

THE USE OF AN EXPERIMENTAL VACCINE IN GESTATING BEEF COWS TO REDUCE THE SHEDDING OF *E. COLI* O157:H7 IN THE NEWBORN CALF¹

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ABSTRACT: One hundred and thirty seven beef cows in the last trimester of pregnancy were used to determine if vaccinating against *E. coli* O157:H7 would increase antibody titers in the serum and also result in the transfer these antibodies to the neonatal calf. Cows calved within a 30 d period starting the end of March. Seventy-one cows were vaccinated with an experimental vaccine (Ft. Dodge Animal Health) and then co-mingled with 66 non-vaccinated control cows. Cows were vaccinated approximately 30 d prior to parturition. Cow fecal and venous blood samples were collected at trial initiation and again at ~14 d after parturition. Calf feces and serum were collected at ~14 d after parturition and again 60 d later at branding. The serum was analyzed for antibody titers to *E. coli* O157:H7 by an ELISA protocol. The prevalence of *E. coli* O157:H7 in feces was determined by collecting a sample on a RAMS swab and submitting all samples to a commercial laboratory for analysis (Food Safety Net, San Antonio, TX). Initial cow antibody titers to O157:H7 were not different ($P=0.50$) between treatments but by parturition the antibody titers for O157:H7 in vaccinated cows were ten times higher ($P<.001$) than for control cows (917 vs. 83). The titers for calves suckling vaccinated cows were similarly higher ($P<0.001$) than control calves (1485 vs.135) at ~14 d after calving. By 60 d, titer levels were still higher ($P<0.001$) for calves suckling vaccinated cows but appeared to decline slightly compared to the titers near parturition. Initial fecal O157:H7 concentrations for cows were negative for both treatments and remained low through parturition. There were no differences in fecal O157:H7 at 60 d post partum among calves; less than 5% of all calves were shedding. Results of this experiment suggest that vaccination of gestating cows for *E. coli* O157:H7 resulted in elevated antibody titers compared to control cows and these antibodies were transferred to the calf.

Key Words: *E. coli* O157:H7, Antibodies, Colostrum, Cattle

Introduction

Escherichia coli O157:H7 is one of several food born pathogens in today's beef industry. *Escherichia coli* is commonly associated with hemorrhagic colitis and has few therapeutic alternatives and poor prognoses for severe sequelae and has led to intensive research targeting elimination or reduction of fecal shedding (Kudva et al., 1999; Lahti et al., 2002). Post-harvest interventions have dealt with controlling food borne pathogens in a "multiple hurdle approach", in order to reduce pathogen contamination in primarily ground beef. More emphasis has been placed on carcasses contaminated by hides and feces; primarily occurring during the slaughter process. The Federal Register (2002) reported: (a) that five multi-state studies showed the apparent prevalence in breeding herds containing one or more infected with *E. coli* O157.H7

were 24%, 61%, 75%, 87% and 100%; (b) three multi-state studies reported the apparent prevalence in feedlots containing one or more cattle infected with *E. coli* O157:H7 was 63%, 100% and 100%; and (c) Elder, et al. (2000) found that 28% of fecal samples from fed-cattle were positive for *E. coli* O157:H7. Similarly, Smith, et al. (2001) reported that the fecal prevalence of *E. coli* O157:H7 in fed cattle was 23%.

As a result, the beef industry has focused more on pre-harvest methods to reduce the incidence of *E. coli* O157:H7 (Elder et al., 2000; Barkocy-Gallagher et al., 2001). Rice et al. (2003) found that the introduction of one animal, which was shedding *E. coli* O157:H7 at high rates quickly infected other cohorts. The beef industry is researching potential interventions at various stages of production. Multiple hurdle interventions have had a positive impact on significantly reducing food born pathogens entering the food supply if the levels of these bacteria allowed into the packing plant were limited (Elder et al., 2000). Recently, Nystrom (August 2004 Agricultural Research magazine, p. 14-15) reported that an experimental vaccine interfered with *E. coli* colonization within the gut of the calf.

The objectives of this research were to determine if an experimental *E. coli* O157:H7 vaccine given to the gestating beef cow would result in transferring antibodies to the newborn calf via colostrum.

Material and Methods

Cow Management. The location of this experiment was the USDA-ARS-LARRL Research Station at Miles City (Ft. Keogh), MT. One hundred thirty seven gestating beef cows in the last trimester of pregnancy were used for this study. Cows were weighed and randomly assigned to the two treatments (Control vs. Vaccinated) approximately 30 d prior to the start of expected calving. The experimental protocol and treatments are described in Table 1. Cows were previously synchronized and artificially inseminated and were expected to calve within a thirty day period starting the end of March. The average weight of the cows approximately 30 d prior to calving was ~567 kg. Seventy-one of the gestating cows were vaccinated with an experimental vaccine, (developed by Fort Dodge Animal Health Laboratories) which was designed to prevent the attachment of *E. coli* O157 to the intestinal wall (Cornick et al., 2002), and then commingled with 66 non-vaccinated control cows. Cows remained in calving pens for the first 15 d and then were placed on native range pasture after parturition.

Sample Collection and Analysis. The cows and calves had 10 ml of whole blood collected from the jugular or tail vein. Blood samples were placed on ice and transported to the laboratory, where they were centrifuged (2,000 rpm for 20 min) and the serum separated for analysis. The analyses of serum for antibody titers *E. coli* O157:H7 were conducted by Fort Dodge Animal Health Laboratories, Ft. Dodge, Iowa. Serum titers against O157:H7 were analyzed using an ELISA (enzyme-linked immunosorbent assay; Widiastih et al., 2004). Samples were dissolved at 1 mg/ml in 1xPBS (phosphate-buffered saline) solution and diluted to 1:100 with the 1xPBS for a concentration of 10 µg/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 were expressed according to the final dilution factor on the plate. (Fort Dodge Animal Health, 2004)

Recovery of E. coli O157:H7. The prevalence of *E. coli* O157:H7 in feces from cows and newborn calves was determined by collecting a fecal sample via a rectoanal mucosal swab (RAMS; Rice et al., 2003). The swab sample was collected from cows on d-30 (before parturition) and 9-14 days after parturition. The calves were also swabbed at approximately 14 d after parturition and again at ~60 d after birth (branding). Fecal samples were obtained by inserting the sterile foam-tipped applicator approximately 2 to 8 cm (Grauke et al., 2002; Naylor et al., 2004) into the anus, and by using a rapid “in and out” motion, the entire mucosal surface of the rectoanal junction was swabbed (Rice et al. 2003). The swab samples were submitted to a commercial laboratory (Food Safety Net, San Antonio, TX) for analysis for *E. coli* O157 (Barkocy-Gallagher et al., 2002).

Statistical Analysis. Data were analyzed using the GLM procedures of SAS (2000). A comparison of control and vaccinated animals for fecal rates were measured by Chi Square analyses. The titer levels were analyzed by one way analysis of variance.

Results and Discussion

The objective of this study was to vaccinate gestating cows with an experimental vaccine to prevent colonization and subsequent shedding of *E. coli* O157:H7 by the cow and the calf.

Initial cow titer levels (prior to vaccination) showed no differences ($P < 0.50$) between treatments for O157:H7 antibody. Similarly, there were no differences in fecal shedding rates of O157:H7 on - 30 d. However, at ~ 14 d after calving, the vaccinated cows had eleven times higher titer levels than that of the control group (917 vs. 83). The fecal samples for the second sample again showed no difference between treatment groups.

Initial titer levels of the calves showed ten fold difference ($P < 0.001$) between treatments (135 vs. 1485 for Control calves vs. calves which suckled vaccinated cows respectively). Titer levels at branding (~60 d) showed a slight decrease in titer levels, however calves from vaccinated cows were still seven times higher ($P < 0.001$) than the control calves.

Fecal samples for the calves were not different with no positive samples detected at 14 d. Similarly, no differences were detected at branding (avg. 3%).

Due to the low number of animals shedding *E. coli* O157:H7 differences were undetectable. This may have been due to the cool spring weather, which could prevent *E. coli* O157:H7 from shedding in greater numbers (Barkocy-Gallagher et al., 2003).

Previous studies have revealed that *E. coli* O157:H7 was pathogenic for neonatal calves (Dean-Nystrom et al., 1997; Widiasih et al., 2004). These studies showed that calves less than 36 h old developed diarrhea and enterocolitis with attaching and effacing lesions. Other, studies have shown that weaned (ruminating) calves dosed with *E. coli* O157:H7 remained clinically healthy, with no evidence of fever or diarrhea (Zhao et al., 1998), compared to the newborn calf.

Implications

A partial reduction in the shedding of *E. coli* O157:H7 will be useful in controlling the contamination of meat. Results of this experiment showed that vaccinating the gestating cow with an experimental vaccine against *Escherichia coli* O157:H7 resulted in increased both cow and suckling calf antibody titers.

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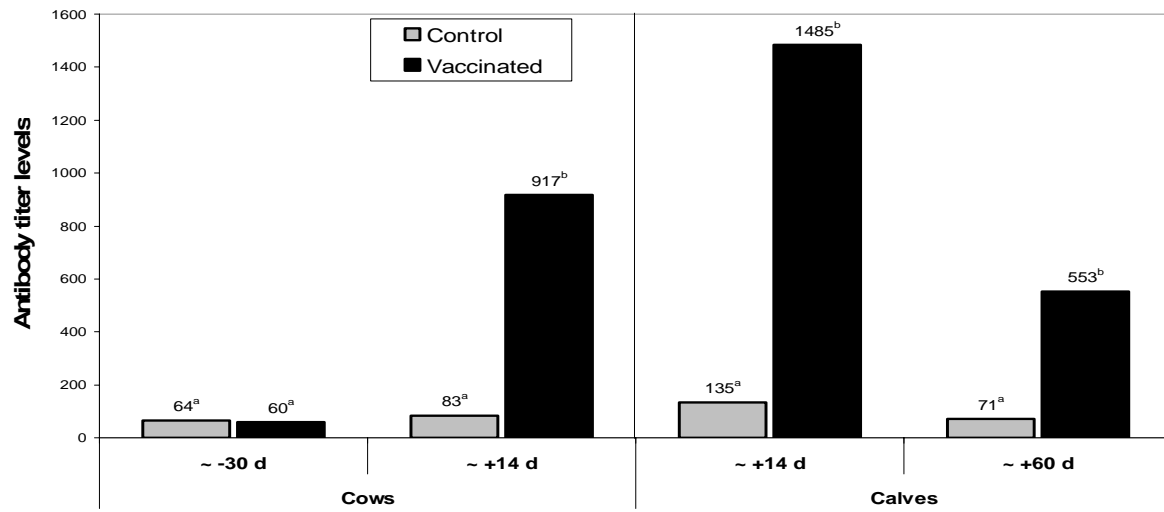
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Table 1. Experimental design and samples collected from cows and calves to determine if vaccinating gestating cows with an experimental *E. coli* O157:H7 vaccine would transfer antibodies to the newborn calf via the colostrums

Treatment:	Samples collected:	Samples analyzed for:
66 Pregnant control cows (not vaccinated)	Cows-Blood and fecal samples at start of trial and at calving. Calves- Blood and fecal samples within 14 days of calving and again at branding (~ 60 days)	Blood: <i>E.coli</i> O157:H7 antibody titers
71 Pregnant vaccinated cows (vaccinated 30 d and 14d prior to calving)		Feces: prevalence of <i>E.coli</i> O157:H7 shedding and enumeration of positive samples

Figure 1. Effect of vaccinating gestating cows with an experimental vaccine against *E. coli* O157:H7 on antibody titers and subsequent titer levels in the neonatal calf.



^{a, b} Unlike means within a time period are different (P<0.05)