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Efficacy of Dose Regimen and Observation of Herd Immunity from a Vaccine against *Escherichia coli* O157:H7 for Feedlot Cattle†

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ABSTRACT

A clinical trial was conducted to test the effect of a vaccine product containing type III secreted proteins of *Escherichia coli* O157:H7 on the probability that feedlot steers shed *E. coli* O157:H7 in feces. Six hundred eight same-source steers were utilized. Of these, 480 steers were assigned randomly to 60 pens (eight head per pen) and to one of four vaccination treatments (120 cattle per treatment, two head per treatment per pen). The four treatments were (i) no vaccination; (ii) one dose, vaccinated once at reimplant (day 42); (iii) two doses, vaccinated on arrival (day 0) and again at reimplant (day 42); and (iv) three doses, vaccinated on arrival (day 0), on day 21, and again at reimplant (day 42). The remaining 128 steers were assigned randomly to 12 pens within the same feedlot to serve as unvaccinated external controls. The probability of detecting *E. coli* O157:H7 among cattle receiving different doses of vaccine was compared with that of unvaccinated external control cattle, accounting for clustering by repeated measures, block, and pen and fixed effects of vaccine, corn product, and test period. Vaccine efficacy of receiving one, two, and three doses of vaccine was 68, 66, and 73%, respectively, compared with cattle in pens not receiving vaccine. Cattle receiving three doses of vaccine were significantly less likely to shed *E. coli* O157:H7 than unvaccinated cattle within the same pen. Unvaccinated cattle housed with vaccinated cattle were 59% less likely to shed *E. coli* O157:H7 than cattle in pens not receiving vaccine, likely because they benefited from herd immunity. This study supports the hypothesis that vaccination with this vaccine product effectively reduces the probability for cattle to shed *E. coli* O157:H7. There was no indication that the vaccine affected performance or carcass quality. In addition, we found that vaccinating a majority of cattle within a pen offered a significant protective effect (herd immunity) to unvaccinated cattle within the same pen.

Beef cattle represent an important reservoir for human exposure to *Escherichia coli* O157:H7, and preharvest strategies to reduce the prevalence of *E. coli* O157:H7 in cattle have been sought as a means to prevent foodborne illness *(1, 2, 15, 24)*. The proportion of cattle carrying *E. coli* O157:H7 in the feces or on hides is correlated with the postharvest rates of carcass contamination with the same organism *(6)*. 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 *(3, 8)*, and this has become a major focus of research groups around the world *(2, 12, 24)*.

The terminal rectum is an important site of intestinal colonization for *E. coli* O157:H7 in cattle *(16, 20)*. Type III secreted proteins are required for the colonization and formation of bacteria-induced attaching-effacing lesions on host cell epithelial surfaces in the terminal recta of naturally and experimentally infected cattle *(17)*. Potter et al. *(19)* showed that vaccinating feedlot cattle with a vaccine product containing type III secreted proteins of *E. coli* reduced the probability that cattle shed *E. coli* O157:H7 in their feces. In that study, cattle were vaccinated three times at 3-week intervals. A three-dose vaccination protocol is of practical concern to feedlot operators who may be challenged to comply with the need to repeatedly vaccinate cattle, and greater numbers of doses increase the cost of feedlot cattle production. Vaccinating cattle once (e.g., at the time growth implants are readministered) or twice (e.g., at initial processing and again at reimplanting) would be easier to implement in current feedlot practices. Therefore, our objective was to evaluate how the number of doses of vaccine affected the probability that cattle would shed *E. coli* O157:H7 in feces.

MATERIALS AND METHODS

Cattle. Six hundred eight steers were obtained in the fall of 2002 and grown in a winter-grazing system until they were placed in their respective treatment pens. To minimize selection bias, all cattle used in this study were obtained during the fall of 2002 and managed as one group from that point forward. Prior to placement in the feedlot, all steers were limit fed a 50% alfalfa hay:50% wet corn gluten feed diet (dry matter [DM]) at 2% body weight (BW) for 5 consecutive days to reduce variation in initial BW due to gut fill. Weights were taken for 2 consecutive days, and initial BW was based on the average of these 2-day weights.

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TABLE 1. *Study design*

Group	Treatment	Description
Vaccinated pens (n = 60 pens of same-source cattle, eight animals per pen)	One dose	n = 120 cattle receiving one dose of vaccine, two animals in each pen
	Two doses	n = 120 cattle receiving two doses of vaccine, two animals in each pen
	Three doses	n = 120 cattle receiving three doses of vaccine, two animals in each pen
	Placebo treated	n = 120 cattle receiving only adjuvant, two animals in each pen
External control pens (n = 12 pens of same-source cattle, 8 or 16 animals per pen, not vaccinated, sampled concurrently)	Unvaccinated	n = 128 cattle in 12 pens, not vaccinated, sampled concurrently

Steers (n = 608) were stratified by BW. The heaviest 128 steers (12 strata) were assigned randomly to 12 pens of unvaccinated steers (external control pens; 8 head in eight pens and 16 head in four pens) and started on feed on 6 May 2003. Four hundred eighty steers were assigned randomly within strata (n = 60) with a random number generator to 60 pens of steers receiving different vaccine treatments (eight head per pen) and started on feed on 9 May 2003. Therefore, a total of 72 pens were included in the study. We designed this experiment without regard for BW because there has been no evidence it is correlated with *E. coli* O157:H7 shedding *(22)*. Weight stratification was primarily used for the nutritional tests superimposed in these pens.

Treatments. All procedures used in this experiment were approved by the University of Nebraska Institutional Animal Care and Use Committee. Cattle within the 60 pens were assigned to one of four vaccination treatments (two head per treatment) within pen (Table 1). The vaccine was a commercial preparation containing type III secreted proteins collected from broth supernatant of *E. coli* O157:H7 similar to methods previously described *(11, 19)*, passed through a 0.2-μm filter, and formulated with the adjuvant Emulsigen D (MVP Laboratories, Omaha, Nebr.). The vaccine (2 ml per dose; Bioniche Life Sciences, Belleville, Ontario, Canada) was administered subcutaneously in the neck with an 18-gauge, 5/8-in. needle. Vaccination treatments included (i) no vaccination; (ii) one dose, vaccinated once at reimplant (day 42); (iii) two doses, vaccinated on day 0 and again at reimplant (day 42); and (iv) three doses, vaccinated on day 0, on day 21, and again at reimplant (day 42). In vaccinated pens, for cattle that were allocated to receive zero, one, or two doses of vaccine, adjuvant (placebo) was given in place of the vaccine following the three-dose vaccination protocol. Adjuvant treatments included (i) not vaccinated, adjuvant given on days 0, 21, and 42; (ii) vaccinated once, adjuvant given on days 0 and 21; (iii) vaccinated twice, adjuvant given on day 21; and (iv) vaccinated three times, no adjuvant given. Cattle within external control pens did not receive any doses of adjuvant.

An unrelated study of the effects of corn product and moisture on cattle performance was superimposed in a balanced design within the sampling blocks of the 60 vaccinated pens of cattle. Three separate corn types were fed with multiple moisture levels for two of the corn types. Corn types and moisture included high-moisture corn at 24% moisture (HMC24) or 30% moisture (HMC30); reconstituted HMC at 28% moisture (RECON28) or 35% moisture (RECON35); and dry-rolled corn at 13% moisture (DRC). There were also three levels of protein balanced across the corn diets (low, medium, and high). The final finishing diets contained 65% test corn, 18% corn bran, 5% grass hay, 3% tallow, 4% DRC, and 5% supplement (DM basis). Pens of steers in vaccinated pens were blocked by three dietary replications; thus, four blocks resulted in 12 dietary replications and four sampling blocks as described above.

Steers in external control pens were on another nutrition study to determine the effects of corn bran and corn steep inclusion on finishing cattle performance. Steers receiving the external control diet received a diet consisting of 75% DRC, 15% corn silage, 5% molasses, and 5% supplement. Corn bran and corn steep replaced DRC and molasses for the remaining three dietary treatments. Corn bran replaced DRC at 30% diet DM for the second dietary treatment. Corn bran and corn steep replaced DRC and molasses at 30 and 15% diet DM, respectively, for the third dietary treatment. The final dietary treatment included 45% corn bran and 15% corn steep as replacements for DRC. Thus, steers in external control pens received DRC as their primary corn product source for the entire study period.

Performance measures collected included average daily gain and carcass characteristics but were only compared on the 60 vaccinated pens to test the effect of vaccination on performance. Gain was based on the final BW minus the initial BW divided by the days fed. Final BW was calculated from hot carcass weight collected at a commercial abattoir the day of slaughter divided by a dressing percentage of 63%. Decision on when cattle were marketed (slaughter) was based on visual appraisal when cattle contained an average of 1.1 to 1.2 cm of back fat for the average of all cattle. On the day of slaughter, hot carcass weight was recorded. Following a 24-h chill, 12th-rib fat was measured, and U.S. Department of Agriculture (USDA) marbling scores were recorded from a trained USDA grader. USDA marbling scores are 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). USDA yield grade measures are based on a scale of 1 to 5. USDA yield grade is an estimate of boneless, closely trimmed retail cuts, with yield grade 1 having more retail yield than yield grade 5, which is much less lean with more fat.

Sampling procedures and management. Steers in vaccinated pens were blocked into four sampling periods so that two blocks could be sampled on 2 consecutive days in 1 week, and two blocks could be sampled on 2 consecutive days the following week. Steers in external control pens were sampled on a contemporaneous 3-week schedule. Vaccination treatments were initiated on day 0, which corresponded to 12 May, 13 May, 19 May, and 20 May for blocks 1, 2, 3, and 4, respectively. Each steer was sampled by rectal fecal grab every 21 days, beginning with day 0 (initial vaccination treatment), resulting in one pretreatment period (day 0), two interim periods (days 21 and 42), and four test period samplings (days 63, 84, 105, and 126).

Feed bunks were evaluated each morning at approximately

6:00 a.m. in order to assign a daily allotment of feed to each pen of cattle. Additionally, personnel visually assessed the health of the animals under study three times per week.

Culture methods. Laboratory personnel were blinded to treatments. Fecal samples were cultured for *E. coli* O157:H7 by procedures previously described *(23)*. Isolates that collectively were sorbitol nonfermenting, lactose fermenting, negative for β-glucuronidase activity, and positive for the O157 antigen were tested in a five-primer-pair multiplex PCR assay *(23)*. The multiplex PCR detected genes for *E. coli* O157 ($rfbE_{O157:H7}$), H7 ($fliC_{h7}$), Shiga toxins 1 (stx_1) and 2 (stx_2), and intimin (eae_{O157}). 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. These criteria allow the detection of *E. coli* O157:H7 strains that have lost *stx* or *eae* genes and also circumvent the ambiguity associated with the nonmotile designation *(7)*.

Indirect ELISA to detect serum antibodies to type III secreted proteins. An indirect enzyme-linked immunosorbent assay (ELISA) to detect serum antibodies against *E. coli* O157:H7 type III secreted proteins and intimin was conducted by a modification of procedures previously described *(19)*. *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, EMD Biosciences-Merck). The region of the *eae* gene cloned into the recombinant intimin construct encodes the 280-carboxyl-terminal amino acids, specific for the γ-intimin subtype. Individual wells of microtiter plates were coated with 100 μl of EspA (150 ng), EspB (250 ng), Tir (100 ng), or intimin (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 triplicate. 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, goat anti-bovine immunoglobulin G (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 determined as the highest dilution having an absorbance (mean minus 1 SEM) greater than the negative control (mean plus 1 SEM), the latter including all reagents except primary serum.

Statistical analyses. Dichotomous outcomes, such as the probability of detecting *E. coli* O157:H7 or probability of grading USDA choice, were tested by the logit link function in a multivariable generalized estimation equation model (Proc GENMOD, SAS Institute, Cary, N.C.). A first-order autoregressive correlation structure defined repeated measures of pens over time, sampling blocks, and pens for the probability of detecting *E. coli* O157:H7. Compound symmetry correlation structure defined steers within pens and pens within blocks for the probability of grading USDA choice at slaughter. The models were fashioned with 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 generalized estimation equation analysis. We tested factors of test period, diet, vaccine treatment, and region within the feedlot to explain the probability of cattle shedding *E. coli* O157:H7 within the 60 pens of vaccinated cattle. We tested vaccine treatment, corn product, and test period to explain the probability of cattle shedding *E. coli* O157:H7 for all 72 pens. Two-way interactions were tested between vaccine treatment and test period. Specific contrasts of vaccine treatment were made of zero, one, two, or three doses of vaccine within pens of vaccinated cattle compared with external control pens and one, two, or three doses of vaccine compared with no dose within vaccinated pens. Least-squared means (LSM) of the logistic parameter estimates (the natural logarithm of the odds) from the multivariable logistic model were used to estimate adjusted probabilities for class variables by the following formula: model-adjusted probability = $e^{(\text{LSM estimate})}/(1 + e^{(\text{LSM estimate})})$, corresponding to the general formula for converting odds to probability. Relative risk values for levels of 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. Each steer's response to vaccination treatment was quantified two ways: (i) by the magnitude of titer change (change in dilutions) and (ii) by the presence or absence of a fourfold or greater seroresponse (two-dilution or greater change in titer). Differences in the magnitude of titer change from the four treatment levels were tested by a generalized linear mixed model with pen as a random effect (Proc MIXED, SAS). If overall treatment effects were significant, then contrasts between treatments (number of doses) were tested. The probability of having a seroresponse for each treatment level was evaluated by the logit link function in a multivariable generalized estimation equation model (Proc GENMOD, SAS), assuming a compound symmetrical correlation of observations within pens.

The effects of vaccine treatment on continuous carcass and performance outcome variables for the 60 pens of vaccinated cattle were tested by generalized linear mixed models with random effects of pen within block.

RESULTS

One steer that received three doses of vaccine was removed from the trial between test periods 4 and 5 because of injury during normal cattle handling activities. Between 8 May and 26 September 2003, steers in treated and external control pens were fed for an average of 138 and 126 days, respectively.

Fecal cultures. In total, *E. coli* O157:H7 was recovered from 845 of 4,253 culture observations. At pretreatment sampling, the proportion of cattle shedding *E. coli* O157:H7 within the pens of vaccinated cattle was 45% and not different ($P > 0.10$) for animals allocated to different numbers of vaccine doses. The proportion of cattle shedding *E. coli* O157:H7 among the external control pens at pretreatment sampling was 30.5% and was significantly lower ($P = 0.05$) than for cattle in vaccinated pens.

Because of the study design, differences existed for BW, region of the feedyard, and diet between the external control pens and the 60 pens of vaccinated steers; however, the 60 pens of vaccinated pens were balanced by treatment for region of the feedyard, BW, and diet, giving us the opportunity to assess the potential for selection bias. For

TABLE 2. *Multivariable logistic regression model of the probability of detecting* E. coli *O157:H7 from feces of steers fed in a research feedlot*[a]

Variable	Unit	Parameter estimate	Odds ratio	95% confidence interval		*P* value
Intercept		0.2788				0.42
Vaccination treatment	0 dose	0.3017	0.331	0.183	0.598	0.0001
	1 dose	0.3034	0.253	0.139	0.458	
	2 doses	0.3096	0.268	0.146	0.492	
	3 doses	0.3264	0.213	0.113	0.405	
	Unvaccinated external control pens	0	1.000	Referent		
Test period[b]	21 dpt	−1.4527	0.23	0.12	0.44	<0.0001
	42 dpt	−1.0914	0.34	0.20	0.55	
	63 dpt	−0.3339	0.72	0.46	1.11	
	84 dpt	0	1.00	Referent		
Corn product[c]	DRC	−0.5179	0.60	0.34	1.03	0.002
	HMC	−0.8867	0.41	0.26	0.64	
	RECON	0	1.00	Referent		

[a] Model fit was slightly underdispersed, evident by deviance per degrees of freedom value of 0.77.
[b] Unit for test period was days posttreatment (dpt).
[c] DRC, dry-rolled corn; HMC, high-moisture corn; RECON, reconstituted (water added) HMC.

the 60 pens of vaccinated cattle, initial BW was not associated with the probability of steers shedding *E. coli* O157:H7 ($P = 0.87$). Region of the feedyard (four alleys within the feedlot for the 60 pens) did not explain the probability of steers shedding *E. coli* O157:H7 ($P = 0.38$). Diet components that were evaluated were corn product and protein content. Protein concentration was not associated with the shedding of *E. coli* O157:H7 ($P = 0.81$). Corn product was associated with the probability of steers shedding *E. coli* O157:H7 ($P = 0.002$). The model-adjusted probability that steers would shed *E. coli* O157:H7 was 0.06 for those fed HMC, 0.09 for those fed DRC, and 0.14 for those fed reconstituted corn. Therefore, we concluded that initial BW and region within the feedlot were not likely to be confounding factors for the external control pens. However, the potential confounding effect of corn product was controlled for by including it as a fixed effect in the full model comparing pens of vaccinated steers with external control pens.

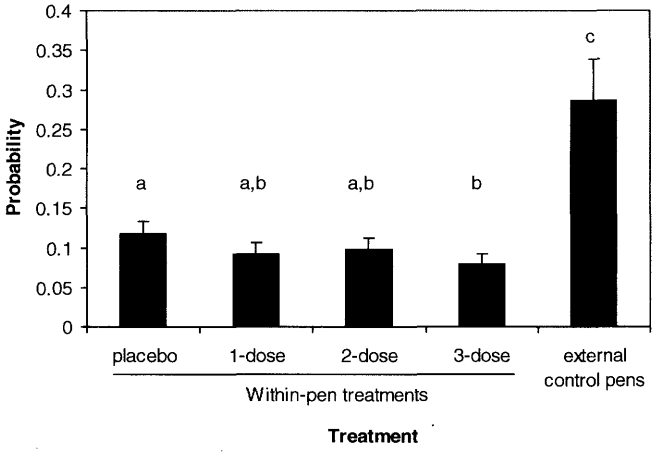

FIGURE 1. *Probability of steers shedding* E. coli *O157:H7 in the feces by treatment adjusted for time and corn product. Number of doses with different letters differ by* $P < 0.05$. *EC, external control; 0, no doses of vaccine; 1, one dose of vaccine administered on day 42; 2, two doses of vaccine administered on days 0 and 42; 3, three doses of vaccine administered on days 0, 21, and 42. Error bars represent 1 standard error.*

The factors explaining the probability that steers would test positive for *E. coli* O157:H7 in the multivariable logistic regression model were vaccination, test period, and corn product (Table 2).

Vaccination reduced the probability of detecting *E. coli* O157:H7 in the feces of steers ($P < 0.0001$; Fig. 1). Accounting for other variables in the model, the probability of detecting *E. coli* O157:H7 from steers in external control pens was 0.29. The probability of detecting *E. coli* O157:H7 from steers receiving zero, one, two, or three doses of vaccine in vaccinated pens was 0.12, 0.09, 0.10, and 0.08, respectively. Therefore, the vaccine efficacy of receiving one, two, and three doses of vaccine was 68, 66, and 73%, respectively, compared with cattle in pens not receiving vaccine. Additionally, within vaccinated pens, cattle receiving three doses of vaccine were 59% less likely ($P = 0.015$) to shed *E. coli* O157:H7 than placebo-treated cattle, exhibiting what is likely herd immunity.

Other variables that contributed to the multivariable logistic model included test period and corn product. The probability that steers would test fecal positive for *E. coli* O157:H7 in both treated and external control pens during test periods 4 to 7 differed significantly by test period ($P < 0.0001$; Fig. 2). Additionally, the type of corn product being fed was significantly ($P = 0.005$) associated with the probability that cattle would shed *E. coli* O157:H7 in the feces (Fig. 3). There was no evidence of a vaccination by corn product interaction.

Serology. Indirect ELISA to detect serum IgG titers against *E. coli* O157:H7 type III secreted proteins (EspA, EspB, and Tir), with intimin as a control, was completed

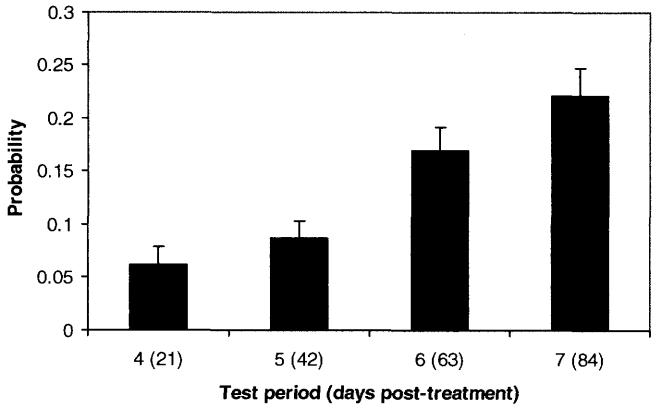

FIGURE 2. *Probability of steers shedding* E. coli *O157:H7 in feces 21, 42, 63, and 84 days posttreatment adjusted for corn product and treatment. Test period was associated* (P < *0.0001) with the probability that steers would shed* E. coli *O157:H7 in feces. Error bars represent 1 standard error.*

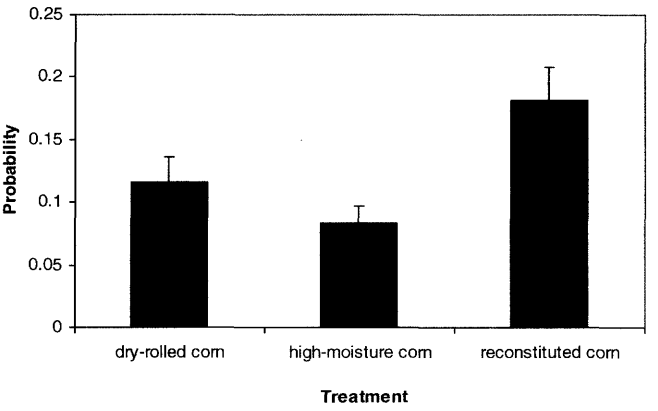

FIGURE 3. *Probability of steers shedding* E. coli *O157:H7 in feces by corn product adjusted for time and treatment. Corn product was associated* (P = *0.01) with the probability that steers would shed* E. coli *O157:H7 in feces. DRC, dry-rolled corn; HMC, high-moisture corn; RECON, reconstituted HMC. Error bars represent 1 standard error.*

on a total of 64 cattle, 16 each receiving zero, one, two, and three doses from within eight pens. Cattle had prevaccination antibody titers to all four proteins; however, significant increases in IgG titers to EspA ($P = 0.01$) and EspB ($P = 0.004$) occurred from prevaccination to 21 days after completion of the vaccine regimen (Fig. 4). For both proteins, the magnitude of titer change was significantly greater if cattle received two or three doses than none ($P < 0.05$). The magnitude of change in titer to intimin ($P = 0.73$) or Tir ($P = 0.24$) from prevaccination to 21 days after completion of the vaccine regimen was not significantly different by dose (Fig. 4). By 84 days after completion of the vaccine regimen, the magnitude of titer change compared with day 0 was not significantly different by dose for any protein. The probability of a fourfold or greater seroresponse did not differ significantly by dose for any of the proteins.

Performance characteristics. Vaccine treatment had no effect on hot carcass weight ($P = 0.38$), fat depth ($P = 0.73$), Longissimus dorsi muscle (ribeye) area ($P = 0.79$), or calculated USDA yield grade ($P = 0.86$). The average daily gain of the steers was not influenced by vaccine treatment ($P = 0.59$). Similarly, vaccination did not significantly affect carcass quality measured as USDA marbling score ($P = 0.07$). Average marbling scores were 389, 404, 385, and 389 for zero, one, two, or three doses, respectively.

DISCUSSION

The most important findings of this study were that cattle vaccinated with one, two, or three doses of vaccine were significantly less likely to shed *E. coli* O157:H7 in their feces than pens of unvaccinated cattle (external controls) and that cattle receiving three doses of vaccine were significantly less likely to shed the organism than placebo-treated cattle in the same pen. Another significant finding was that the placebo-treated cattle were less likely to shed *E. coli* O157:H7 than the external controls. We interpret this observation as evidence of herd immunity (i.e., the placebo-treated steers had a reduced probability to shed *E. coli* O157:H7 because of a reduction in fecal shedding of the organism by vaccinated penmates). The concept of herd immunity has also been termed herd protection *(18)*. Other explanations for this observation are possible, but less likely. For example, the placebo-treated cattle (and vaccinated

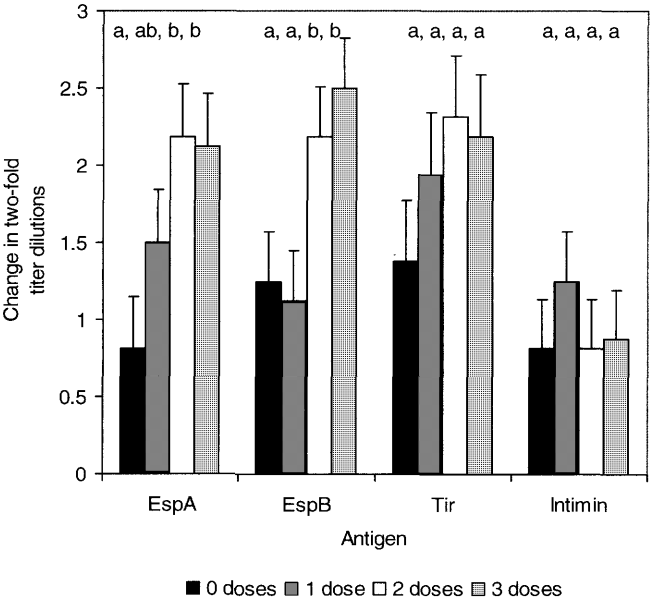

FIGURE 4. *Change in serum IgG titer to* E. coli *O157:H7 type III secreted proteins and intimin, as determined by indirect ELISA, from prevaccination to 21 days after completion of the vaccine regimen.* E. coli *O157:H7 His-tag purified recombinant EspA, EspB, Tir, and intimin (C-terminal 280 amino acids) were coated onto polyvinyl chloride microtiter plate wells, and serum dilutions from steers given zero, one, two, or three doses of vaccine were added. Binding of antibodies to the immobilized proteins was detected by goat anti-bovine IgG labeled with horseradish peroxidase and* o*-phenylenediamine substrate, and reactions were read at 490 nm. Serum antibody titers were* log_2 *transformed prior to statistical analysis. Different letters within antigen groups differ significantly* (P < *0.05). Error bars represent 1 standard error.*

cattle) differed from the unvaccinated cattle by the alley of the feedyard, by their incoming BW, and by their having received adjuvant. However, cattle in this study did not differ in the probability to shed *E. coli* O157:H7 by feedyard alley or BW. Also, it is unlikely that adjuvant treatment alone would affect fecal shedding, and the potential for confounding by the corn product fed was controlled for in the statistical analysis.

In this study, the three-dose regimen was numerically most effective, although not significantly different from the one- or two-dose regimen. The results of this study indicate that the vaccine has the potential to improve food and environmental safety by reducing the probability that live cattle will shed the organism. Further study will be necessary to determine the number of doses for optimum effectiveness.

Compared with placebo-treated penmates, vaccinated cattle developed significant serum IgG responses to the *E. coli* O157:H7 type III secreted proteins EspA and EspB by day 21 postvaccination, and a dose effect was observed in the face of preexisting titers. Responses due to prior *E. coli* O157:H7 exposure may influence antibody titers to *E. coli* O157:H7 virulence factor antigens; therefore, intimin was included as a control to monitor this effect. As hypothesized, we observed IgG responses to *E. coli* O157:H7 intimin; however, the response was nearly identical, regardless of treatment. Although the dose-dependent responses to Tir were not statistically significant, the pattern of response was more similar to that of EspA and EspB than intimin.

Other variables such as test period also explained fecal shedding. This observation is consistent with results from previous longitudinal studies, which show that the proportion of cattle shedding *E. coli* O157:H7 varies greatly over the course of the feeding period *(9, 19)* and is related to conditions of the environment that affect the ability of the organism to survive and be ingested by another host *(23)*. In studies designed to describe and explain the ecology of *E. coli* O157:H7 by time and place in commercial beef feedlots, there were large differences in the proportion of pens of cattle classified as positive for *E. coli* O157:H7 by feeding season, weeks within season, and feedyard *(9, 23)*. The pretreatment prevalence of *E. coli* O157:H7 shedding was greater in pens of vaccinated cattle than in external control pens; however, the pattern was reversed after the vaccination treatments were applied. The proportion of cattle shedding *E. coli* O157:H7 among the external control pens was higher than in the treated pens for the entirety of the response period.

The probability of detecting *E. coli* O157:H7 in the feces of cattle was different for cattle fed different corn products. This finding was unexpected, because in one of our previous studies, the probability that cattle would shed *E. coli* O157:H7 in the feces was unaffected when corn bran and wet corn gluten feed or HMC and wet corn gluten feed were substituted for DRC *(13)*. However, the results of other studies suggest that dietary components play a role in *E. coli* O157:H7 shedding *(4, 5, 10, 14)*, although the explanation for dietary effects is not clear. Other researchers have suggested that feed is an important source of *E. coli* O157:H7 exposure for cattle, especially if it is contaminated with the organism prior to arrival at the feedbunk *(5, 21)*. In this case, the reconstituted corn could have been contaminated by the water that was added, but we cannot substantiate this.

In conclusion, this study supports the hypothesis that vaccination with one, two, or three doses of this vaccine product effectively reduces the probability that cattle will shed *E. coli* O157:H7. Cattle receiving three doses of vaccine were significantly less likely to shed *E. coli* O157:H7 than unvaccinated cattle within the same pen. There was no indication that the vaccine affected performance or carcass quality. In addition, we found that vaccinating a majority of cattle within a pen offered a significant protective effect (herd immunity) to unvaccinated cattle within the same pen.

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REFERENCES

1. Armstrong, G. L., J. Hollingsworth, and J. G. Morris. 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.* 18:29–51.
2. Callaway, T. R., R. C. Anderson, T. S. Edrington, K. J. Genovese, K. M. Bischoff, T. L. Poole, Y. S. Jung, R. B. Harvey, and D. J. Nisbet. 2004. What are we doing about *Escherichia coli* O157:H7 in cattle? *J. Anim. Sci.* 82(E-Suppl.):E93–E99.
3. Cray, W. C., and H. W. Moon. 1995. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 61:1586–1590.
4. Dargatz, D. A., S. J. Wells, L. Thomas, D. D. Hancock, and L. P. Garber. 1997. Factors associated with the presence of *Escherichia coli* O157 in feces of feedlot cattle. *J. Food Prot.* 60:466–470.
5. Dodd, C. C., M. W. Sanderson, J. M. Sargeant, T. G. Nagaraja, R. D. Oberst, R. A. Smith, and D. D. Griffin. 2003. Prevalence of *Escherichia coli* O157 in cattle feeds in Midwestern feedlots. *Appl. Environ. Microbiol.* 69:5243–5247.
6. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
7. Gannon, V. P., S. D'Souza, T. Graham, R. K. King, K. Rahn, and S. Read. 1997. Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains. *J. Clin. Microbiol.* 35:656–662.
8. Hancock, D. D., T. V. Lynn, and T. E. Besser. 1997. Feasibility of preharvest food safety control. *Compend. Cont. Educ. Pract. Vet.* 19:S200–S207.
9. Khaitsa, M. L., D. R. Smith, J. A. Stoner, A. M. Parkhurst, S. Hinkley, T. J. Klopfenstein, and R. A. Moxley. 2003. Incidence, duration, and prevalence of *Escherichia coli* O157:H7 fecal shedding by feedlot cattle during the finishing period. *J. Food Prot.* 66:1972–1977.
10. Kudva, I. T., P. G. Hatfield, and C. J. Hovde. 1995. Effect of diet on the shedding of *Escherichia coli* O157:H7 in a sheep model. *Appl. Environ. Microbiol.* 61:1363–1370.
11. Li, Y., E. Frey, A. M. Mackenzie, and B. B. Finlay. 2000. Human response to *Escherichia coli* O157:H7 infection: antibodies to secreted virulence factors. *Infect. Immun.* 68:5090–5095.

12. Loneragan, G. H., and M. M. Brashears. 2005. Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. *Meat Sci.* 71:72–78.
13. Loy, T., C. Wilson, D. Bailey, M. Blackford, T. Klopfenstein, R. A. Moxley, D. R. Smith, and S. Younts. 2001. Influence of restricted intake and reduced dietary starch on colonic pH and E. coli prevalence. Nebraska Beef Cattle Rep. MP 76-A:86–88. University of Nebraska–Lincoln.
14. Lynn, T. V., D. D. Hancock, T. E. Besser, J. H. Harrison, D. H. Rice, N. T. Stewart, and L. L. Rowan. 1998. The occurrence and replication of *Escherichia coli* in cattle feeds. *J. Dairy Sci.* 81:1102–1108.
15. Moxley, R. A. 2004. *Escherichia coli* O157:H7: an update on intestinal colonization and virulence mechanisms. *Anim. Health Res. Rev.* 5:15–33.
16. Naylor, S. W., J. C. Low, T. E. Besser, A. Mahajan, G. J. Gunn, M. C. Pearce, I. J. McKendrick, D. G. Smith, and D. L. Gally. 2003. Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infect. Immun.* 71:1505–1512.
17. Naylor, S. W., A. J. Roe, P. Nart, K. Spears, D. G. Smith, J. C. Low, and D. L. Gally. 2005. *Escherichia coli* O157:H7 forms attaching and effacing lesions at the terminal rectum of cattle and colonization requires the LEE4 operon. *Microbiology* 151:2773–2781.
18. Paul, Y. 2004. Herd immunity and herd protection. *Vaccine* 22:301–302.
19. Potter, A. A., S. Klashinsky, Y. Li, E. Frey, H. Townsend, D. Rogan, G. Erickson, S. Hinkley, T. Klopfenstein, R. A. Moxley, D. R. Smith, and B. B. Finlay. 2004. Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. *Vaccine* 22:362–369.
20. Rice, D. H., H. Q. Sheng, S. A. Wynia, and C. J. Hovde. 2003. Rectoanal mucosal swab culture is more sensitive than fecal culture and distinguishes *Escherichia coli* O157:H7-colonized cattle and those transiently shedding the same organism. *J. Clin. Microbiol.* 41: 4924–4929.
21. Sargeant, J. M., M. W. Sanderson, D. D. Griffin, and R. A. Smith. 2004. Factors associated with the presence of *Escherichia coli* O157 in feedlot-cattle water and feed in the Midwestern USA. *Prev. Vet. Med.* 66:207–237.
22. Smith, D. R., M. P. Blackford, S. M. Younts, R. A. Moxley, J. T. Gray, L. L. Hungerford, C. T. Milton, and T. J. Klopfenstein. 2001. Ecological relationships between the prevalence of cattle shedding *Escherichia coli* O157:H7 and characteristics of the cattle or conditions of the feedlot pen. *J. Food Prot.* 64:1899–1903.
23. Smith, D. R., R. A. Moxley, S. L. Clowser, J. D. Folmer, S. Hinkley, G. E. Erickson, and T. J. Klopfenstein. 2005. Use of rope devices to describe and explain the feedlot ecology of *Escherichia coli* O157: H7 by time and place. *Foodborne Pathog. Dis.* 2:50–60.
24. Stevens, M. P., P. M. van Diemen, F. Dziva, P. W. Jones, and T. S. Wallis. 2002. Options for the control of enterohaemorrhagic *Escherichia coli* in ruminants. *Microbiology* 148:3767–3778.