Nebraska Beef Cattle Reports   Animal Science Department

1-1-2008

Vaccination for *Escherichia coli* O157:H7 in Feedlot Cattle

Rodney A. Moxley
University of Nebraska - Lincoln, rmoxley1@unl.edu

David R. Smith
University of Nebraska - Lincoln, dsmith8@unl.edu

Karen Hansen
University of Nebraska - Lincoln

Matthew K. Luebbe
University of Nebraska - Lincoln, mluebbe2@unl.edu

Galen E. Erickson
University of Nebraska - Lincoln, gerickson4@unl.edu

See next page for additional authors

Moxley, Rodney A.; Smith, David R.; Hansen, Karen; Luebbe, Matthew K.; Erickson, Galen E.; Klopfenstein, Terry J.; and Rogan, Dragan, "Vaccination for *Escherichia coli* O157:H7 in Feedlot Cattle" (2008). *Nebraska Beef Cattle Reports.* Paper 38.
http://digitalcommons.unl.edu/animalscinbcr/38

Authors

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Terry J. Klopfenstein
Dragan Rogan[1]

Summary

A clinical trial conducted during the summer of 2006 evaluated the effects of two- and three-dose regimens of an Escherichia coli *O157 vaccine product on the probability of detecting* E. coli *O157:H7 in feces and on colonization of the terminal rectum. The three-dose regimen significantly reduced the probability for cattle to shed* E. coli *O157: H7 in feces by 63% compared to placebo treated cattle. A dose-effect was demonstrated because a two-dose regimen of the vaccine product was intermediate in effect. These results are consistent with previous estimates of vaccine efficacy against fecal shedding and agree with our previous finding that efficacy is related to the number of doses.*

Introduction

Vaccination of feedlot cattle against type III secreted proteins of *Escherichia coli* O157:H7 as a strategy to reduce the probability for cattle to shed the organism and effects of dose regimen has been reported in several beef reports (*2006 Nebraska Beef Report*, pp. 68-69; *2006 Nebraska Beef Report*, pp. 70-71; *2005 Nebraska Beef Report*, pp. 61-63).

The issue of how many vaccine doses to administer for effectiveness is an important one for efficiency of cattle handling. Our 2003 study tested the number of doses of vaccine administered to cattle within pens (*2005 Nebraska Beef Report*, pp. 61-63). In that study, vaccination of a majority of cattle within the pen conferred protection to nonvaccinated animals within pens indicating a pen-level effect of vaccination. In order to clarify the effect of the number of vaccine doses, a clinical trial was conducted that compared the effects of two and three doses of vaccine against nonvaccinated animals, with vaccination treatment being applied to the pen rather than the individual animal as in the previous study.

Procedure

The clinical trial was conducted from June to October of 2006 at the beef research feedlot of the University of Nebraska Agricultural Research and Development Center at Ithaca, Neb. Four hundred eighty medium weight steers were stratified by BW and randomly assigned to 60 pens (8 steers/pen). Twenty pens were assigned to each of three treatments: 1) three doses of placebo given at days 0, 21, and 42; 2) two doses of vaccine given at days 0 and 42; and 3) three doses of vaccine given at days 0, 21, and 42. Pens were managed in 4 blocks to account for differences in date of first vaccination, vaccination-to-slaughter intervals (15 or 16 weeks) and location within the feedlot facility. All animals were fed similar high-moisture corn (HMC) diets with 20% wet corn gluten feed, 7.5% roughage, and 5.0% supplement. Blocks 1 and 2 were comprised of 18 pens each, with each block treated and sampled at different times (block 2 was vaccinated one week later and slaughtered 1 week later than block 1). Block 3 was composed of 9 pens with 3 pens per vaccine treatment. Block 4 was composed of 15 pens with 5 pens per treatment. Blocks 3 and 4 were treated and sampled on the same days but were in different locations within the feedlot. Vaccine and placebo treatments were coded by the manufacturer (Bioniche Life Sciences Inc.) so that researchers and feedlot personnel were blinded to treatment.

Fecal samples were obtained from the rectum on days 0, 63, 77, 91 and the day prior to harvest (i.e., 1 pre-treatment period and 4 test period samplings), and a sample of the terminal rectum mucosa (TRM) was collected at harvest. Fecal cultures from all steers were collected by block as stated above within the same test period. Fecal samples were labeled with a bar-code and TRM samples were numbered in order of harvest, which blinded laboratory personnel to animal identification. All samples were transported to the laboratory within a few hours of collection. Standard broth enrichment and plate culture methods (*2006 Nebraska Beef Report,* pp. 68-69) with modifications were used to yield a positive or negative result and determination of the probability (prevalence) of *E. coli* O157:H7 fecal shedding or TRM colonization. Serial dilution of weighed samples followed by direct plating (i.e., without broth enrichment) and standard culture methods (*2006 Beef Report,* pp. 68-69) with modifications were used to quantify *E. coli* O157:H7 CFU per gram of feces or TRM.

The effect of vaccine treatment on the probability of detecting *E. coli* O157:H7 from feces was modelled using multi-level logistic regression (GENMOD, SAS Institute, Cary, N.C.). Least squared means of the parameter estimates from the multi-variate analysis were used to estimate adjusted probabilities for vaccine treatment. Relative risk (RR) values for each vaccine treatment were calculated from the adjusted probabilities and vaccine efficacy was calculated as 1-RR.

Results

Prevalence of E. coli O157:H7 fecal shedding

In this study *E. coli* O157:H7 was detected in 236 of 2,387 fecal samples (10%); 117 of 478 pre-treatment (24%) and 119 of 1,909 post-treatment fecal samples (6%) were culture positive. The prevalence of fecal shedding among all treatment groups declined during the feeding period, with a very slight increase at harvest (Figure 1).
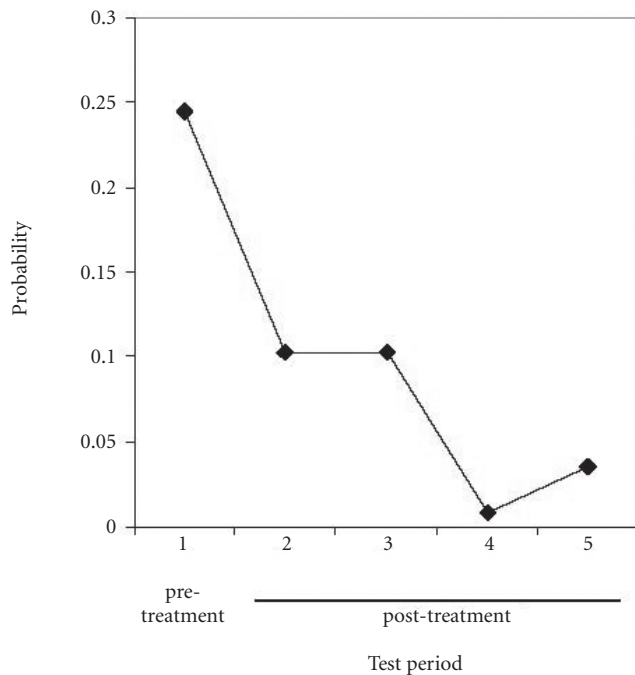
Figure 1. Unadjusted prevalence of *E. coli* **O157:H7 fecal shedding as determined by enrichment culture, plotted by test period.**
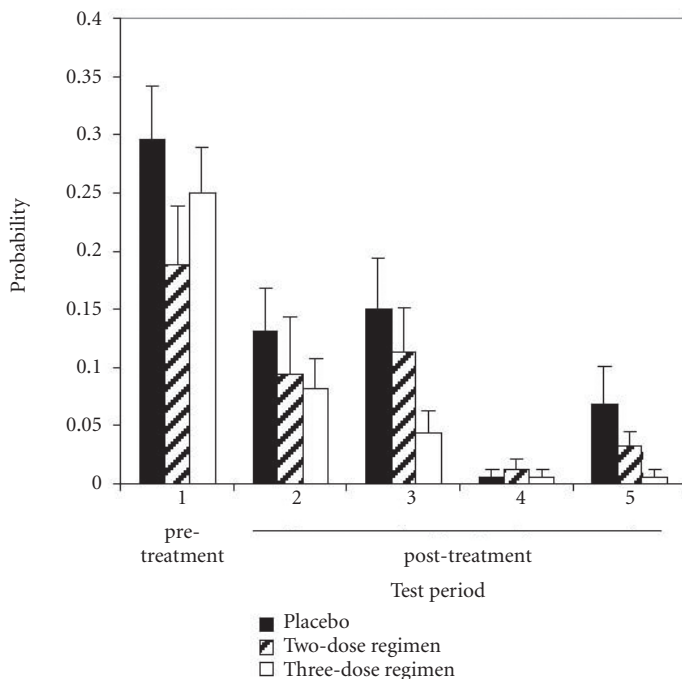

Figure 2. Unadjusted mean pen-level prevalence of *E. coli* **O157:H7 fecal shedding as determined by enrichment culture, plotted by treatment and test period. Error bars represent one SEM.**

The unadjusted mean prevalence of shedding by treatment and test period is summarized in Figure 2. The pen-level prevalence of *E. coli* O157:H7 fecal shedding during the post treatment period was 9%, 6% and 3% for placebo-treated, 2-, and 3-dose vaccine regimens, respectively.

Quantification of E. coli *O157:H7 shed in feces*

Direct plating for quantification of *E. coli* O157:H7 was completed on a total of 1,961 pre- and post-treatment fecal samples. The organism was detected in 29 of 1,961 samples (1.5%).

E. coli O157:H7 was detected in 16 of 415 pre-treatment samples (3.9%); 7 were from placebo-treated, 2 were from 2-dose-treated, and 7 were from 3-dose-treated pens of cattle. Mean (SEM) $\log_{10}$ bacterial counts (CFU/g) were 3.0 (0.38), 3.5 (0.50) and 3.2 (0.41) for placebo-treated, 2-dose, and 3-dose treatment groups, respectively, suggesting that in cattle that were shedding, relatively similar amounts of the organism were shed. *E. coli* O157:H7 was detected in 13 of 1,546 post-treatment samples (0.8%); 5 from placebo-treated, 4 from 2-dose, and 4 from 3-dose treated pens of cattle. Mean (SE) $\log_{10}$ bacterial counts (CFU/g) were 2.6 (0.29), 4.1 (0.55), and 3.7, (0.64) for placebo-treated, 2-dose, and 3-dose treatment groups, respectively.

Prevalence of E. coli *O157:H7 Colonization*

At harvest, a total of 5 of 380 (1.3%) steers were colonized; 3 were placebo-treated and 2 had been vaccinated 3 times. Only 2 steers were culture positive from direct plating, both were from the placebo-treated group.

Statistical tests of efficacy

Statistical tests of colonization and fecal quantification from direct plating were not done because there were too few positive observations among placebo-treated cattle to make valid inferences regarding vaccine efficacy. Pre-treatment pen-level prevalence of fecal shedding did not differ between pens of cattle in the three treatment groups, based on either a generalized linear mixed model with block as random effect, or a generalized estimation equation model defining correlation of pens within block. Post-treatment vaccine efficacy was tested using three multilevel modeling approaches. Estimates of vaccine efficacy from each of the three models were nearly identical, based on least square means of the three model estimates. The significance of within-treatment differences was nearly identical for each of the three models. The

outcome of the first model is shown graphically in Figure 3.

The three-dose regimen significantly reduced the probability for cattle to shed *E. coli* O157:H7 in the feces 63% compared to placebo treated cattle. A dose-effect was demonstrated in that a 2-dose regimen of the vaccine product was intermediate in effect between placebo and a 3-dose regimen. These results are consistent with previous estimates of efficacy for fecal shedding and agree with our previous finding that efficacy of 2 or 3 doses of vaccine exhibit a dose-response.

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[1]Rodney A. Moxley, professor; David R. Smith, professor; and Karen Hansen, graduate student; Department of Veterinary and Bio-medical Sciences, Lincoln. Matt K. Luebbe, research technician; Galen E. Erickson, associate professor; Terry J. Klopfenstein, professor, Animal Science, Lincoln. Dragan Rogan, vice-president, Research and Development, Bioniche Animal Health, Bioniche Life Sciences, Belleville, Ontario, Canada.
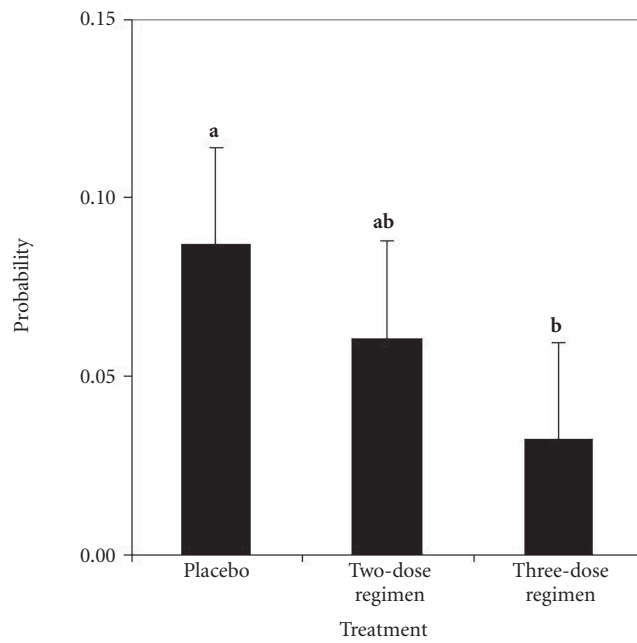

Figure 3. **Model-adjusted post-treatment probabilities for cattle to shed *E. coli* O157:H7 in feces, determined by enrichment culture, by treatment. Error bars represent one SEM. Treatments with different superscripts differ, *P* < 0.05.**