A clinical trial of vaccination and direct-fed microbials to control *Escherichia coli* o157:h7 in feedlot cattle

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Summary
We conducted a clinical trial to test the effect of vaccination (VAC) and feeding a direct fed microbial (DFM) product on the proportion of steers shedding *Escherichia coli* O157:H7 in feces in conditions of Midwest US beef feedyards. The prevalence of *E. coli* O157:H7 shedding observed was typical of that previously observed in both research (4) and commercial (5) feedyards.

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Materials and Methods

Study design Three hundred eighty-four steers were allocated to 3 weight blocks, and assigned randomly to 16 pens within each block. The finishing diet of 54.5% HMC, 35% WCGF, 5% corn silage, 2.5% alfalfa hay, 2% supplement, and 1% water was identical for all treatments and contained a minimum of 12.5% CP, 0.7% calcium, 0.65% potassium, and 0.3% phosphorus. DFM and VAC treatments were randomly allocated to 4 pens within each of the 3 weight blocks in a 2 x 2 factorial design. The VAC, designed to immunize against specific proteins of *E. coli* O157:H7 attachment, was administered 3 times at 3 week intervals to cattle within assigned pens beginning 9, 17, and 23 days after cattle arrived in the feedyard for blocks 1, 2, and 3 respectively. A *Lactobacillus acidophilus* DFM product was fed with the ration continuously from d-24 of the trial. Samples of rectal feces were collected for bacterial culture. Each block was sampled every three weeks for the entire (May-September) feeding period resulting in 1 pre-treatment and 5 test-period samplings.

Preparation of vaccine. Supernatant proteins containing type III secreted proteins (Esps and Tir) were prepared from *E. coli* O157:H7 as described (1) and formulated with the adjuvant VSA3 (2) such that the protein concentration was 25 :g/ml. One dose of vaccine consisted of 2 ml administered by the subcutaneous route in the neck with a 1.9 cm needle.

Direct fed microbial. The DFM product consisted of *L. acidophilus* strain NPC747 (Nutrition Physiology Corp., Indianapolis, IN). For those animals in DFM treatment groups, the DFM was added to the water component of the diet. In this case, the DFM was mixed with water, applied to the feed truck mixing box, and fed at

a rate of 1 X 10^9 colony-forming units per steer per day from day-24 until the completion of the study. Animals in non-DFM treatment groups received the 1% water component in the ration with no added DFM. Two separate trucks were used to mix and distribute feed to the cattle (one to deliver feed with DFM and the other feed without DFM).

Fecal culture for O157:H7 Fecal samples were cultured for *E. coli* O157:H7 as previously described (5) with modifications. Ten g fecal samples were incubated 6 h in 90 ml Gram-negative (GN) broth containing 8 :g/ml vancomycin, 0.05 :g/ml cefixime, and 10 :g/ml cefsulodin. One ml of this culture was subjected to O157 immunomagnetic separation (Dynal, Lake Success, NY), and 20 :l of the final washed bead-bacteria mixture was spread onto sorbitol-MacConkey plates containing cefixime (0.05 :g/ml) and potassium tellurite (2.5 :g/ml; CT-SMAC) and cultured overnight. One to three isolated sorbitol-nonfermenting colonies were subcultured for isolation on CT-SMAC plates, and an individual sorbitol-nonfermenting colony from each subculture plate was inoculated onto both MacConkey and Fluorocult (EM Science, Gibbstown, NJ) agars. Isolates that fermented lactose but not sorbitol within 24 h and were negative for ∃-glucuronidase activity were streaked for isolation on blood agar plates. Following overnight incubation, one colony per isolate from a blood agar plate was tested for O157 and H7 antigens by latex agglutination (Remel, Lenexa, KS). One to three *E. coli* O157 latex agglutination-positive isolates per culture were stocked and further tested by multiplex PCR.

Multiplex PCR Isolates that were positive for *E. coli* O157 antigen by latex agglutination (regardless of H7 results) were tested in a five primer-pair multiplex polymerase chain reaction (PCR) assay that detected genes for *E. coli* O157 ($rfbE_{O157:H7}$), H7 ($fliC_{h7}$), Shigatoxins 1 (stx_1) and 2 (stx_2), and *E. coli* O157 intimin (eae_{O157}). For PCR amplification, cells from an individual colony on blood agar were transferred into a 1.5 ml Eppendorf tube containing 50 :l sterile nuclease-free water and incubated at 100ºC for 6 minutes. The tube was then centrifuged at 2000 rpm for 2 min in a microfuge and the supernatant used as template. For each assay, template prepared from *E. coli* O157:H7 strain EDL933 (ATCC #43895) was used as a positive control, and one lacking any template was used as a negative control. The PCR was conducted in a 50-:l reaction mixture containing 2 :l template DNA, 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 200 :M of each dNTP (Promega, Madison WI), and 1.5 U *Taq* DNA polymerase (Promega). Oligonucleotide primers were used at the following concentrations: 0.5 :M for those targeting the eae_{O157}, stx_1, stx_2, and $rfbE_{O157:H7}$ genes, and 0.2 :M for those targeting the $fliC_{h7}$ gene. Samples were amplified in a PTC-200 DNA engine (MJ Research Inc., Incline Village, NV) under the following cycling conditions. Conditions included an initial denaturation step of 5 minutes at 94ºC, followed by 34 cycles with denaturing at 94ºC for 45 sec, annealing at 50ºC for 30 sec, and extension at 72ºC for 90 sec. The final cycle consisted of extension for 10 min at 72ºC, ramping to 50ºC at 0.1ºC/sec, holding 50ºC for 5 min, ramping to 4ºC at 0.2ºC/sec, and soaking at 4ºC. Samples were analyzed by standard agarose gel electrophoresis (10 :l per sample) on a 1.3% gel, stained with ethidium bromide, and then visualized under UV illumination. DNA molecular size standards (PCR marker, Promega, Madison WI) and positive and negative controls were included in every run. Isolates with a positive PCR reaction for both $rfbE_{O157:H7}$

and *fliC*$_{h7}$, and at least one of the virulence factor genes were designated enterohemorrhagic *E. coli* O157:H7 (3). Using these criteria allows for detection of *E. coli* O157:H7 strains that have lost *stx* or *eae* genes, and also circumvents the ambiguity associated with the non-motile designation (3).

Statistical analysis Outcome measures were pen-level performance and the proportion of animals per pen culture-positive for *E. coli* O157:H7. Feedlot performance and *E. coli* O157:H7 outcomes were analyzed using mixed modeling procedures (SAS PROC MIXED) accounting for repeated sampling for O157:H7.

Results

Treatment groups did not differ in performance (ADG, DMI, gain to feed, marbling score, fat thickness, or yield grade). The pre-treatment prevalence of *E. coli* O157:H7 averaged 31%, and did not differ significantly between treatments (p=0.19). The average proportion of cattle shedding *E. coli* O157:H7 differed (p=0.01) over the 5 test-periods (18.5%, 10.2%, 11.7%, 4.4%, and 18.8%, respectively); however, no interaction was observed between treatments or between treatment and time. The average proportion of cattle shedding *E. coli* O157:H7 for treatments of control, DFM alone, VAC alone, and DFM with VAC were 21.3%, 13.3%, 8.8%, and 7.7%, respectively. Adjusting for the effect of DFM and block, the proportion of cattle shedding O157:H7 in VAC treated pens was significantly less than non-VAC pens (p=0.03). The decrease in prevalence observed in DFM treated pens was not significant (p=0.21)

Discussion

This clinical trial was conducted under conditions of natural exposure in an environment typical of feedyards in the Midwestern U.S. The prevalence of *E. coli* O157:H7 shedding observed was typical of that previously observed in both research (4) and commercial (5) feedyards. These and previous results indicate that virulence factors secreted by the type III system can be effective vaccine components for the reduction of colonization of cattle by *E. coli* O157:H7 (6). The vaccine is relatively simple to prepare and economical, essential requirements for any product intended for use in food animals. These advantages, and the favorable results, emphasize the feasibility and utility of vaccination of cattle for *E. coli* O157:H7 as a pre-harvest intervention strategy aimed at reducing the risk of human infections.

Acknowledgement

This work was supported by the Nebraska Beef Council, Nutrition Physiology Corporation, and Nebraska State Legislature LB1206.

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