E. coli O157:H7 SRP antibodies (SRPAb) in circulation at branding time (P < 0.001). Upon entry to the feedlot, overall fecal E. coli O157:H7 prevalence was 23% among calves, with 25% in the CON treatment group, 19% in the CALFVAC group, 32% in the COWVAC group, and 15% in the BOTH group (P > 0.05). Fecal shedding of E. coli O157 on arrival to the feedlot was not correlated with fecal shedding at slaughter (Spearman’s rho = −0.02; P = 0.91). No significant effects of cow or calf E. coli O157:H7 SRP vaccination treatment were found on feedlot calf health or performance (P > 0.05), prevalence of lung lesions or liver abscess (P > 0.05), or morbidity, retreatment, or mortality numbers (P > 0.05). The findings of this study indicate that the timing of vaccination of calves against E. coli O157:H7 may be an important consideration for maximizing the field efficacy of this vaccine.

Enterohemorrhagic Escherichia coli O157:H7 is one of the most problematic pathogens in the United States and around the world. E. coli was first recognized as a human foodborne pathogen in the early 1980s after two infection outbreaks were linked to undercooked hamburgers (23). Three manifestations of E. coli O157:H7 infections occur in humans: hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (12). Signs of human infection occur 3 to 5 days after ingestion of the organism. Most cases are self-limiting; only 5% of cases result in the severe complications of hemolytic uremic syndrome or thrombotic thrombocytopenic purpura (2). The groups of people most at risk of developing the severe complications are children younger than 5 years and elderly and immunocompromised individuals. The largest numbers of reported cases of E. coli O157:H7 infections have occurred in the United States, Canada, and the United Kingdom (12). The 2006 incidence of E. coli O157 infections in the United States reported by the Centers for Disease Control and Prevention FoodNet was 1.31 per 100,000 individuals (5), which is half of the incidence reported just 10 years before. Much of the decrease is likely related to peri- and postharvest intervention strategies implemented through the hazard analysis and critical control point systems in all harvest facilities regulated by the U.S. Food and Drug Administration.

A major development in the ecology and epidemiology of E. coli O157 was the establishment of cattle as the reservoir for E. coli O157:H7 (22). This discovery spurred a large amount of research focused specifically on E. coli O157:H7 and its association with beef cattle. Early estimates of the herd level prevalence of E. coli O157 were around 8 to 16% (2). The development of testing procedures such as immunomagnetic separation has increased the sensitivity of detection of this pathogen by 10- to 100-fold (7) and resulted in more accurate and higher estimates of the true prevalence of E. coli O157:H7 in U.S. beef herds. Elder et al. (8) found a feedlot-level prevalence of 63% and an individual animal-level prevalence of approximately 28%. The prevalence of E. coli O157 is affected by season, the testing frequency, the timing of sampling, the treatment of

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the samples before isolation, and the geographic location (2, 21). In the United States, the summer months of May through September tend to be associated with the highest prevalence estimates of E. coli O157:H7 in cattle feces, and in the winter months the prevalence is lowest (29).

Various preharvest E. coli O157:H7 intervention strategies have been reported. In two reviews of preharvest controls for E. coli O157:H7 (14, 24), the reviewers found that a probiotic containing Lactobacillus acidophilus NP51 and Propionibacterium freudenreichii was the only intervention strategy that was significantly and consistently proven in field trials to increase animal resistance to colonization with E. coli O157. Sargeant et al. (24) concluded that sodium chloride in the feed or water also increased animal resistance to colonization with E. coli O157; however, sodium chloride has not been labeled for use in the United States. In neither of the reviews was the vaccine technology utilizing E. coli O157:H7 siderophore receptor and porin proteins (SRP) considered for preharvest control of E. coli prevalence in cattle. This technology has recently received conditional licensure in the United States and has been extensively studied for control of E. coli O157:H7 prevalence and levels in feeder cattle feces (9, 27, 28, 33). The majority of existing research and strategies have been focused on control of E. coli O157:H7 at the feedyard and harvest stages of production. These approaches solely address the problem postcolonization.

The objectives of this study were to determine the E. coli O157:H7 shedding characteristics, health, and performance of beef calves born to dams vaccinated with E. coli O157:H7 SRP vaccine versus unvaccinated dams. The effects of a series of E. coli O157:H7 SRP vaccinations in beef calves before and at the time of arrival at the beef finishing facility also were examined in terms of E. coli O157:H7 shedding characteristics, health, and performance of these calves throughout their lives.

MATERIALS AND METHODS

General overview. This study was conducted under conditions approved by the Kansas State University Institutional Animal Care and Use Committee. In January 2009, 437 cows from two commercial cow-calf herds associated with Kansas State University were stratified by age and assigned randomly to either E. coli SRP vaccine or placebo treatment groups. Blood samples were collected from cows before the initial vaccination to ensure an E. coli O157:H7 SRP antibody-free status. Cows were vaccinated with their assigned vaccine treatments at 60 and 30 days before the projected start of the calving season. To create a 2 x 2 factorial treatment structure, calves were stratified by dam treatment and assigned randomly to E. coli SRP vaccine or placebo treatment groups. Treatments were administered at branding, preconditioning, and entry to the feedlot for a total of four treatments groups: (i) neither cows nor calves vaccinated with E. coli O157:H7 SRP (CON), (ii) cows vaccinated with E. coli O157:H7 SRP prepartum but their calves not vaccinated (COWVAC), (iii) cows not vaccinated but calves vaccinated with E. coli O157:H7 SRP (CALFVAC), (iv) cows vaccinated with E. coli O157:H7 SRP prepartum and their calves also vaccinated (BOTH). Within each ranch, an equal number of calves was assigned to each of the four treatment groups. The two vaccine treatments were prepared by the sponsoring company and labeled A and B to blind study personnel. All blood and fecal samples were labeled with sequential numbers to blind laboratory personnel.

Cows and calves were managed according to normal ranch management conditions at each unit throughout the study. While on pasture, cows and calves from the four treatment groups were allowed to commingle, i.e., vaccinates with placebo animals. During the preconditioning and feedlot phases of the trial, calves were penned according to sex and vaccine treatment group. Calves were gathered while on grass at the traditional branding time of approximately 90 days of age. Calves were given a seven-way clostridial vaccine and their first assigned SRP vaccine treatment, blood and fecal samples were collected, and males were castrated. Calves were returned to their dams and turned out to grass pastures until weaning. Calves were abruptly weaned and transported to preconditioning pens at each of the two ranches, where they were processed and given the second dose of SRP vaccine, fecal samples were collected, and calves were provided ad libitum access to fresh grass hay and water. At the end of the 30-day preconditioning period, calves were transported by truck 3 h to an auction facility, where they remained overnight, and then were reloaded and transported to the feedyard. Calves were processed on arrival to the feedyard and given their third and final SRP vaccine dose if assigned to that treatment. Feedlot pen groups were created by combining a matched (by sex and treatment assignment) preconditioning pen group from each of the two ranches to create a single pen of either vaccinated or unvaccinated calves. Calves remained in these pens for the remainder of the study. Health and performance data were collected monthly.

Serology. Serum antibody response was determined by enzyme-linked immunosorbent assay (ELISA). E. coli O157 SRP antigen was coated on a Nunc Maxisorp 96-well plate (Nalge Nunc International, Rochester, NY) at 250 ng per well in carbonate coating buffer (pH 9.6), covered, and incubated overnight at 4°C. Plate solutions were then dumped, reactions were blocked with 1% polyvinyl alcohol–phosphate-buffered saline (PVA-PBS), and plates were covered and incubated at 37°C for 1 h. Dilutions of the serum samples were prepared in 1% PVA-PBS at 1:500 in duplicate. Plates were covered, incubated at 37°C for 1 h, and then washed three times with 0.05% PBS–TWEEN 20. Following the wash step, a 1:1,600 dilution of the conjugate, horseradish peroxidase sheep anti-bovine immunoglobulin G (The Binding Site, San Diego, CA), in 1% PVA-PBS was applied to the plate, which was then covered and incubated at 37°C for 1 h. The wash step was repeated, and the reaction was developed with 2,2’-azino-di-3-ethyl-benzthiazoline-6-sulfonate (KPL, Inc., Gaithersburg, MD). The absorbance of the wells was read at 405 to 490 nm with an ELISA reader. The average of the optical density of the negative control was calculated and subtracted from all values as a reagent blank. Results for sample duplicates were averaged and divided by the positive control average, yielding the sample:positive titer.

Fecal culture. Fecal samples were obtained directly from the rectum of each animal as it was restrained at each handling time. The samples were placed into collection vials labeled with sequential numbers to blind treatment assignments, placed on ice, and sent overnight to the Epitopix LLC testing laboratory. Upon arrival, fecal samples were processed for isolation of E. coli O157 by immunomagnetic separation. The same procedure previously described by Wileman et al. (33) was used for the branding, preconditioning, and feedlot arrival samples. The preslaughter fecal samples were processed with the same procedure.
except samples underwent a 6-h rather than overnight enrichment to improve laboratory processing flow (3). Samples were weighed, and approximately 2 g of each fecal specimen was placed into a Whirl-Pak filter bag (Nasco, Modesto, CA). Gram-negative broth containing cefixime (12.5 μg/ml), cefsulodin (2.5 μg/ml), and vancomycin (8 mg/ml) (GNccv) was used to get the fecal specimen in a liquid state for sample processing. Samples were normalized by weight so each sample was present at a ratio of 1 g of feces per 10 ml of GNccv broth. Samples were incubated at 37°C overnight (6 h for simulated slaughter samples) for enrichment of *E. coli* O157. After enrichment, 1 ml of each sample was added to a 96-well plate containing 20 μl of magnetic anti-O157 Dynabeads (Invitrogen, Carlsbad, CA). The enriched cultures were allowed to incubate with the magnetic beads on a shaker at room temperature for at least 15 min. Magnetic particles were recovered and washed with an eight-channel magnetic PikPen (Bio-Nobile, Turku, Finland). After the final wash, the particles were released into 100 μl of wash buffer (PBS containing 0.05% Tween 20) in a 96-well plate for analysis of *E. coli* O157 that was bound to the magnetic particles. For plating, 50 μl of solution was plated onto a sorbitol MacConkey agar plate containing cefixime and tellurite (CT-SMAC; BD, Franklin Lakes, NJ), and 50 μl was plated onto a Chromagar-O157 plate (Chromagar, Paris, France). The inoculum was spread onto each agar plate, and the plates were incubated at 37°C overnight. The plates were observed for suspect *E. coli* O157 colonies, which were then tested for O157 agglutination with an O157 test kit (Remel, Lenexa, KS). Positive samples were subcultured to Chromagar-O157 or CT-SMAC to acquire a pure culture of *E. coli* O157.

**Data analysis.** Data were recorded and summarized with Excel (Microsoft, Redmond, WA). For feed efficiency and dry matter intake, the pen was the experimental unit; for all other factors, the calf was the experimental unit. The majority of the heifers enrolled in this study were placed onto a heifer development trial; therefore, only data from steers and cull heifers destined for slaughter (n = 252) were used for the feedlot entry and slaughter statistics. Data were analyzed using the GLIMMIX and Mixed procedures in SAS version 9.1 (SAS Institute Inc., Cary, NC). Lung lesions, liver abscesses, morbidity, and mortality data were given a binomial score, summarized, and modeled using an events-trial format with feedlot entry as the trial variable. Model adjusted risk probabilities were then calculated for these outcomes. Cow vaccination, calf vaccination, ranch of origin, preconditioning trial treatment, and interactions of these variables were presented as possible variables in each model. P values of ≤0.05 were considered significant.

**RESULTS**

The final number of cattle in each treatment group was 105 CON, 103 CALFVAC, 100 COWVAC, and 108 BOTH, for a total of 416 head. None of the calves were shedding *E. coli* O157, based on fecal cultures at the time of initial vaccination at branding. Calves born to vaccinated cows had significantly higher titers of anti-*E. coli* O157 SRP antibodies (SRPAb) in circulation at branding (P < 0.001). Only three calves were shedding *E. coli* O157 at weaning, and all three were placebo calves. At weaning, calves from ranch 1 had significantly higher SRPAb titers than did calves from ranch 2 (P = 0.009). All four treatment groups (CON, CALFVAC, COWVAC, and BOTH) had significantly different titers (P < 0.001) from each other during the feedlot phase; CALFVAC calves had the highest average titer, followed by BOTH calves (Fig. 1). Because of the study design, when a longitudinal data analysis of SRPAb was performed accounting for the repeated samples from the same animal over time, a significant three-way interaction between time, cow treatment, and calf treatment was identified (P = 0.008; Fig. 1).

Upon feedlot entry, the overall fecal *E. coli* O157:H7 prevalence was 23% among calves. The group fecal *E. coli* O157 prevalences at entry were 25% for CON, 19% for CALFVAC, 32% for COWVAC, and 15% for BOTH calves; these prevalences were not significantly different from one another (P > 0.05; Fig. 2). *E. coli* O157 fecal prevalence based on just the calf vaccination treatment was 17% for vaccines (CALFVAC and BOTH) and 31% for placebo calves (CON and COWVAC). At slaughter, overall fecal *E. coli* O157 prevalence decreased slightly to 27%. Group fecal *E. coli* O157 prevalences at slaughter were 22% for CON, 32% for CALFVAC, 15% for COWVAC, and 39% for BOTH calves; these prevalences were not significantly different from one another (P > 0.05; Fig. 2). *E. coli* O157 fecal prevalence based on just the calf vaccination treatment was 36% for vaccines (CALFVAC and BOTH) and 18% for placebo calves (CON and COWVAC).

Unequal distribution of shedding prevalence was noted between pens. Calves in three of the eight pens (two vaccine pens and one placebo calf pen) had fecal *E. coli* O157 prevalences greater than 45%. In the remaining five pens, calves from one pen had a prevalence of 13% and those from the remaining four pens had a prevalence of 4% or less. Seventeen percent (11 of 65) of the calves shedding *E. coli* O157 at slaughter also were shedding the pathogen at feedlot entry. Nearly all (10 of 11) of these calves were from
one of the three very high prevalence pens (Fig. 3). Fecal shedding of *E. coli* O157 on arrival at the feedlot was not correlated with fecal shedding at slaughter (Spearman’s rho = −0.02; *P* = 0.91). No significant effects of cow or calf *E. coli* O157:H7 SRP vaccination treatment on feedlot health or performance of calves were found (*P* > 0.05; Table 1). No vaccination effects on the prevalence of lung lesions or liver abscess (*P* > 0.05; Table 1) or on the number of morbid, retreated, or dead calves (*P* > 0.05; Table 1).

**DISCUSSION**

Nearly all of the previously reported studies for preharvest *E. coli* O157 control have been performed on cattle in the feedlot (14, 15, 24). This is the first study in which SRP vaccination of the dam to prevent colonization of the calf preharvest (through passive transfer) has been evaluated. Our laboratory had previously reported the successful passive transfer of *E. coli* O157:H7 SRP–specific antibodies in a subsample of calves from the current study (33). Protection of neonates from disease through manipulation of colostral components via vaccination of the dam prepartum is a long-used method for prevention of pathogenic viral and bacterial diseases (1, 10, 30). However, protection of calves against a relatively nonpathogenic commensal organism through passive immunization has not been as well explored. In a small study conducted in Japan, passive transfer of *E. coli* O157 antibodies to calves was successful, but the researchers did not follow the calves to determine whether these antibodies were protective (32). In a study in Argentina in which the ability of bovine colostrum–derived antibody to inhibit hemolytic activity of attaching and effacing *E. coli* was examined, the researchers found that colostral lactoferrin also was very important for inhibition of hemolytic activity (31). Lactoferrin is an iron-binding glycoprotein in colostrum and milk that has inflammatory, antimicrobial, and immunomodulatory functions (4). Thus, lactoferrin and the SRP-based technology combat *E. coli* by using its need for iron against itself (18). The assumption is that the combination approach of antibodies against the iron acquisition proteins and lactoferrin sequestering of free-iron stores additively inhibit colonization of young cattle by *E. coli* O157. However, throughout the feedlot phase of the current study, fecal *E. coli* O157 prevalence remained at high levels.

A significant increase in the prevalence of *E. coli* O157 was noted from the time of weaning (0.6% prevalence) until the time of harvest (27% prevalence). The cause of this increase is not known, but this finding is similar to those of other researchers who reported that prevalence of *E. coli* O157 was low (0 to 1.9%) in calves on pasture (11, 22, 25). In a similar longitudinal trial, Gannon et al. (11) found an apparently cyclic pattern of *E. coli* shedding, where fecal prevalence was 25% from 1 to 7 weeks of age, declined to 0% after the animals were turned out to pasture, and increased to 6 to 14% by 2 weeks postweaning. In the present study a similarly increasing prevalence was found, with a branding time (already on grass) prevalence of 0%, a

**TABLE 1. Summary of feedlot performance and carcass effects by calf vaccination treatment**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vaccinate calves</th>
<th>Placebo calves</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wean wt (lb)</td>
<td>488</td>
<td>487</td>
<td>15</td>
<td>0.96</td>
</tr>
<tr>
<td>Feedlot entry wt (lb)</td>
<td>528</td>
<td>527</td>
<td>15</td>
<td>0.99</td>
</tr>
<tr>
<td>Final body wt (lb)</td>
<td>1,128</td>
<td>1,134</td>
<td>10</td>
<td>0.67</td>
</tr>
<tr>
<td>Feedlot ADG (lb/day)</td>
<td>3.06</td>
<td>2.91</td>
<td>0.16</td>
<td>0.50</td>
</tr>
<tr>
<td>Weaning through slaughter ADG (lb/day)</td>
<td>2.79</td>
<td>2.65</td>
<td>0.15</td>
<td>0.50</td>
</tr>
<tr>
<td>Hot carcass wt (lb)</td>
<td>750</td>
<td>757</td>
<td>13</td>
<td>0.70</td>
</tr>
<tr>
<td>Marbling</td>
<td>44.47</td>
<td>43.85</td>
<td>0.90</td>
<td>0.64</td>
</tr>
<tr>
<td>Back fat (in.)</td>
<td>0.44</td>
<td>0.42</td>
<td>0.025</td>
<td>0.70</td>
</tr>
<tr>
<td>Ribeye area (in.)</td>
<td>12.67</td>
<td>12.74</td>
<td>0.12</td>
<td>0.70</td>
</tr>
<tr>
<td>Kidney, pelvic, and heart fat (%)</td>
<td>2.96</td>
<td>3.15</td>
<td>0.26</td>
<td>0.61</td>
</tr>
<tr>
<td>Yield grade</td>
<td>2.97</td>
<td>2.99</td>
<td>0.13</td>
<td>0.93</td>
</tr>
<tr>
<td>Dry matter intake (lb/day)</td>
<td>22.48</td>
<td>22.59</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Feed:gain</td>
<td>6.35</td>
<td>6.51</td>
<td>0.14</td>
<td>0.44</td>
</tr>
<tr>
<td>Liver abscess (risk)</td>
<td>0.28</td>
<td>0.26</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Lung lesion (risk)</td>
<td>0.37</td>
<td>0.42</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>First pull (risk)</td>
<td>0.12</td>
<td>0.09</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Retreatment (risk)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Mortality (risk)</td>
<td>0.03</td>
<td>0.01</td>
<td>0.42</td>
<td></td>
</tr>
</tbody>
</table>

*Vaccinate and placebo are calf vaccine treatments. No cow vaccine treatment effects were significant (P > 0.10).*

*SEM, standard error of the mean.*

*ADG, average daily gain.*

**FIGURE 3.** Fecal *E. coli* O157:H7 prevalence in feedlot pens by calf treatment at feedlot entry and slaughter.
weaning prevalence of 0.6%, a feedlot entry prevalence of 23%, and a harvest prevalence of 27%. Unlike in the Gannon et al. study, samples were not collected from the calves in the present study before 7 weeks of age, but the calves were followed through harvest. The prevalence estimates reported for the feedlot and harvest phases of this study are within many of the previously reported ranges of feedlot prevalence (6, 8, 13, 20, 26).

The pen-level fecal *E. coli* O157 prevalence at feedlot arrival was not correlated with fecal prevalence at harvest. Figure 3 illustrates the range of fecal prevalences observed in this study, with three pens (one of placebo calves and two of vaccinates) with a very high prevalence compared to the other pens. Ten of the 11 calves that were shedding *E. coli* O157 at entry to the feedyard and at harvest were from these three pens. These 11 calves may have been super shedders and thus largely responsible for the increased prevalence in these pens, but unfortunately quantitative analysis of fecal samples was not conducted, so this hypothesis could not be addressed. Presence of super shedders could explain the large increases in shedding in these three pens from entry to harvest and the finding that these animals shed *E. coli* O157 on two occasions 168 days apart. In previous studies, the amount and duration of fecal shedding of *E. coli* O157 increased in super shedders and was likely a significant contributor to transmission between animals (16, 17, 19). Therefore, the possibility of a large, persistent challenge in these pens may have resulted in the lack of difference between placebo and vaccinate groups. However, Fox et al. (9) found that the SRP vaccine given three times was effective for stopping super shedders from shedding *E. coli* O157. The three doses administered in that trial were all given during the feedyard phase. The results from the present study combined with those of Fox et al. suggest that vaccination should occur during the feedyard phase of production.

In this study, the *E. coli* O157:H7–specific antibody titers peaked shortly after animals arrived at the feedlot and were lower at the time of harvest (Fig. 1). Although evaluation of serum titers was beyond the scope of this study, the serum titers may have dropped below the level of protection that would have resulted in a significant difference in fecal shedding between vaccinates and placebo calves. In previously reported studies (9, 27) of the same vaccine, all yearling cattle had a final booster vaccination within 60 days of harvest, and all vaccinations were administered within 100 days of harvest. In the present study, the first vaccination was given at approximately 75 days of age, the second was given at 187 days of age, and the final was given at 217 days of age. This protocol resulted in 112 days between the first and second vaccination and 168 days between the final vaccination and harvest. This approach was selected to reflect standard industry handling times for administration of the SRP vaccine because of its three-dose protocol. Administration of the three doses at normal handling time points would be preferred to addition of another handling time during the feedlot phase of the production cycle when performance of the cattle is critical. Comparison of the previously reported results and intervals from final vaccination to harvest with those of the present study reveals that the timing of the final vaccination relative to the time of harvest is important.

No adverse performance or health effects in this study were associated with *E. coli* O157:H7 SRP vaccination. No difference between SRP vaccinated cattle and placebo cattle was noted with regard to fecal *E. coli* O157 prevalence upon entry to the feedyard and at harvest based on a modified three-dose regimen. However, SRP vaccination did result in a significant increase in serum antibody titers in these calves compared with placebo calves. SRP vaccination is an effective strategy for the reduction of fecal shedding of *E. coli* O157:H7 in feedlot cattle based on previous studies with traditional vaccination administration times (9, 27, 28). The timing of vaccination appears to be a major determinant of its efficacy for reduction of fecal *E. coli* O157:H7 at the time of harvest and warrants further study. Inclusion of *E. coli* O157:H7 SRP vaccine into a multiple hurdle food safety scheme will help maintain beef as a safe and nutritious food source.

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