

VACCINATION AS AN INTERVENTION STRATEGY FOR REDUCTION OF *ESCHERICHIA COLI* O157:H7 IN CATTLE FECES¹

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ABSTRACT: Three hundred sixty-seven recently weaned steers were used in a growing (45 d) and finishing (189 d) experiment to determine if vaccination with an experimental *E. coli* O157:H7 vaccine would reduce fecal shedding and elevate antibody titers. Treatments (Trt.) compared were: Group 1) received two doses of the *E. coli* vaccine in the growing phase only; Group 2) received two doses of the *E. coli* vaccine during the growing phase and a third dose on d 100 of the finishing phase; Group 3) received two doses of the *E. coli* vaccine; one on d 21 of the finishing phase and a second dose on d 100 of the finishing phase. Treatment group 4 served as the control (no vaccination). On d 0 and 45 of the growing phase and d 21, 100 and 162 of the finishing phase, fecal grab and venous blood samples were collected. Fecal samples were analyzed for *E. coli* O157:H7 by Food Safety Net Services. Blood samples were sent to Fort Dodge Laboratories for analysis of antibody titers against *E. coli* O157:H7. Prevalence of fecal *E. coli* O157:H7 was not different ($P>0.05$) at any sampling period among treatment groups. The initial and final prevalence rates during the feedlot phase for Trt. 1 were one and 10%; Trt. 2 were 3 and 8%; Trt. 3 were 3 and 17% and Trt. 4 (Control) were 5 and 8%, respectively. Serum titers did show an elevated immune response. During the 45 d growing phase, the vaccine increased ($P<0.001$) blood titers from an average of 62 to 2,217. During the finishing period vaccinated calves had higher titers compared to control calves (avg. of Trt 1, 2, 3 vs. Trt 4 ($P<0.05$)). Vaccination during the 45 d growing phase and then again on d 100 of the finishing phase (Trt. 2) resulted in the greatest ($P=0.012$) pre-slaughter titer level, but did not affect ($P>0.05$) *E. coli* O157:H7 prevalence. At harvest, Trt 1. steer's titre levels had returned to near pre-vaccination levels indicating that the immune levels did decline over time. Although an immune response was generated by this vaccine, the limited number of animals shedding *E. coli* O157:H7 warrants additional research in calves with higher levels of shedding.

Key words: *E. coli* O157:H7; Cattle; Vaccination

Introduction

During the past few years, significant investment ¹dollars have been allocated towards research investigating intervention strategies to reduce contamination of beef carcasses with *E. coli* O157:H7. Both pre- and post-harvest methods have been evaluated. Post-harvest interventions have dealt with controlling foodborne pathogens in a "Multiple Hurdle Approach" including washing cattle prior to harvest followed by pre-evisceration wash with water and an organic acid, steam vacuuming, hot water wash and proper carcass chilling. Recently more emphasis has been placed on attacking pathogens at their source. These "pre-harvest" methods

¹ Research supported by National Cattlemen's Beef Association through their \$1.00 Checkoff and Bair Ranch Foundation

investigated have included dietary changes, direct fed microbials, water treatments and the addition of antibiotics to rations prior to slaughter. The direct fed microbials are currently the only products commercially available claiming to reduce *E. coli* O157:H7 in live cattle. Many of the attempted pre-harvest interventions however, have met with challenges including ethics, low overall effectiveness and/or production feasibility.

Initial experiments have developed hypothesis towards the possibility of vaccination against enterohemorrhagic *E. coli* (McKee et al. 1995, Dean-Nystrom et al. 1998 and Cornick et al. 2002). These experiments revealed the basic mechanisms by which the pathogenic *E. coli* attach to the epithelial cells of the gastrointestinal tract. Immunity was first used to protect livestock (pigs; Dean-Nystrom et al., 2002) from asymptomatic infection with enterohemorrhagic *E. coli*. Two vaccines have been developed and are currently awaiting government approval for use in beef cattle. One of these vaccines has been successfully tested (Potter et al. 2004; Peterson et al. 2005).

The objective of this study was to evaluate the effectiveness of vaccinating live cattle prior to harvest, as a method of pre-harvest reduction in the shedding of *E. coli* O157:H7.

Materials and Methods

Three hundred sixty-seven steers were transferred from a 45 d pre-conditioning experiment where 183 of the 367 steers had received vaccination against *E. coli* O157:H7 on d 0 and 21 of the 45 d pre-conditioning period.

Pre-conditioning Experiment Background. Three hundred eighty-nine heifers and three hundred sixty-seven steers were selected from a single herd in central MT. The selected herd was composed of Angus and Simmental genetics and primarily calves in February and March. The calves selected were removed randomly from their mothers (weaned) over a three week period in October of 2003 with approximately one third of the calves being weaned each week. On the day of weaning, calves were transported from pasture to a receiving yard consisting of six, 150 hd pens and an appropriate cattle handling facility. Upon arrival at the receiving yard, calves were sorted by gender (steers vs. heifers), placed in holding pens and then processed separately. At processing all calves received an individual electronic identification tag, and an individual panel tag each with a unique number and a single dose of: 1) Nasalgen IP (Schering Plough); 2) Pyramid 4 + Presponse (Fort Dodge Animal Health); 3) Vision 7 + Somnus (Intervet); and 4) Cydectin pour-on (Fort Dodge Animal Health). The Pyramid 4 + Presponse and Vision 7 + Somnus were administered subcutaneously on the right side of the animal in the lateral neck and boosted on d 21.

A systematic randomization scheme was utilized so that every other animal through the chute would be given a dose of the experimental *Escherichia coli* vaccination (E-vac) designed to prevent the attachment of *Escherichia coli* O157:H7 to the intestinal wall of cattle. The *E. coli* vaccine was administered sub-cutaneously in the left neck at a dose of 2 ml / head and was boosted with a second dose on d 21. Following processing on d 0, calves were systematically allocated to pens as they exited the chute; resulting in pens with a final disposition of approximately 50% E-vac and 50% control within gender. Calves were fed a grass hay based diet combined with a protein supplement that contained additional minerals and vitamins. The

response variables measured were initial weight (d 0), final weight (d 45), average daily weight gain, blood antibody titers (d 0 and 45) and individual fecal prevalence of *E. coli* O157:H7 (d 0 and 45).

Finishing Phase Experimental Design. Only steers (n = 367) from the pre-conditioning experiment were utilized in the finishing experiment. Prior to shipping from the pre-conditioning facility to the feedlot, steers were individually weighed (feedlot initial weight), treated again for internal and external parasites with Cydectin pour-on, boosted with Pyramid 4 + Prespense and randomly sorted four ways. The randomization for the sort was conducted by sorting steers only (in a database) by previously vaccinates and previously controls. All sorted steers were then assigned a random number using Microsoft Excel's random number generator. Steers which were previously controls were sorted by random number in ascending order and steers from previously vaccinates were sorted by random number in ascending order. The first 92 steers from the previously vaccinated group were assigned into finishing group 1; the second 92 steers from the previously vaccinated group were assigned into finishing group 2; the first 91 steers from the previously control group were assigned into finishing group 3 and; the remaining 92 steers from the previously control group were assigned into finishing group 4. Four feedlot pens were utilized and pens were assigned by alternating pen numbers 1 – 4 throughout the list of steers in each finishing group until all steers were assigned to a pen. The pen allotment was designed to provide for equal finishing group representation in each pen.

At shipping to the feedlot each of the four sorts were placed on a separate truck and upon feedlot arrival each truck was placed in a separate pen as was previously assigned in the randomization. All steers were fed a similar high energy ration throughout the experiment, were individually weighed on d 0, 21, 100 and prior to harvest on d 162 and given a Synovex Choice implant on d 21 and 100.

Treatments were assigned to each finishing group as follows (Table 1.): Group 1 received two doses of the *E. coli* vaccine in the pre-conditioning phase only; Group 2 received two doses of the *E. coli* vaccine during the pre-conditioning phase and a third on d 100 of the finishing phase; Group 3 received two doses of the *E. coli* vaccine one on d 21 and one on d 100 of the finishing phase; and Group 4 never received any *E. coli* vaccine (Control).

Sample Collection. On d 0 and 45 of the pre-conditioning experiment and on d 21, 100 and 162 of the finishing phase a fecal grab and venous blood sample was collected from all calves. The fecal samples were collected aseptically via rectal palpation using a new OB glove for each animal and then placing approximately 50 g of feces into a new screw top cup (Fisher Scientific). Cups were labeled with barcodes and then placed in shipping containers with ice packs to prevent temperature abuse. Samples were shipped overnight to Food Safety Net Services (San Antonio, TX) for analysis.

Recovery of E. coli O157:H7. Samples were analyzed for the presence of *E. coli* O157 using the previously described (Barkocy-Gallagher et al., 2002) MARC MRU method. The method involved enrichment in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.), immunomagnetic separation, and selective plating.

Antibody Titre Analysis. Ten milliliters of whole blood were collected from the jugular or tail vein. Blood samples were placed on ice and transported to the Montana State nutrition laboratory, where they were centrifuged (2,000 rpm for 20 min) and the serum separated for analysis. The analysis of serum for antibody titres *E. coli* O157:H7 were conducted by Fort Dodge Animal Health Laboratories, Ft. Dodge, Iowa. Serum titres against *E. coli* O157:H7 were analyzed using an ELISA (enzyme-linked immunosorbent assay; Widiasih et al. 2004). Samples were dissolved at 1mg/ml in 1xPBS (phosphate buffered saline) solution and diluted to 1:100 with the 1xPBS for a concentration of 10µg/ml (Cray et al. 1995). Microtiter plates were coated with diluted LPS solution. The samples were placed through a series of similar steps of washing and coating the plates. Results were then read by a spectrophotometer at 405 nm, approximately 10 – 30 min after the addition of a substrate solution. The results are expressed according to the final dilution factor on the plate. (Fort Dodge Animal Health, 2004).

Statistical Analysis. The odds of a vaccinated animal shedding *E. coli* O157:H7 was compared to that of unvaccinated control cattle, accounting for repeated measures and pen using the GENMOD procedure of SAS (SAS Inst., Inc., Cary, NC). Odds ratios (OR) and their 95% confidence limits are reported. Antibody titre data was evaluated using the MIXED procedure of SAS accounting for repeated measures and pen. Feedlot performance was evaluated using the MIXED procedure of SAS accounting for pen.

Results and Discussion

E. coli O157:H7. In total, *E. coli* O157:H7 was recovered from 101 of 1,835 (5.5%) fecal samples. Table 1. shows the odds ratios (odds of a vaccinated animal shedding *E. coli* O157:H7 compared with controls), the 95% confidence limits and the probability that differences were due to vaccination. The fifth sampling period was the only period used in the overall analysis due to it being the only period following all vaccination as was designed. Results presented in Figure 1 show the prevalence of control cattle that were at or below the levels of all vaccinated groups resulting in unfavorable odds ratios relative to vaccine efficacy. The high level of variation in the 95% confidence limits of the odds ratios indicates that more post vaccination samplings would have been beneficial. These design flaw conclusions are substantiated by the results reported by Peterson et al., (2005) where four post treatment test periods were used to evaluate vaccine efficacy and resulted in a significant reduction in shedding. Moreover, point in time estimates of *E. coli* O157:H7 may misrepresent the true prevalence of *E. coli* O157:H7 associated with cattle (Smith et al., 2005). Additionally, in a similar experimental design where 75% of cattle in a pen received vaccination, Peterson et al. (2005) reported that unvaccinated cattle commingled with vaccinated cattle were less likely to shed *E. coli* O157:H7 than cattle in pens not receiving vaccine (external controls). Therefore, the low probability to detect *E. coli* O157:H7 in the current experiment may be a result of herd immunity. Never the less these data suggest ($P = 0.2742$) that the vaccine was unsuccessful in reducing the prevalence of *E. coli* O157:H7 in the feces of cattle under the conditions of this experiment.

Table 1. Experimental design and odds of detecting *E. coli* O157:H7 in cattle feces at harvest with 95% confidence limits by group.

Day of Trial / Phase	Treatment Groups			
	1	2	3	4
- 45 / Pre-Conditioning	Sampled / Vaccinated	Sampled / Vaccinated	Sampled / Control	Sampled / Control
- 24 / Pre-Conditioning	Vaccinated	Vaccinated	Control	Control
0 / Finishing	Sampled	Sampled	Sampled	Sampled
Feedlot Arrival				
21 / Finishing	Sampled / Control	Sampled / Control	Sampled / Vaccinated	Sampled / Control
100 / Finishing	Sampled / Control	Sampled / Vaccinated	Sampled / Vaccinated	Sampled / Control
183 / Finishing	Sampled	Sampled	Sampled	Sampled
Harvest				
	Odds Ratio	95% Confidence Limits		P-value
		Lower	Upper	
Treatment Group				
1	1.29	0.46	3.61	0.2742
2	1.02	0.34	3.05	
3	2.37	0.92	6.13	
4	1.00	1.00	1.00	

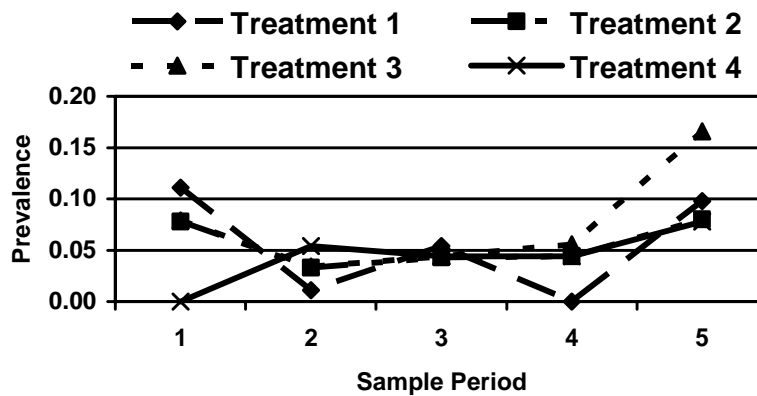


Figure 1. The proportion of steers shedding *E. coli* O157:H7 by sample period and treatment group. Where: Treatment 1 = vaccination during pre-conditioning only, Treatment 2 = vaccination during pre-conditioning and on d 100 of the finishing phase, Treatment 3 = vaccination on d 21 and 100 of the finishing phase and Treatment 4 = controls (no vaccination).

Antibody Titers. Venous blood samples were collected on d 0 and 45 of the pre-conditioning experiment and on d 21, 100 and 162 of the finishing phase. Blood titer levels were

elevated after vaccination ($P < 0.0001$; Figure 2). There were no differences in pre-vaccination blood titer levels ($P = 0.25$). Vaccination during the pre-conditioning phase (groups 1 and 2) did increase blood titers ($P < 0.0001$) compared with controls (groups 3 and 4). The blood titer levels of steers that were vaccinated only during the pre-conditioning phase (group 1) returned to approximately pre-vaccination levels by the fourth sampling period indicating that effects of vaccination may have a defined effectiveness. The group that maintained the highest overall immune response were those vaccinated during pre-conditioning and again on d 100 of the finishing phase (Group 2). The steady decline of titer levels by Groups 1 and 2 following test period 2 indicate that the feedlot dose may have been delayed in Group 2 and may have been more beneficial at d 21. This observation if substantiated could prove beneficial to the overall acceptance of the vaccine in production systems if vaccination during pre-conditioning and again upon feedlot entry proved to be the most efficacious, however further experiments are required to confirm these observations. The Group 3 steers were only vaccinated on d 21 and 100 of the finishing phase and overall did not respond as well as those steers vaccinated during the pre-conditioning phase. At harvest all vaccinated groups continued to have higher ($P \leq 0.02$) titer levels compared with controls, but these effects on shedding of *E. coli* O157:H7 were limited due to low prevalence.

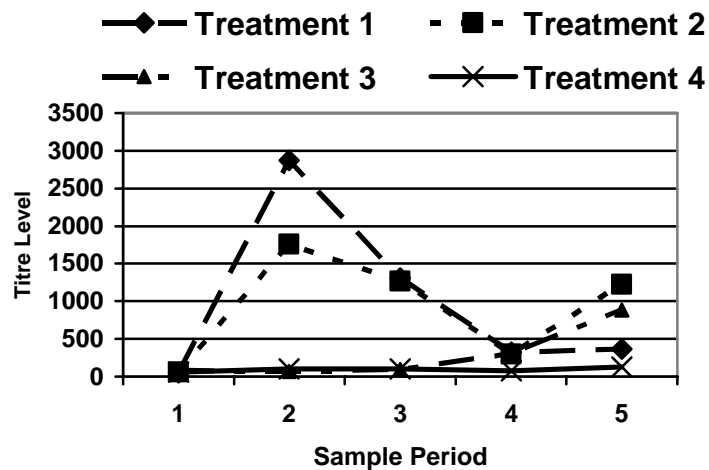


Figure 2. *E. coli* O157:H7 antibody titre levels by sample period and treatment group (Vaccination effect - $P = 0.0001$; Interaction effect - $P = 0.0001$). Where: Treatment 1 = vaccination during pre-conditioning only, Treatment 2 = vaccination during pre-conditioning and on d 100 of the finishing phase, Treatment 3 = vaccination on d 21 and 100 of the finishing phase and Treatment 4 = controls (no vaccination).

Feedlot Performance. Least squares means for finishing performance measures are presented in Table 2. There were no differences ($P > 0.10$) in finishing performance for steers receiving the *E. coli* vaccine in three different regimes. These data suggest vaccinating cattle against Type III secretory proteins of enterohemorrhagic *Escherichia coli* will have no considerable impact on finishing performance.

Table 2. Effects of Vaccination against *E. coli* O157:H7 on finishing performance of steers

Item	Treatment Group ^a				SEM ^b	VAC ^c
	1	2	3	4		
Steers	92	92	91	92		
Initial BW, kg	274	264	270	273	3.28	0.16
Final BW, kg	525	511	512	520	5.38	0.19
Daily gain, kg	1.55	1.52	1.49	1.53	0.02	0.48

^a1=vaccination during pre-conditioning only; 2=vaccination during pre-conditioning and on d 100 of finishing; 3=vaccination on d 21 and 100 of the finishing phase; 4=no vaccination (control)

^bStandard error of least squares means

^cMain effect of vaccination treatment

Implications

In conclusion an effect of vaccination on *Escherichia coli* O157:H7 shedding was not detected, however, elevated blood titres against *Escherichia coli* O157:H7 indicate that an immune response was generated. The limited number of animals found positive for *Escherichia coli* O157:H7 in this experiment coupled with limited post vaccination test periods and an elevated immune system warrant additional research of this vaccine. The blood titer levels may be beneficial in the development of further experiments targeting practical use of this vaccine.

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