January 2006

Large-scale Clinical Trial to Evaluate an Experimental *Escherichia coli* Vaccine

Robert E. Peterson
University of Nebraska-Lincoln

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

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

Terry J. Klopfenstein
University of Nebraska-Lincoln, tklopfenstein1@unl.edu

Susan Hinkley
University of Nebraska-Lincoln

See next page for additional authors

Large-scale Clinical Trial to Evaluate an Experimental *Escherichia coli* Vaccine

Robert E. Peterson
David R. Smith
Rodney A. Moxley
Terry J. Klopfenstein
Susan Hinkley
Galen E. Erickson*1*

Summary

A clinical trial was conducted within 19 Nebraska feedlots to evaluate effects of an E. coli *vaccine on the probability to detect* E. coli *O157:H7 on ROPES or for cattle to be colonized by* E. coli *O157:H7 at the terminal rectum. Vaccinated pens of cattle were less likely to test ROPE-positive than nonvaccinated pens of cattle and a lower probability for* E. coli *O157:H7 colonization among vaccinated cattle compared with nonvaccinated cattle was observed. The vaccine was effective at reducing* E. coli *O157:H7 in the feedlot pen environment and colonization at the terminal rectum of cattle.*

Introduction

Research reported in the previous article of this report indicates several benefits of vaccination for *E. coli* O157:H7 in market ready beef cattle (*2006 Nebraska Beef Report*). However, vaccination has not been evaluated in a large-scale study that accounted for multiple factors known to influence the probability to detect *E. coli* O157:H7 in the feedlot environment. For example, time of year, pen condition, and feedlot have all been identified as factors that explain the variability in the prevalence of *E. coli* O157:H7 associated with feedlot cattle. Therefore, there was a need to evaluate vaccination as a pre-harvest intervention strategy in a large-scale commercial feedlot study.

Procedure

The study was a large-scale clinical trial designed to test the effect of a two-dose vaccination regimen on the probability to detect *E. coli* O157:H7 on pen-test devices (ROPES) and from mucosal cells of the rectoanal junction of cattle at harvest. Commercial feedlots were classified as either feeding or not feeding a direct-fed microbial (DFM) product. Pens of vaccinated and nonvaccinated cattle within feedlots were matched by time of sampling, reprocessing schedule, and estimated days to finish weight. Vaccine was given to all cattle within treated pens at initial processing and again at reimplant. Pair-matched nonvaccinated pens of cattle were sampled on the same days. Research personnel responsible for vaccinating cattle and collecting samples and other data from the cattle were blinded to microbiological results. Research personnel working in the microbiological laboratory were blinded to treatment assignments.

Each pen of cattle enrolled in the study was sampled for *E. coli* O157:H7 starting at least one week after the second dose of vaccine was given (untreated pens of cattle were sampled on the same day as the pair-matched vaccinated pen) and continued every three weeks for four test period samplings. Pens were tested for *E. coli* O157:H7 by hanging seven ropes from the neckrail of the feedbunks where cattle could easily lick, chew, or rub on them. Pens were classified ROPES-positive if *E. coli* O157:H7 was recovered from at least one rope-device. *E. coli* O157 was isolated and identified by standard methods involving selective enrichment, immunomagnetic separation, agar plating, biochemical and immunological testing and PCR confirmation.

The outcome variable (Yes/No) defined if pens tested ROPES-positive for *E. coli* O157:H7. The binomial probability of detecting *E. coli* O157:H7 from at least one ROPES within a pen was modeled with a Generalized Estimating Equations (GEE) model using the GENMOD procedure of SAS accounting for a correlated data structure with repeated measure of pens (test periods), and clustering of matched pairs of pens within feedlot.

The variable of interest was vaccination (Yes/No). Additional specific contrasts were vaccination versus not vaccinated and short revaccination period (13-45 days) versus long revaccination period (45-100 days). Potential confounders tested in the GEE model were feeding a DFM, region of the state (defined as East or West of a North/South line extending through Grand Island, Neb.), month of sampling, the condition of the pen floor (dry and dusty, wet and muddy, ideal condition), number of cattle in the pen (145 cattle or less, greater than 145), cleanliness of the cattle, and test period. An interaction between vaccination and test period was tested. Additionally, the variable representing direct-fed microbial feeding was forced in the model as a fixed effect because of its importance as a potential confounder. Other variables remained in the model if they contributed to the model fit and significantly explained the probability for ROPES-positive pens ($\alpha \leq 0.05$).

Twenty one pens of cattle on the study (11 vaccinated, 10 not vaccinated) were followed to the packing plant so samples could be collected to test effect of the vaccine on probability for colonization of mucosal cells of the terminal rectum. Cattle were systematically selected for sampling from within each pen. The sample size for each pen was calculated so that we would be 95% confident to estimate EC prevalence at 50% with a 15% precision. Terminal rectum mucosal cells (TRM) were collected by scraping the mucosa of the terminal rectum 1-2 inches proximal to the rectoanal juncture. The TRM were cultured using standard methods involving selective enrichment, immunomagnetic separation, agar plating, biochemical and immunological testing, and PCR confirmation as previously described.

The outcome of interest was the probability of detecting *E. coli* O157:H7 from TRM, analyzed using a generalized linear mixed model. Differences in the mean days from reprocessing to slaughter for vaccinated and not vaccinated pens was tested by the Student's t test assuming equal variances.

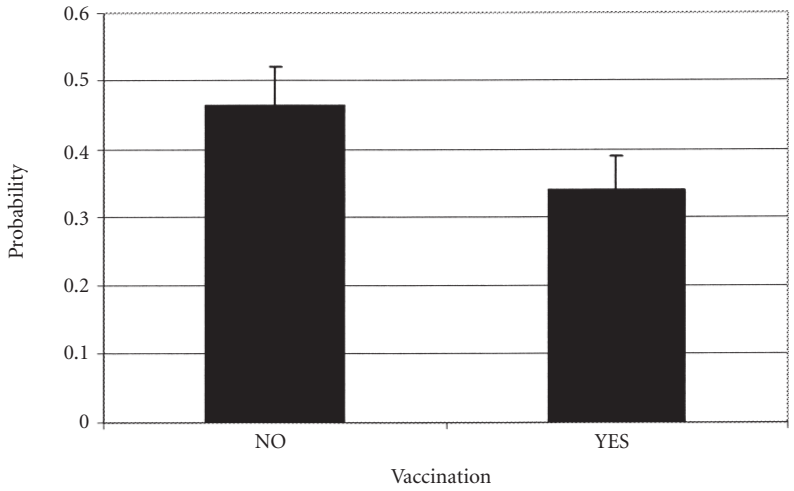

Figure 1. Adjusted probabilities for vaccinated and unvaccinated pens to test ROPES-positive for *E. coli* O157:H7.

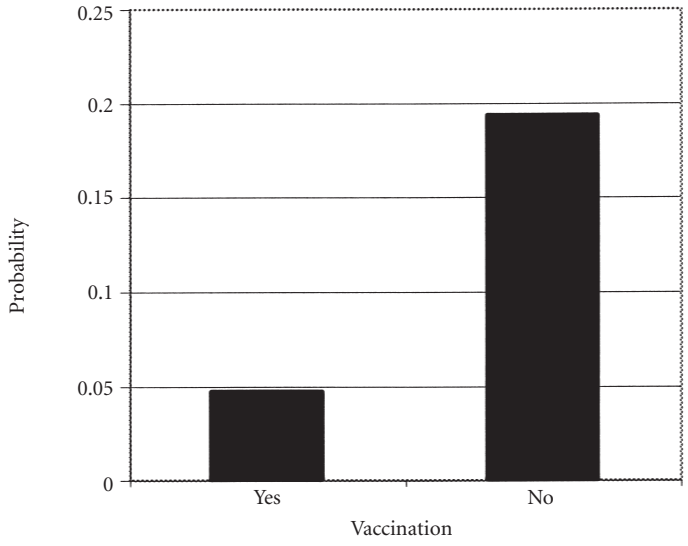

Figure 2. Probabilities for *E. coli* O157:H7 colonization of the rectoanal junction at slaughter for vaccinated and unvaccinated cattle.

Results

One-hundred forty eight pens of cattle (n=21,691 hd of cattle) within 19 commercial feedlots in Nebraska were enrolled in this study. However, two matched pairs of cattle pens were not reprocessed until October and November leaving no usable observations during the study period ending October 31 and cattle from two pairs of pens were not revaccinated; therefore, the data analyzed were from 140 pens of cattle within 19 feedlots representing 20,566 cattle.

Data were not collected from all four periods for all pens of cattle either because some pens of cattle were marketed before all four test periods were completed, or because some test periods fell outside of the study period (after October 31). In total, 86 pair- matched pens of cattle were in feedlots feeding a direct-fed microbial (DFM) and 54 pair- matched pens of cattle were in feedlots not feeding a DFM. The time interval between initial process (vaccination) and reprocessing (revaccination) averaged 54.2 (13-104) days. There were 485 pen observations and each observation had complete dependent and independent data. The number of cattle per pen averaged 146.8 (53-300) head.

ROPES

Nonvaccinated pens of cattle were more likely to test ROPES-positive than matched vaccinated pens of cattle (OR (odds ratio) =1.68, $P = 0.0035$), accounting for other variables in the model (Figure 1). There was no significant interaction between vaccination treatment and test period ($P = 0.94$), demonstrating efficacy of the vaccine did not change over time after revaccination.

The variables representing month of the year, region of the state, and the number of cattle within the pen remained in the model because they significantly explained the probability for pens of cattle to test ROPES-positive. Condition of the pen floor was retained in the model because the variable approached significance and has previously been demonstrated to explain the probability for pens of cattle to test ROPES-positive.

Terminal Rectum Mucosa

Terminal rectum mucosal (TRM) samples were collected from 720 cattle; 382 vaccinated cattle from within 11 pens and 338 nonvaccinated cattle from 10 pens. Four-hundred forty-one cattle were from within 13 pens fed DFM and 279 cattle were from within 8 pens of cattle not fed DFM.

Probability for *E. coli* O157:H7 colonization of the mucosal cells of the terminal rectum at slaughter among vaccinated cattle was lower (4.7%) compared with nonvaccinated cattle (19.5%). Vaccination reduced the probability for cattle within a feedlot to be colonized with *E. coli* O157:H7 at slaughter (OR=0.20; $P = 0.03$). Vaccine efficacy was 76% (Figure 2).

Vaccination of cattle within commercial feedlots was effective for reducing the probability of detecting *E. coli* O157:H7 from ROPES and the vaccine reduced, at slaughter, *E. coli* O157:H7 colonization of the terminal rectum mucosal cells of cattle fed in a commercial system.

Acknowledgments

The authors of this report would like to express their sincere appreciation to the feedlots that so graciously participated in the study. Without the outstanding cooperation from these feedlots this study would not have been possible.

[1]Robert Peterson, research technician; Dave Smith, Rod Moxley, professors, Veterinary and Biomedical Sciences; Terry Klopfenstein, Galen Erickson, professors, Animal Science; Susan Hinkley, professor, Veterinary and Biomedical Sciences.