

Vaccination on the ranch as an intervention strategy to reduce the probability of detecting *E. coli* O157:H7 associated with commercial feedlot cattle

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ABSTRACT: A clinical trial was conducted to test the effect of vaccinating freshly weaned Montana calves against *Escherichia coli* on the probability to detect *E. coli* O157:H7 (EC) in feces or on RAMS. Two cow/calf sources (1 Central MT; 1 Southeast MT), two feedlots (1 Central NE; 1 Western NE), and five packing plants (2 CO, 1 KS, 2 NE) participated in the study. Steer and heifer calves (N=1001) were weaned during the months of September and October 2003 and systematically allocated to treatment so that one-third of the calves would receive vaccine (VAC; vaccinated at weaning, 21 d post weaning, and 80-100 d prior to harvest) and two-thirds would not (NOVAC). Following weaning and a backgrounding period at the ranch of origin, calves were transported to NE feedlots. During finishing, the distribution of treatments within a pen was maintained as 1/3 VAC calves and 2/3 NOVAC calves. Each calf was sampled four times for 1 pre-treatment period (d 0; weaning; fecal), 2 interim periods (21 d post weaning; 80-100 d prior to harvest; fecal), and 1 test-period sampling (harvest; Rectoanal Mucosa Swab, RAMS). In total, 3595 individual fecal or RAMS samples were collected from 1003 calves. The odds for a VAC animal to shed EC were compared to that of NOVAC cattle accounting for repeated measures, feedlot, pen and the time of marketing. Overall pre-treatment probability of detecting EC averaged 0.40%. The probability of detecting EC for samples two, three, and four averaged 0.0, 0.40, and 1.24%, respectively, and were not different (OR=0.82; P>0.10) between vaccination treatments. The highest probability for detecting EC was observed at harvest for both NOVAC (1.37%) and VAC (1.00%) cattle, and were not different (OR=0.67, P>0.10). Because of the low probability of detecting EC shedding throughout the entire production period, this study could not determine the effect of vaccination on the ranch as a pre-harvest food safety intervention strategy.

Key Words: Cattle, *E. coli* O157:H7, Vaccination

Introduction

Human exposure to *Escherichia coli* O157:H7 can cause severe diarrhea (hemorrhagic colitis), and in a small percentage of cases, hemolytic-uremic syndrome (HUS). Beef cattle populations are important reservoirs for *Escherichia coli* O157:H7 and this pathogen causes important economic losses to the beef industry. Historically, the beef industry has focused their efforts to control this pathogen at the post-harvest stage of beef production. However, management practices aimed at controlling food borne pathogens prior to harvest have been suggested as potential pre-harvest food safety interventions to reduce the prevalence of *E. coli* O157:H7 in the feces and on the hides of beef feedlot cattle; including diet change, feeding direct-fed microbial products, sodium chlorate, antibiotics, water trough treatment, and vaccines (Callaway et al., 2004).

In an earlier study we found that vaccinating feedlot cattle against Type III secretory proteins of *Escherichia coli* O157:H7 reduced the probability for cattle to shed the organism in feces by 59% (Potter et al., 2004). In that study, cattle were vaccinated three times at three-week intervals with the first dose of vaccine given when cattle would normally enter the feedlot for the finishing phase of beef production. The use of a three-dose vaccination protocol is of practical importance because feedlot operators may be challenged to comply with the need to repeatedly vaccinate cattle. However, if vaccination could be implemented into pre-existing pre-conditioning programs at the ranch of origin, one or two doses of vaccine could be given to cattle before they are ever sent to a feedlot for the finishing phase of production.

Our objective was to evaluate the effectiveness of vaccinating calves against Type III secretory proteins of *E. coli* O157:H7 at the ranch (1 dose given at weaning and a 2nd dose given 14-21 d post weaning) and at the feedlot (reimplanting) on the probability to detect *E. coli* O157:H7 at the rectoanal juncture in cattle at harvest.

Materials and Methods

Source of Cattle

Spring born steer and heifer (n=1001) calves were weaned, and subsequently enrolled for study, starting September 29, and ending October 15, 2003. Calves originated from two sources in Montana. Source 1 enrolled 438 steers and source 2 enrolled 290 steers and 298 heifers. Calves were pre-conditioned at the ranch of origin for an average of 45 and 105 d for sources 1 and 2 respectively. At weaning, cattle were weighed, individual fecal samples collected, and were allocated to treatment.

Treatments

The vaccine (2ml/dose; Bioniche Life Sciences) was administered subcutaneously in the neck using an 18 ga x 5/8-inch needle. The vaccine contained supernatant proteins prepared from *E. coli* O157:H7 as previously described (Li et al., 2000), and formulated with the adjuvant VSA3 such that the protein concentration was 50µg/dose. Vaccination treatments included vaccination and no vaccination. Cattle were systematically allocated to treatment so that one-third of the calves would receive vaccine (VAC) and two-thirds would not (NOVAC). For VAC cattle, the first dose of vaccine was given at weaning, a second dose given 14-21 d later following the pre-conditioning protocol already in place at each respective ranch, and a third dose of vaccine given at reimplant time during the finishing phase of beef production (80-100 d prior to harvest).

Each animal was aseptically sampled by rectal fecal grab at weaning, at the end of the preconditioning period at the ranch of origin, and again at reimplant time, resulting in one pretreatment sample and two interim samplings. At harvest, each animal was aseptically sampled by swabbing the rectoanal juncture using Rectoanal Mucosal Swabs (RAMS) prior to the bunting bench at commercial beef packing plants.

Cattle were marketed at the discretion of the respective feedlot manager where the cattle were finished. Because we did not want to interfere with the normal cattle marketing activities at each feedlot, cattle were harvested at five different packing plants (2 CO, 1 KS, 2 NE).

Microbiological measurements

Laboratory personnel were blinded to treatments. Fecal samples were collected directly from the rectum of each animal and shipped by overnight delivery to the UNL *E. coli* lab and analyzed for presence of *E. coli* O157:H7 using procedures previously described (Smith et al., 2001) with modifications. Briefly, ten-gram fecal samples were incubated for 6 hr in Gram Negative (GN) broth containing vancomycin, cefixime, and cefsoludin. An aliquot of culture material was then subjected to immunomagnetic bead separation and plated onto sorbitol-MacConkey agar containing cefixime and tellurite (CT-SMAC). After 18-24 hr incubation, three non-sorbitol-fermenting colonies were picked and subcultured onto CT-SMAC to ensure purity then were subcultured onto MacConkey and Flourocult agars. After 18-24 hr incubation, lactose-fermenting colonies that yielded a negative MUG (4-methylumbelliferyl- β -D-glucuronide) reaction were streaked for isolation on blood agar. After an overnight incubation, one colony per isolate on blood agar was tested for *E. coli* O157 and H7 antigens by latex agglutination. Isolates that were positive for both the O157 and H7 antigens were tested in a 5-primer-pair multiplex polymerase chain reaction (PCR) assay that detected genes for *E. coli* O157, H7, Shigatoxins 1 and 2, and intimin. Detection of genes for O157, H7, and at least one other target in the assay was considered to be confirmation of an isolate as *E. coli* O157:H7.

RAMS samples were collected directly from the rectoanal juncture of each animal at harvest, placed in 3 ml of TSB in a 19 ml Falcon tube, and taken directly to the UNL *E. coli* lab and analyzed for presence of *E. coli* O157:H7 using procedures previously described (Rice et al., 2003). Briefly, each sample was vortexed for 20 sec and 1 ml of vortexed suspension was transferred to a 5 ml tube. An aliquot of the vortexed solution (100 μ l) was pipetted into a tube containing 900 μ l of PBS buffer, and mixed well. This dilution was repeated once more. An aliquot (100 μ l) of both the 10 and 100 x dilutions were then plated on CTVM SMAC and aseptically spread using the standard spread plate method. Plates were then incubated for 18-24 hr. After 18-24 hr incubation, RAMS samples were treated the same as fecal samples in order to determine presence of *E. coli* O157:H7.

Statistical Analysis

The effect of vaccine was tested by modeling the probability of detecting *E. coli* O157:H7 from feces or RAMS. Treatment differences were considered significant at $\alpha \leq 0.05$. Pre-treatment *E. coli* O157:H7 data were analyzed using a generalized linear mixed model (GLMM) with a logit link function accounting for vaccination treatment and source. Interim *E. coli* O157:H7 data were analyzed using a GLMM with a logit link function accounting for vaccination, source, and sex as fixed effects in the model and repeated measures within pen as random effects. Final *E. coli* O157:H7 data were analyzed using a GLMM with a logit link function accounting for vaccination, source, and sex as fixed effects in the model and sale period and pen as random effects.

Results

We collected a total of 3595 individual fecal or RAMS samples from the 1001 calves housed in 9 commercial feedlot pens during this study. One entire pen of cattle (n=72 hd) was not sampled at harvest because we were not notified that the feedlot had marketed that pen of

cattle. Portions of the 1001 cattle original enrolled in the study were held back from the feedlot for various reasons. Additionally, interim fecal samples were missed at each feedlot due to cattle being held in sick pens or not located in the pen of interest on the day of sampling. In total, 901 individual RAMS samples were collected from 8 pens of cattle from 2 sources and 2 commercial feedlots at harvest. Cattle on this study were harvested at five different commercial beef packing plants (2 CO, 1 KS, 2 NE).

There were no factors that explained the probability to detect *E. coli* O157:H7 in the feces of cattle. The pre-treatment probability of detecting *E. coli* O157:H7 in the feces of calves was not different ($P=0.62$) between vaccination treatments and averaged 0.40% at weaning. The odds of detecting *E. coli* O157:H7 in the feces of calves at source 2 (0.71%) were 3.63 times greater than detecting *E. coli* O157:H7 in the feces of calves at source 1 (0.00%; $P=0.053$).

The probability of detecting *E. coli* O157:H7 for samples two, three, and four averaged 0.00, 0.40, and 1.24%, respectively, and the probability to detect *E. coli* O157:H7 was not different between vaccination treatment ($OR=0.82$; $P>0.10$). During test periods two, three, and four, the probability to detect *E. coli* O157:H7 for source 1 was 0.00, 0.34, and 0.49%, respectively. For source 2, the probability to detect *E. coli* O157:H7 for test periods two, three, and four was 0.00, 0.43, and 1.87%, respectively. The probability to detect *E. coli* O157:H7 from the feces of calves was not different ($OR=3.75$; $P=0.07$) between sources for test periods two, three, and four.

The highest probability to detect *E. coli* O157:H7 was observed at harvest for both NOVAC (1.37%) and VAC (1.00%) cattle. There were no differences in the probability to detect *E. coli* O157:H7 at harvest by treatment ($OR=0.68$, $P=0.60$). Additionally, the probability to detect *E. coli* O157:H7 by source ($OR=4.20$; $P=0.28$) and gender ($OR=0.45$; $P=0.56$) was not different at harvest.

Discussion

We have demonstrated that vaccinating feedlot cattle against Type III secretory proteins of *E. coli* O157:H7 reduced the probability for cattle to shed the organism in feces by 59% (Potter et al., 2004). In a follow up study we showed dose response and herd immunity effects in response to vaccinating feedlot cattle against *E. coli* O157:H7 (Peterson et al., 2005). The current study was designed to evaluate the effects of vaccinating cattle at the ranch and at the feedlot under a commercial management environment.

We chose to use the individual animal as the experimental unit and co-mingled VAC and NOVAC cattle within pen. Research has shown when the majority of cattle are vaccinated within a pen vaccinated pen mates confer protection to non vaccinated cattle within the same pen (Peterson et al., 2005). We tried to off-set any herd immunity effects by only vaccinating 1/3 of the animals enrolled in the trial and maintaining 1/3 to 2/3 ratio of VAC to NOVAC cattle within a pen throughout the production period. However, due to the low probability to detect *E. coli* O157:H7 in the feces it is reasonable to suggest that we may not have completely limited the effects of herd immunity in this trial.

Lab personnel were blinded to treatment and we systematically allocated cattle to treatment within source and day of weaning. Lab personnel had no knowledge concerning which treatment each sample was collected from. Additionally, when enrolling cattle for the study, a systematic treatment allocation scheme was used so that every third animal through the chute at processing would be assigned to the VAC treatment. This allocation scheme should eliminate selection bias between treatments. If selection bias was to occur, it would usually occur before the study begins. A selection bias would be expected if steers selected for VAC and NOVAC treatments originated from a different management background, and therefore different opportunities for exposure. In our study all cattle were allocated to treatment within source and day of weaning, resulting in an equal distribution of source and day of weaning to vaccination treatment.

Research indicates that *E. coli* O157:H7 infection occurs in cattle before weaning, prior to entry to feedlots (Leigreid et al., 1999). More specifically, *E. coli* O157:H7 is widely dispersed at low prevalence in prefeedlot, weaned calves (Dunn et al., 2004). In our study, *E. coli* O157:H7 was isolated from feces at weaning from only one of the two sources that participated in the project, and the overall prevalence of *E. coli* O157:H7 in the feces of freshly weaned calves was very low (0.40%). However, it should be noted that the majority of the samples were collected during the winter months. Research has documented the seasonal patterns associated with *E. coli* O157:H7 infection in cattle (Garber et al., 1999; Van Donkersgoed et al., 1999; Smith et al., 2005) and similar seasonal patterns for human cases of the illness (Mead et al., 1999; Wallace et al., 2000; Chapman et al., 2001). Nevertheless, the probability to detect *E. coli* O157:H7 in feces of these freshly weaned Montana calves was low.

The observed low probability to detect *E. coli* O157:H7 observed at weaning persisted throughout the entire production period. The probability of detecting *E. coli* O157:H7 in feces of cattle on this study never reached 1.0%.

We collected RAMS samples at harvest for two primary reasons. First, it has been suggested that RAMS provide a superior sampling method when compared to collecting individual fecal samples (Rice et al., 2003) because *E. coli* O157:H7 colonizes in cattle 3-5 cm proximal to the rectoanal juncture (Naylor et al., 2003). Secondly, we wanted to collect our final sample at the point of harvest without causing considerable disruption to normal packing plant operations. Although we were able to collect RAMS samples at chain speed in each of the packing plants the cattle in this study were harvested at, recent paired testing research conducted at the University of Nebraska suggests that fecal samples are a more sensitive sampling technique compared with RAMS under the conditions this study was conducted (Moxley, 2005).

The probability to detect *E. coli* O157:H7 associated with cattle was the highest at slaughter. Other research has documented similar results (Potter et al., 2004; Peterson et al., 2005). However, the cattle on this study were sent to slaughter primarily during the summer months, when one would expect higher levels of *E. coli* O157:H7 infection in cattle (Garber et al., 1999; Van Donkersgoed et al., 1999; Smith et al., 2005).

The probability to detect *E. coli* O157:H7 in VAC was numerically lower at harvest (1.00%) compared with the probability to detect *E. coli* O157:H7 associated with NOVAC cattle (1.37%). Although not statistically significant, it would be hard to rule out vaccination as an

intervention strategy from the results of this experiment because the probability of detecting *E. coli* O157:H7 associated with cattle was so uncharacteristically low. The low sensitivity of RAMS compared with feces (Moxley, 2005) may help explain the low probability to detect *E. coli* O157:H7 at harvest associated with these Montana cattle. The results of paired testing comparing RAMS and feces at harvest suggest that fecal samples are more sensitive (Moxley, 2005). Given the results of that study, it is reasonable to suggest that the probability to detect *E. coli* O157:H7 at harvest would have been higher had we collected individual fecal samples instead of RAMS. When the probability to detect *E. coli* O157:H7 in the feces is high, research has shown vaccine to be 59% and 70% effective compared with non vaccinated cattle (Potter et al., 2004; Peterson et al., 2005).

Implications

Because of the low prevalence of *E. coli* O157:H7 shedding throughout the entire production period, this study could not determine the effect of vaccination as a pre-harvest food safety intervention strategy. Additional research should evaluate pre-harvest interventions with methodology to detect colonization in the individual animal or assign treatments to the pen.

Literature Cited

- Callaway, T. R. R. C. Anderson, T.S. Edrington, K.J. Genovese, K.M. Bischoff, T.L. Pool, 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.
- Chapman, P.A., A.T. Cerdan-Malo, M. Ellin, R. Ashton, and M. Harkin. 2001. *Escherichia coli* O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK. *International Journal of Food Microbiology.* 64:139-150.
- Dunn, J.R., J.E. Keen, R. Del Vecchio, T.E. Wittum, and A. Thompson. 2004. *Escherichia coli* O157:H7 in a cohort of weaned, preconditioned range beef calves. *J. Food Prot.* 67:2391-2396.
- Garber, L., S. Wells, L. Schroeder-Tucker, and K. Ferris. 1999. Factors associated with fecal shedding of verotoxin-producing *Escherichia coli* O157 on dairy farms. *J. Food Prot.* 62:307-312.
- Hancock, D. D., T. E. Besser, D. H. Rice, D. E. Herriott, and P. I. Tarr. 1997. A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. *Epidemiol. Infect.* 118:193-195.
- Laegreid, W.W., R.O.Elder and J.E. Keen. 1999. Prevalence of *Escherichia coli* O157:H7 in range beef calves at weaning. *Epidemiol. Infect.* 123:291-298.
- Mead, P.S., L. Slutsker, V. Dietz, L.F. McCraig, J.S. Bresee, C. Shapiro, P.M. Griffin, and R.V. Tauxe. 1999. Food-related illness and death in the United States. *Emerging and Infectious Diseases* 5:1-35.
- Moxley, R.A. 2005. Personnel communication.

- Naylor, S. W., J. Christopher Low, T. E. Besser, A. Mahajan, G. J. Gunn, M. C. Pearce, I. J. McKendrick, D. G. E. 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.
- Peterson, R.E., T. J. Klopfenstein, R. A. Moxley, G. E. Erickson, S. Hinkley, D. Rogan and D. R. Smith. 2005. Dose response and herd immunity of beef feedlot cattle to a vaccine against *Escherichia coli* O157:H7. In preparation.
- 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.
- Rice, D. H., H. Q. Sheng, S. A. Wynia, and C. J. Hovde. 2003. Recto anal 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.
- Smith, D.R., M. Blackford, S. Younts, R. Moxley, J. Grey, L. Hungerford, T. Milton, and T. 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.
- 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 Pathogens and Disease.* 2(1):50-60.
- Van Donkersgoed, J., T. Graham, and V. Gannon. 1999. The prevalence of verotoxins, *Escherichia coli* O157:H7, and *Salmonella* in the feces and rumen of cattle at processing. *Can Vet. J.* 40:332-340.
- Wallace, D.J., T. Van Gilder, S. Shallow, T. Fiorentino, S.D. Seagler, K.E. Smith, B. Shiferaw, R. Etzel, W.E. Garthright and F.J. Angulo. 2000. Incidence of foodborne illnesses reported by the foodborne diseases active surveillance network (FoodNet)-1997. FoodNet Working Group. *J. Food Prot.* 63:807-809.