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Effect of a Vaccine Product Containing Type III Secreted Proteins on the Probability of *Escherichia coli* O157:H7 Fecal Shedding and Mucosal Colonization in Feedlot Cattle†

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ABSTRACT

Preharvest intervention strategies to reduce *Escherichia coli* O157:H7 in cattle have been sought as a means to reduce human foodborne illness. A blinded clinical trial was conducted to test the effect of a vaccine product on the probability that feedlot steers, under conditions of natural exposure, shed *E. coli* O157:H7 in feces, are colonized by this organism in the terminal rectum, or develop a humoral response to the respective antigens. Steers (n = 288) were assigned randomly to 36 pens (eight head per pen), and pens were randomized to vaccination treatment in a balanced fashion within six dietary treatments of an unrelated nutrition study. Treatments included vaccination or placebo (three doses at 3-week intervals). Fecal samples for culture (n = 1,410) were collected from the rectum of each steer on pretreatment day 0 and posttreatment days 14, 28, 42, and 56. Terminal rectum mucosal (TRM) cells were aseptically collected for culture at harvest (day 57 posttreatment) by scraping the mucosa 3.0 to 5.5 cm proximal to the rectoanal junction. *E. coli* O157:H7 was isolated and identified with selective enrichment, immunomagnetic separation, and PCR confirmation. Vaccinated cattle were 98.3% less likely to be colonized by *E. coli* O157:H7 in TRM cells (odds ratio = 0.014, $P < 0.0001$). Diet was also associated with the probability of cattle being colonized ($P = 0.04$). Vaccinated cattle demonstrated significant humoral responses to Tir and O157 lipopolysaccharide. These results provide evidence that this vaccine product reduces *E. coli* O157:H7 colonization of the terminal rectum of feedlot beef cattle under conditions of natural exposure, a first step in its evaluation as an effective intervention for food and environmental safety.

Escherichia coli O157:H7 is an important foodborne, zoonotic pathogen of humans *(1, 3, 60)*. This organism is the prototype of the enterohemorrhagic *E. coli* class, organisms that characteristically cause hemorrhagic colitis and hemolytic uremic syndrome in humans *(32)*. Enterohemorrhagic *E. coli* strains produce a type of Shiga toxin (Stx), which has a principal function in the causation of vascular damage *(60)*. In addition, these strains produce attaching-effacing lesions in the intestines, which contribute to the pathogenesis *(26, 39)*. Stx is produced not only by serotype O157:H7, but also by many other serotypes of *E. coli* that are consequently referred to as Shiga toxin–producing *E. coli (31, 48)*. Nearly 500 O serotypes of Shiga toxin–producing *E. coli* have been isolated from humans with disease; however, <10 cause the majority of cases *(21)*. Hence, based on the importance of Stx as a virulence factor *(31, 60)*, Shiga toxin–producing *E. coli* is considered a potential pathogen for humans, although it is likely that not all strains have the ability to cause disease, because the pathogenesis is multifactorial and involves multiple virulence factors *(21, 31, 48)*.

In the United States, ground beef is the most common vehicle associated with foodborne outbreaks of *E. coli* O157:H7 *(2, 7)*. Cattle are an asymptomatic reservoir of this microorganism and shed it in the feces *(38, 55, 59)*. Fecal material is an important source of infection for humans, who most often acquire the organism in contaminated food and water or by direct contact with carrier animals *(1, 8, 52, 55)*. The proportion of cattle carrying *E. coli* O157:H7 in the feces or on hides is correlated with postharvest rates of carcass contamination with the same organism *(16)*. Preharvest strategies to prevent foodborne illness in humans, e.g., reduction of the prevalence of cattle shedding *E. coli* O157:H7 in their feces, have been proposed early on to prevent foodborne illness *(10, 22)*, and this has become a major focus of research groups around the world *(6, 35, 59)*.

Bacterial virulence mechanisms important in intestinal colonization are a logical target for intervention strategies. The outer membrane protein intimin (Eae) and the type III secreted proteins, including but not limited to the translocated-intimin receptor (Tir) and *E. coli*–secreted proteins ([Esp] A and B), are bacterial proteins that mediate the formation of attaching-effacing lesions *(23)*. These proteins are encoded on a pathogenicity island called the locus of enterocyte effacement (LEE) *(17)*. After *E. coli* O157:H7 contacts a mucosal epithelial cell in the intestine, it delivers

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Tir, EspB, and other type III secreted proteins into the cell through a transiently produced translocation tube *(44)*, which structurally consists of an assembly of EspA subunits *(17)*. A series of cytoskeletal alterations resulting in effacement of the microvilli and pedestal formation at the site of bacterial attachment ensues *(53)*. Subsequently, intimin is bound to Tir, which is integrated into the host cell membrane, and this effects intimate attachment *(13)*.

The relationship and relevance of attaching-effacing lesions to intestinal colonization have been demonstrated by mutagenesis studies. Inactivation of the gene encoding intimin (*eae*) in *E. coli* O157:H7 has significantly reduced enteritis and bacterial fecal shedding in experimentally infected neonatal calves *(11)* and colonization and fecal shedding in yearling cattle *(9)*. Similarly, experimental challenge of calves with an *E. coli* O157:H7 strain containing a deletion in the operon (LEE4) that encodes the type III secreted proteins resulted in reduced colonization and fecal shedding *(42)*.

Human patients with *E. coli* O157:H7–associated disease (e.g., hemolytic uremic syndrome) were found to develop a robust serum antibody response to Tir *(34, 65)*, and based on this observation, the hypothesis was raised that type III secreted proteins would make a suitable vaccine target for this pathogen in humans and cattle *(34)*. Subsequent studies demonstrated that a vaccine prepared from culture supernatant materials enriched with type III secreted proteins has significant potential as a preharvest intervention strategy for *E. coli* O157:H7 *(50)*. However, to our knowledge, the effect of this vaccine on colonization of the terminal rectum has not previously been tested.

The reduction in colonization and fecal shedding in experimentally infected calves that result from the deletion of the LEE4 operon in the *E. coli* O157:H7 strain was concluded to provide a proof of concept for the utilization of type III secreted proteins as a vaccine strategy *(42)*. However, some studies have not found vaccination to be efficacious. One of these utilized type III secreted proteins as the vaccine antigen, although they were formalin treated, which may have altered the immunogenicity of the vaccine *(63)*. A recent study involved vaccinating young calves <1 month old by the intramuscular route with subunit intimin$_{280\text{-}\gamma}$ Efa-1′, and enterohemorrhagic *E. coli* bacterin, singularly or in combination, and then boosting by intranasal exposure with the same antigens with cholera toxin B subunit as an adjuvant *(62)*. Although the calves developed humoral antibodies and some developed salivary immunoglobulin A (IgA) antibodies to the homologous antigen, they were not protected against intestinal colonization by *E. coli* O157:H7 following experimental challenge *(62)*.

The objectives of this clinical trial were to test, under conditions of natural exposure, the effect of a vaccine containing *E. coli* type III secreted proteins on (i) the probability that feedlot steers shed *E. coli* O157:H7 in the feces, (ii) the probability that feedlot steers are colonized by this organism in the terminal rectum, (iii) the growth performance of cattle, and (iv) the humoral immune responses to the respective antigens.

MATERIALS AND METHODS

Source of cattle. Two hundred eighty-eight crossbred steers were obtained in the fall of 2003 and grown in a winter-grazing system until they were placed in their respective treatment pens on day 1 of the trial (19 May 2004). Prior to day 1 of the trial, steers were limit fed a 50% alfalfa hay:50% wet corn gluten feed diet on a dry matter (DM) basis at 2% body weight (BW) for 5 consecutive days to reduce variation in initial weight due to gut fill. Initial BWs (352 ± 11 [SD] kg) were calculated as the average of weights taken the last 2 days of the 5-day limit-fed period.

Study design. Upon completion of the first weigh day, steers were stratified by BW so that the heaviest 36 cattle could be assigned within their weight strata to 36 pens with a random number generator. This randomization process was repeated seven times so that each pen housed a total of eight steers with initial BW distributed evenly among the 36 pens.

Six pens were assigned to each of six dietary treatments with a random number generator in a balanced fashion. Six levels of dietary treatments evaluated the level of wet distillers' grains plus solubles (DG) on cattle performance as follows: (i) 0% DG (00DG), (ii) 10% DG (10DG), (iii) 20% DG (20DG), (iv) 30% DG (30DG), (v) 40% DG (40DG), and (vi) 50% DG (50DG), all included in the rations as a percentage of DM. Alfalfa hay was included in all diets at 5.0% of DM, and high-moisture corn and dry-rolled corn were fed at a 1:1 ratio (DM basis). DG replaced corn in each treatment diet.

Vaccination treatments were assigned randomly within dietary treatment in a balanced fashion, so that within each group of six pens of a dietary treatment, three were randomly assigned to receive one of two vaccine treatments (total of 18 pens per vaccination treatment). The vaccine (2 ml per dose; Bioniche Life Sciences, Belleville, Ontario, Canada) was administered subcutaneously in the neck with an 18-gauge, 1.6-cm needle. The vaccine contained secreted proteins prepared from *E. coli* O157:H7 as previously described *(34, 50)* and was formulated with the adjuvant Emulsigen D (MVP Laboratories, Omaha, Nebr.) such that the protein concentration was 66.1 μg per dose. Vaccination treatments were coded A (vaccination) and B (placebo), so that all feedlot and laboratory personnel were blinded to treatments until after completion of the study. All steers received three doses of the vaccine or placebo (adjuvant only) at 21-day intervals on days 28, 49, and 70 of the 126-day feeding period. The study timeline is summarized in Table 1.

Management, sampling procedures, and carcass data collection. Feedlot personnel visually evaluated feedbunks each morning to assign a daily allotment of feed to each pen of cattle. Additionally, experienced animal health personnel visually appraised cattle three times each week to evaluate health status.

Feces were collected per rectum from each steer on day 28 of the feeding period and every 14 days following completion of the three-dose regimen, resulting in one pretreatment sample (day 28 of the feeding period) and four test-period samplings at days 84, 98, 112, and 126 of the feeding period (14, 28, 42, and 56 days following completion of the three-dose regimen). Individual BWs were collected concurrently with fecal sampling.

Serum samples were collected from cattle on days 28, 84, and 126 of the feeding period (pretreatment and 14 and 56 days following completion of the three-dose regimen). Enzyme-linked immunosorbent assay (ELISA) titers were determined, as described below, from two systematic-randomly selected steers within each pen (72 cattle in total). The systematic-random selection

TABLE 1. *Study timetable*

Date (2004)	Feedyard days	Treatment days	Posttreatment days	Description	Samples collected
19 May	1	—	—	Placed into feedyard	
15 Jun.	28	0	—	First treatment dose	Blood, feces
6 Jul.	49	21	—	Second treatment dose	
27 Jul.	70	42	0	Third treatment dose	
10 Aug.	84	—	14	Test period 1	Blood, feces
24 Aug.	98	—	28	Test period 2	Feces
7 Sep.	112	—	42	Test period 3	Feces
21 Sep.	126	—	56	Test period 4	Blood, feces
22 Sep.	—	—	57	Harvest	Terminal rectum mucosa
23 Sep.	—	—	58	Completion of harvest	Terminal rectum mucosa

process was to randomly assign the eight steers from each pen a value of one to eight and then select serum from animals with the same two randomly selected values (e.g., steers 3 and 6 from each pen).

Samples of terminal rectum mucosal (TRM) cells were collected at the abattoir. Following evisceration of the animal, an abattoir worker removed the terminal 20 to 30 cm of the large intestine and placed it into a plastic bag, along with an identification tag. Minutes later, at a separate location in the abattoir, laboratory workers made an incision lengthwise along the excised large intestinal segment to expose the mucosal surface and removed the contents with a sterile wooden tongue depressor. Following removal of all visible material from the lumen and mucosal surface, a 2.5-cm-wide circumferential band of TRM cells, extending from 3.0 to 5.5 cm proximal to the rectoanal junction, was obtained by scraping with a sterile glass microscope slide (Fig. 1). Approximately 1.5 g of TRM cells was harvested from each animal and placed in 15 ml of chilled, modified Cary-Blair transport media (ParaPak C & S, Meridian Bioscience, Inc., Cincinnati, Ohio). Transport media vials were submitted to the laboratory on ice and refrigerated (4°C) until TRM cells were cultured. TRM cells were cultured within 24 h after collection from the animal. Sample collectors and laboratory personnel were blinded to the treatment of the animals.

Feedlot performance outcomes of average daily gain, daily DM intake, and feed efficiency were calculated from individual (average daily gain) and pen-level measures. Carcass characteristic outcomes were obtained from individual cattle at harvest and included the hot carcass weight, 12th-rib fat thickness, U.S. Department of Agriculture (USDA) marbling score, and USDA yield grade. Carcass weights were recorded immediately prior to washing and placement of the carcass in the cooler. After the carcass was chilled for 24 h, fat thickness, marbling score, and yield grade were recorded. Marbling score is indicative of intramuscular fat at the 12th rib and recorded on a scale of 300 = slight marbling (USDA select quality grade) and 400 = small marbling (USDA low choice quality grade). Yield grade measures are based on a scale of 1 to 5. Yield grade is an estimate of boneless, closely trimmed retail cuts, with a yield grade 1 having more retail yield than a yield grade 5, which is much less lean with more fat.

Culture methods. Samples of feces and TRM cells were cultured to assess for shedding and intestinal colonization, respectively. Fecal samples were cultured for *E. coli* O157:H7 by procedures that have been previously described *(57).* For each fecal sample, a 10-g aliquot was added to 90 ml of gram-negative broth containing vancomycin at 8 μg/ml, cefixime at 50 ng/ml, and cefsulodin at 10 μg/ml and incubated at 37°C for 6 h. After incubation, 1 ml of culture broth was removed and subjected to *E. coli* O157 immunomagnetic separation (Dynal, Lake Success, N.Y.). Twenty microliters of the final immunomagnetic separation suspension was plated on sorbitol MacConkey agar containing cefixime (0.05 μg/ml) and tellurite (2.5 μg/ml). Three isolated, non–sorbitol-fermenting colonies per plate were picked and reisolated on plates containing sorbitol MacConkey agar containing

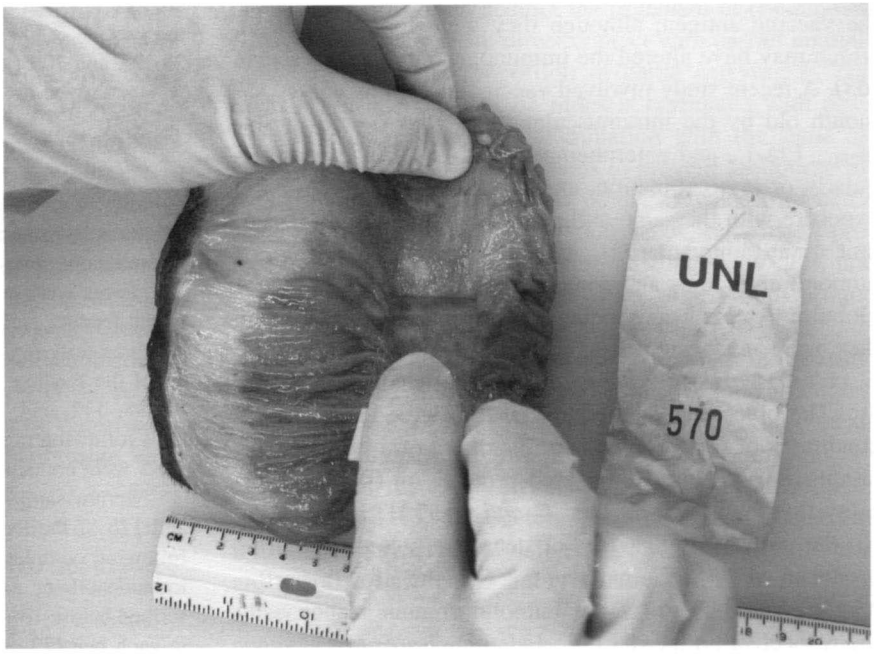

FIGURE 1. *Use of a sterile glass microscope slide to obtain terminal rectum mucosal (TRM) cells from a 2.5-cm-wide circumferential band extending from 3.0 to 5.5 cm proximal to the rectoanal junction.*

cefixime (0.05 μg/ml) and tellurite (2.5 μg/ml) to ensure the purity of isolates. One well-isolated nonsorbitol-fermenting colony was picked from each subculture and inoculated onto Fluorocult (EM Science, Gibbstown, N.J.) and MacConkey agars and into MacConkey broth and *E. coli* broth, the last to which 4-methylumbelliferyl-β-D-glucuronide had been added. Isolates that fermented lactose but were negative for β-glucuronidase activity both by Fluorocult and *E. coli* broth culture were subjected to latex agglutination tests for *E. coli* O157 and H7 antigens (Remel, Lenexa, Kans.). Isolates that agglutinated anti–O157-coated latex beads, regardless of H7 agglutination results, were tested further by multiplex PCR. The multiplex PCR was a five-primer-pair assay that has been previously described *(50)* but detects the presence of $rfbE_{O157:H7}$ *(46)*, $fliC_{h7}$ *(18)*, stx_1 *(45)*, stx_2 *(47)*, and eae_{O157} *(19)*. The $rfbE_{O157:H7}$ gene encodes an enzyme required for the synthesis of the *E. coli* O157 antigen *(4, 46)*; the eae_{O157} gene encodes intimin of *E. coli* O157:H7 origin and is relatively O serogroup–specific *(19)*; and the $fliC_{h7}$ gene encodes the structural protein of the H7 flagellum *(18)*. Confirmation of an isolate being *E. coli* O157:H7 was dependent on a positive reaction for both $rfbE_{O157:H7}$ and $fliC_{h7}$ and at least one of the virulence factor genes (i.e., stx_1, stx_2, or eae_{O157}). Use of these criteria allows the detection of *E. coli* O157:H7 strains that have lost *stx* or eae_{O157} genes and also circumvents the ambiguity associated with the nonmotile designation *(18)*.

TRM cell suspensions in transport media were cultured by enrichment by adding 5 ml of suspension to 45 ml of gram-negative broth containing vancomycin at 8 μg/ml, cefixime at 50 ng/ml, and cefsulodin at 10 μg/ml and incubating for 6 h at 37°C. Procedures to isolate and identify *E. coli* O157:H7 from the TRM cell enrichment cultures were identical to those described above for the fecal cultures. Fecal and TRM isolates that, collectively, were sorbitol nonfermenting, lactose fermenting, negative for β-glucuronidase activity, and positive for the O157 antigen were tested in the five-primer-pair multiplex PCR assay, as described above. As noted above, detection of genes for O157, H7, and at least one other target in the assay was considered confirmation of an isolate as *E. coli* O157:H7.

Indirect ELISA to detect serum antibodies to type III secreted proteins. Serum antibody titers to *E. coli* O157:H7 antigens, including EspA, EspB, translocated intimin receptor (Tir), intimin, and O157 lipopolysaccharide (LPS), were determined on two steers from each of the 18 pens in the A and B treatment groups. The indirect ELISA was conducted by a modification of procedures previously described *(34)*. *E. coli* BL21 λDE3 lysogens transformed with recombinant pET28a His-tag expression vectors (Novagen, EMD Biosciences-Merck, San Diego, Calif.) containing cloned *tir-cesT, eae, espA,* or *espB* genes were provided by Dr. B. Brett Finlay. Purified His-tagged proteins were obtained from these strains by standard procedures (Novagen His-Bind Kit). The region of the *eae* gene cloned into the recombinant intimin construct encoded the 280–carboxyl-terminal amino acids, specific for the γ-intimin subtype. The source of *E. coli* O157 LPS was *E. coli* O157:H7 strain 86-24, obtained by phenol-water extraction and purified by standard methods *(24)*. Individual wells of microtiter plates were coated with 100 μl of EspA (150 ng), EspB (250 ng), Tir (100 ng), intimin (200 ng), or O157 LPS (200 ng) in a 0.1 M carbonate solution and incubated overnight at 4°C. Wells were washed four times, blocked overnight at 4°C with 60°C heat-treated 1% bovine serum albumin in 0.1 M carbonate solution, and washed as described above. Twofold serial dilutions of sera from 1:100 to 1:204,800 were made, and 100 μl of each dilution was added to wells in duplicate. Plates were incubated for 3 h at 37°C and washed and blocked as described above. One hundred microliters of a 1:5,000 dilution of affinity-purified, horseradish peroxidase–conjugated, H+L goat anti-bovine IgG (Jackson ImmunoResearch Laboratories, West Grove, Pa.) was added to each well, and then plates were incubated for 1 h at 37°C and washed four times. One hundred microliters of orthophenylenediamine (1 mg/ml) was added, and plates were incubated at room temperature for 20 min. Fifty microliters of 3 M *o*-phosphoric acid was added, and A_{492} was measured. The antibody titer was the highest dilution having an absorbance (mean minus 1 SEM) greater than the negative control (mean plus 1 SEM), the latter of which included all reagents except primary serum.

Statistical analyses. The effect of vaccine treatment on the probability to detect *E. coli* O157:H7 from feces was tested by multivariable modeling of the number of culture-positive individuals per number of individuals within the pen with a logit link, binomial distribution, generalized estimation equation (GEE) model (Proc GENMOD, SAS Institute, Cary, N.C.). A first-order autoregressive correlation structure was defined to account for clustering by repeated measures of pens over time. The models were fashioned by a manual forward selection process with the opportunity for subsequent backward elimination so that variables in the final model were significant at $\alpha \leq 0.05$ with the score statistic for type 3 GEE analysis. Fixed effects tested in the model were vaccine treatment, dietary treatment, and test period. Two-way interactions between these variables were also tested. Model fit was assessed by evaluating the deviance parameter.

The effect of vaccine treatment on the probability of colonization was tested by multivariable modeling of the number of TRM culture-positive individuals per number of individuals within the pen with a logit link function, binomial distribution, GEE model (Proc GENMOD, SAS). Compound symmetry was the defined correlation structure to account for the clustering of animals within a pen. The model was fashioned by a manual forward selection process with subsequent backward elimination so that variables in the model were significant at $\alpha \leq 0.05$ by the score statistic for type 3 GEE analysis. Fixed effects tested in the model were vaccine and dietary treatment. Model fit was assessed by evaluating the deviance parameter.

Least-squared means of the parameter estimates from logistic regression models were used to estimate model-adjusted probabilities of *E. coli* O157:H7 outcomes for the levels of each class variable in the model. Relative risk values for vaccine treatment were calculated from the model-adjusted probabilities, and vaccine efficacy was calculated as 1 minus relative risk.

Serum antibody titers were $\log_2$ transformed prior to statistical analysis. The effect of vaccination on the magnitude of titer change from prevaccination until either (i) 14 days after the vaccine regimen was completed or (ii) the day of harvest (56 days after the vaccine regimen was completed) was tested in a generalized linear mixed model (Proc MIXED, SAS) with pen as a random effect. The effect of vaccination on the probability of a fourfold (2 $\log_2$) or greater titer change from prevaccination until either (i) 14 days after the vaccine regimen was completed or (ii) the day of harvest was tested in a logit link function, binomial distribution, GEE model (Proc GENMOD, SAS), accounting for a compound symmetry correlation structure by pen. Statistical significance was at $\alpha \leq 0.05$. Model fit was assessed by evaluating the deviance parameter.

The effect of vaccination on feedlot performance outcomes of average daily gain, daily DM intake, and feed efficiency was evaluated in generalized linear mixed models (Proc MIXED, SAS). Pen was the experimental unit. Fixed effects were vacci-

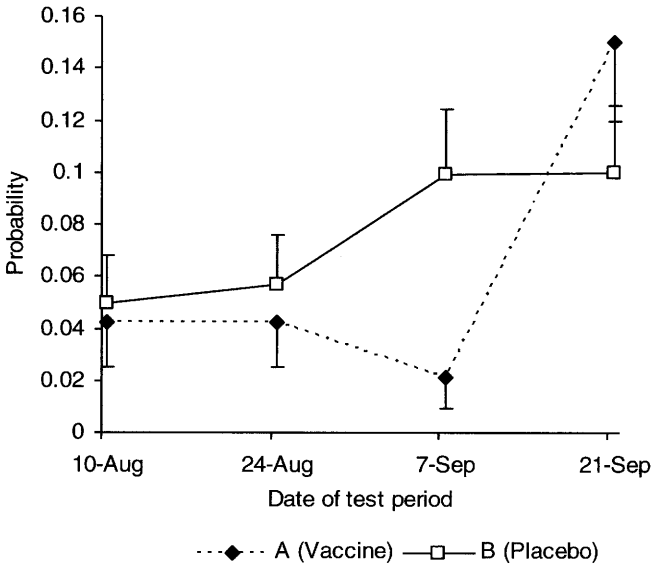

FIGURE 2. *Mean probability that cattle will shed* E. coli *O157:H7 in feces at each of the posttreatment test periods for vaccinated (solid diamond) and placebo-treated (hollow square) pens of cattle. The greatest probability of shedding in either treatment group occurred simultaneously with a 6.4-cm rain event on 21 September, 1 day prior to harvest. Error bars represent 1 standard error.*

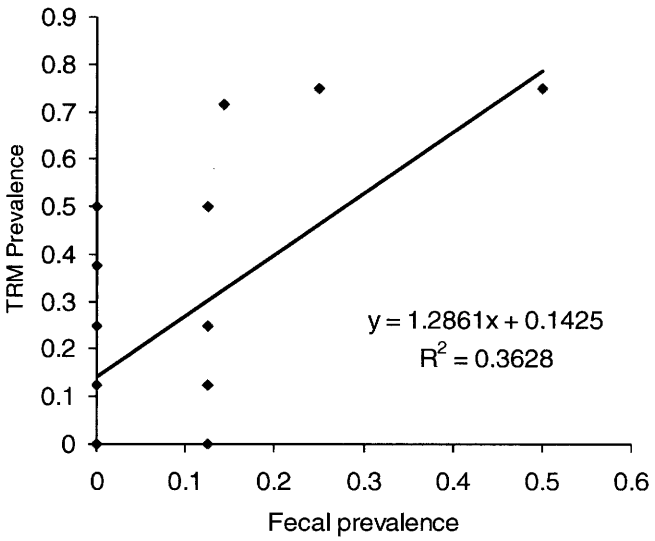

FIGURE 3. *Correlation between prevalence of* E. coli *O157 in feces and terminal rectum mucosa 1 day later from pens of placebo-treated cattle. Prevalence of the two measures was positively correlated (*r *= 0.60,* P *= 0.008). The solid line represents the least-squares linear regression.*

nation and dietary treatment. Treatment means were considered significantly different at $\alpha \leq 0.05$.

The effect of vaccination on individual carcass characteristics of hot carcass weight, 12th-rib fat thickness, marbling score, and yield grade was evaluated in a generalized linear mixed model (Proc MIXED, SAS). Fixed effects were vaccination and dietary treatment. Treatment means were considered significantly different at $\alpha \leq 0.05$. Pen was included in the model as a random effect. Observations from cattle that were held 1 additional day before harvest were excluded from carcass data analysis.

RESULTS

Five steers were removed ($n = 2$ vaccinated, $n = 3$ placebo) from the trial prior to the first test period sampling because of receiving the wrong vaccine ($n = 3$) or factors unrelated to the study ($n = 2$). All steers on trial were fed for 126 days and were sent to harvest on 21 September 2004; however, a scheduling error at the abattoir prevented all from being harvested on the same day. Two hundred forty-seven steers were harvested on 22 September, and the remaining 36 were harvested on 23 September. All cattle harvested on the second day were vaccinated steers. TRM cell samples were not collected from two vaccinated steers harvested on 22 September, most likely because the identification tags were lost.

Fecal cultures. *E. coli* O157:H7 was recovered from 90 (6.4%) of 1,410 fecal samples. During the pretreatment sampling period, the mean pen-level prevalences of steers shedding *E. coli* O157:H7 within the vaccinated and placebo-treated pens were 6.3 and 1.4%, respectively ($P = 0.07$). The probability that steers would shed *E. coli* O157:H7 at the start of the trial was not significantly different by dietary treatment assignments ($P = 0.92$). *E. coli* O157:H7 was cultured from 79 of 1,127 fecal samples obtained during the postvaccination test periods. The mean pen-level prevalence of *E. coli* O157:H7 isolated from feces was 4.6, 5.0, 6.0, and 12.4%, respectively, for test periods corresponding to 14, 28, 42, and 56 days after completion of the three-dose regimen. A total of 36 (6.4%) of 564 samples were from vaccinated steers, and 43 (7.5%) of 563 were from placebo-treated steers. At each of the three posttreatment test periods prior to the last sampling date, the posttreatment prevalence of fecal shedding was lower among vaccinated cattle than placebo-treated cattle ($P = 0.07$). However, this picture changed during the last sampling period in the feedyard (Fig. 2). At the last test period, the prevalence of fecal shedding among vaccinated cattle was greater than during previous periods. A major rain event (6.4 cm of rain) occurred in the early hours of 21 September prior to testing. On the day prior to sampling (20 September), the pen surface condition score of every pen on study was recorded as dry and dusty, but prior to sampling cattle on 21 September, the pen surface conditions for all pens were recorded as extremely wet and muddy.

When all four posttreatment periods were considered in the analysis, neither vaccination treatment (odds ratio [OR] = 0.81, $P = 0.57$), diet ($P = 0.41$), nor test week ($P = 0.58$) significantly explained the probability that cattle would shed *E. coli* O157:H7 in the feces.

Culture of TRM cells. Samples of TRM cells were collected from 281 steers at harvest. A total of 1 (0.71%) of 140 TRM cell samples collected from vaccinated cattle tested positive for *E. coli* O157:H7, whereas 38 (27.0%) of 141 TRM cell samples collected from placebo-treated cattle tested positive. Within placebo-treated pens, the prevalence of fecal shedding prior to loading cattle for harvest on 21 September correlated with the prevalence of colonization at harvest 1 day later ($r = 0.60$, $P < 0.008$; Fig. 3).

TABLE 2. *Multivariable logistic regression model of the probability of culturing* E. coli *O157:H7 from samples of terminal rectum mucosal cells from steers vaccinated against* E. coli *O157:H7 or treated with placebo and fed in a research feedlot*[a,b]

Variable	Unit	Parmeter estimate	Odds ratio	95% confidence interval		*P* value
Intercept		−0.914				0.03
Vaccination	Vaccine	−4.2881	0.014	0.002	0.085	<0.0001
	Placebo	0	1.00	Referent		
Diet	10DG	−1.4544	0.23	0.03	1.57	0.04
	20DG	−1.4994	0.22	0.07	0.75	
	30DG	−2.192	0.11	0.02	0.70	
	40DG	0.945	2.57	0.92	7.17	
	50DG	1.3413	3.82	0.98	14.99	
	00DG	0	1.00	Referent		

[a] 10DG, 10% distillers' grains in finishing diet; 20DG, 20% distillers' grains in finishing diet; 30DG, 30% distillers' grains in finishing diet; 40DG, 40% distillers' grains in finishing diet; 50DG, 50% distillers' grains in finishing diet; 00DG, no distillers' grains in finishing diet.

[b] Deviance parameter divided by degrees of freedom = 0.78, indicating slight underdispersion in the model.

The factors explaining the probability that TRM cell samples from steers would test culture positive for *E. coli* O157:H7 in the multivariable logistic regression model (Table 2) were vaccination treatment (OR = 0.014, $P < 0.0001$) and diet ($P = 0.04$). There was no significant interaction between diet and vaccination treatment, vaccination and test period, or diet and test period. The model-adjusted probability of *E. coli* O157:H7 colonization in the terminal rectum was 0.003 and 0.199 for vaccinated and placebo-treated steers, respectively. Vaccine efficacy for preventing colonization was 98.3%.

Serology. At the time of the first vaccination, all cattle in the study were seropositive for each of the *E. coli* O157:H7 antigens tested, but the titers were not significantly different between the two treatment groups. From prevaccination to 14 days after completion of treatment regimen (Fig. 4), the magnitudes of the seroresponse (change in $\log_2$ titer values) of vaccinated cattle were 0.89 and 3.03 to Tir ($P = 0.04$) and O157 LPS ($P < 0.0001$), respectively. Similarly, during the same period, vaccinated cattle had significantly greater probabilities for a fourfold or greater seroresponse to Tir (OR = 2.7, $P = 0.04$) and O157 LPS (OR = 8.0, $P < 0.0001$) than placebo-treated cattle. From prevaccination to harvest (Fig. 4), vaccinated cattle had a significantly greater magnitude of seroresponse ($P = 0.0008$) and greater probability of a fourfold or greater seroresponse (OR = 4.9, $P = 0.003$) to O157 LPS. However, neither the magnitude of seroresponse to Tir ($P = 0.23$) nor the probability of a seroresponse ($P = 0.46$) was significantly different from prevaccination to harvest. From prevaccination to 14 days after treatment regimen, the magnitudes of the seroresponse of vaccinated cattle were 0.44, 0.58, and 0.22 greater than those of placebo-treated cattle to EspA ($P = 0.30$), EspB ($P = 0.10$), and intimin ($P = 0.57$), respectively. Similarly, the probabilities of a fourfold or greater seroresponse to EspA (OR = 1.15, $P = 0.81$), EspB (OR = 2.07, $P = 0.17$), and intimin (OR = 0.63, $P = 0.46$) in vaccinated cattle were not significant compared with placebo-treated cattle.

Feedlot performance and carcass characteristics. There was no interaction between diet and vaccination treatment for feedlot performance or carcass characteristic

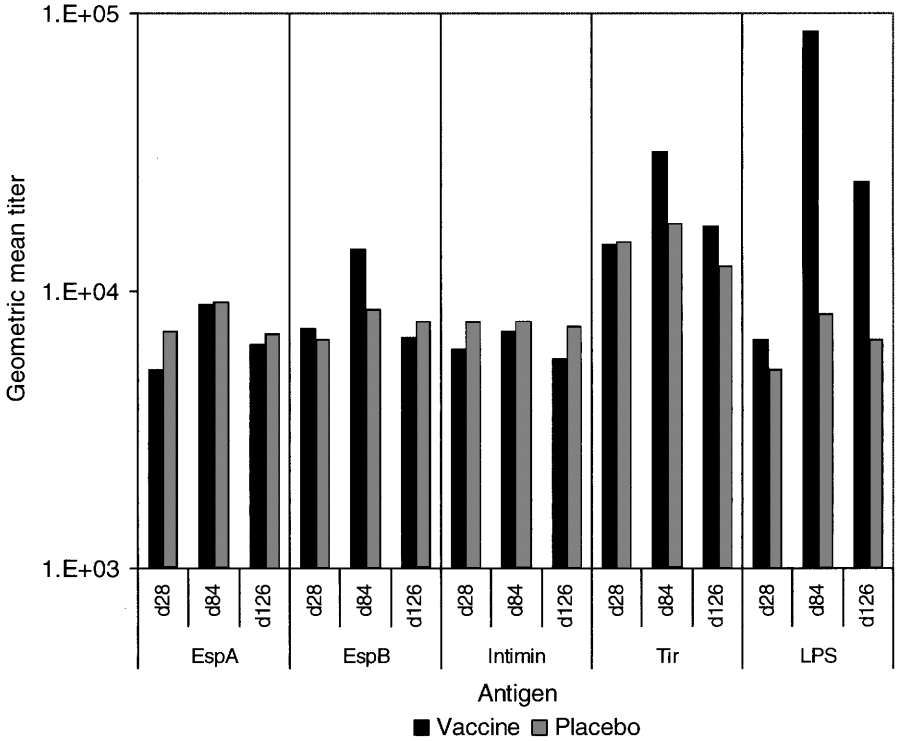

FIGURE 4. *Geometric mean antibody titers against specific antigens measured from serum collected from a subset of vaccinated and placebo-treated cattle at various days in the feedyard. Feedlot days 28, 84, and 126 correspond to the day of first treatment, 14 days after completing the treatment regimen, and the last day in the feedyard (56 days after completing the treatment regimen), respectively.*

outcomes. Feedlot performance, adjusted for dietary treatment, was not significantly different among vaccinated and placebo-treated pens of steers. Initial trial BW averaged 352 kg ($P = 0.89$). Final live BW was not different ($P = 0.92$) and averaged 584 kg for vaccinated and placebo-treated steers. Daily DM intake averaged 11.00 kg for vaccinated cattle and 11.15 kg for placebo-treated cattle ($P = 0.24$). Average daily gains were 1.84 and 1.85 kg ($P = 0.87$) for vaccinated and placebo-treated pens of steers, respectively. Feed efficiency averaged 0.167 for vaccinated pens of steers and 0.165 ($P = 0.45$) for placebo-treated pens of steers.

Carcass characteristics of hot carcass weight, 12th-rib fat thickness, and USDA yield grade for vaccinated and placebo-treated steers, adjusting for diet, were not significantly different for vaccinated or placebo-treated steers. Hot carcass weight averaged 366 kg for vaccinated cattle and 369 kg for placebo-treated cattle ($P = 0.32$). Fat thickness averaged 1.26 cm for vaccinated cattle and 1.22 cm for placebo-treated cattle ($P = 0.40$). Yield grade averaged 2.57 for vaccinated cattle and 2.69 for placebo-treated cattle ($P = 0.16$). Paradoxically, the USDA marbling score did differ between treatment groups. The marbling score averaged 405 for vaccinated cattle and 423 for placebo-treated cattle ($P = 0.05$).

DISCUSSION

The most important finding of this study was that vaccinated cattle were less likely to be colonized at the TRM. We assessed vaccine efficacy by determining the extent to which colonization of the TRM was prevented, because this is an important site of colonization *(41, 42)*. To our knowledge, this is the first report to evaluate a preharvest intervention for the control of *E. coli* O157:H7 with colonization of TRM as the outcome variable. This finding is consistent with the hypothesized mode of action of this vaccine. It was unfortunate, but unavoidable, that problems at the plant required a delay in harvesting TRM cells from some cattle. However, the data provide no evidence of bias resulting from this delay.

Vaccines designed to protect humans and animals against *E. coli* and other enteric infections are, in general, aimed at preventing intestinal colonization and often require the ability to induce a significant mucosal immune response to be efficacious *(33, 43)*. The primary targets of *E. coli* vaccines that attempt to prevent intestinal colonization are bacterial adherence factors *(61)*. For *E. coli* O157:H7, proof-of-concept studies have shown that antibodies directed against intimin, an adherence factor known to play a significant role in colonization in different species, including adult cattle *(9, 37)*, reduce bacterial adherence to epithelial cells, both in vitro and in vivo *(12, 20)*. LEE-encoded type III secreted proteins, including Tir, are also important for colonization of the bovine intestine *(42)*, and Tir is thought to mediate its effect on colonization primarily by binding to the C-terminal region of intimin *(64)*. Further, antibodies to EspA, another type III secreted protein involved in initial adherence and attaching-effacing lesion development, block development of these lesions *(30)*.

In a previous study, a vaccine containing type III secreted proteins effectively reduced the probability that feedlot steers would test positive for *E. coli* O157:H7 in the feces *(50)*. In the present study, however, we were unable to observe significant differences between vaccinated and placebo-treated cattle in the probability of detecting *E. coli* O157:H7 from feces. However, the overall probability of detecting *E. coli* O157:H7 among placebo-treated cattle in this study was very low compared with our previous observations of untreated cattle in research and commercial feedyards *(27, 50, 57)*. The present study was designed with sufficient power to detect differences similar to those observed in an earlier study *(50)* in the same feedlot during a similar time of year; however, the probability of detecting *E. coli* O157:H7 in the feces of placebo-treated cattle (21.3%) in that study was greater than what was observed in the present study (7.5%). Other researchers who investigated a vaccine containing type III secreted proteins in a large-scale commercial feedyard study did not find significant differences in the fecal shedding of *E. coli* O157:H7 between vaccinates and nonvaccinates, possibly because of low overall shedding prevalence, large within-pen variability, and a different vaccine formulation (viz. formalin-treated antigen) *(63)*.

Regardless of the overall low probability of cattle shedding *E. coli* O157:H7 in feces throughout the study, a weather event may have had the greatest impact on our ability to observe treatment differences in fecal shedding. Immediately prior to harvest, vaccinated and placebo-treated cattle were suddenly exposed to wet and muddy pen conditions, which have been shown to provide a greater opportunity for fecal-oral transmission of *E. coli* O157:H7 *(57, 58)*. We suspect the organisms we isolated from the feces were simply passing through the intestinal tract, and the rain event serendipitously provided an opportunity to witness the colonization outcome following the sudden oral challenge. In the feedlot, prior to loading, cattle from both treatment groups shed organisms in feces at the highest rate of the study; however, at harvest, vaccinated cattle were significantly less likely to be detected as colonized than placebo-treated controls. This finding is consistent with the proposed mechanism of action of this vaccine product *(50)*, previous longitudinal studies showing a wide variation in shedding prevalence over time *(27, 50)*, and the relationship between weather-related factors and the feedlot occurrence of *E. coli* O157:H7 *(58)*.

The vaccine used in this study was prepared from culture supernatant, and the primary intended antigens were type III secreted proteins, although O157 LPS was also present. Although we did not attempt to measure antibodies to type III secreted proteins in intestinal secretions, we did determine that vaccination induced a significant IgG seroresponse to Tir and O157 LPS. IgG1 plays a highly significant role in mucosal immunity in cattle and other ruminants, in addition to secretory IgA; in cattle, IgG1 is actively secreted across epithelial surfaces, including the intestinal mucosa *(5)*. In cattle, subcutaneous immunization induces significant IgG1, IgM, and IgG2 seroresponses *(51)*. In adult humans, immunization with an investigational

vaccine consisting of *E. coli* O157 O-specific polysaccharide–*Pseudomonas aeruginosa* recombinant exoprotein A conjugate induced high titers of serum bactericidal activity that roughly correlated with the serum IgG and IgM antibody levels *(28).*

We speculate that, in vaccinated cattle, antibodies to type III secreted proteins and possibly other bacterial components (e.g., O157 LPS) were transported into the intestinal lumen and blocked colonization. We further speculate that colonization was prevented or reduced by interference with attaching-effacing lesion formation and possibly other adherence mechanisms. In two previous studies, we found that this vaccine product reduced the probability of *E. coli* O157:H7 fecal shedding in cattle *(49, 50).* Antibodies to O157 LPS are also known to mediate bactericidal activity against *E. coli* O157:H7 through complement-mediated lysis and could play a role in mucosal immunity by killing bacteria in the intestinal lumen and on the surfaces of epithelial cells *(28, 40).* An additional role for anti-O157 antibodies could be to enhance the opsonization of bacteria. However, a previous study had reported that serum antibody titers to O157 LPS were not correlated with reduced fecal shedding or protection against repeat challenge in cattle *(25).*

The probability of detecting *E. coli* O157:H7 in TRM cells from cattle was different for cattle fed different levels of DG. In a previous study, dietary substitution of dry-rolled corn with corn bran and wet corn gluten feed did not affect the probability that cattle would shed *E. coli* O157:H7 in the feces *(36).* However, more recently, we found that the corn component of the diet affected the probability that cattle would test positive for *E. coli* O157:H7 in feces *(49).* A variety of diets and dietary components have been associated with the probability that cattle and sheep would shed *E. coli* O157:H7 in feces *(14, 15, 29, 54).* However, the mechanisms by which diet or diet components might affect *E. coli* O157:H7 fecal shedding patterns are unclear.

Feedlot performance and carcass characteristics did not differ between vaccinated and placebo-treated cattle with the exception of marbling score. It is unclear why, in this study, marbling was decreased among vaccinated cattle without the observation of other carcass or performance effects. Further, in two other studies with larger numbers of cattle, we did not observe an effect of vaccination on marbling score *(49, 56).* Therefore, we believe the observation in the present study is a spurious result from examining multiple carcass outcomes.

Results from this study suggest that this vaccine product effectively reduces the probability that cattle will become colonized by *E. coli* O157:H7 in TRM cells under conditions of natural exposure, a first step in its evaluation as an effective intervention for food and environmental safety.

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