December 2005

Food-Bourne Pathogens – Control Through Management

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

David R. Smith
University of Nebraska - Lincoln, dsmith@cvm.msstate.edu

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

Robert Peterson
University of Nebraska - Lincoln

Galen E. Erickson
University of Nebraska - Lincoln, gerickson4@unl.edu

Proceedings, The Range Beef Cow Symposium XIX
December 6, 7 and 8, 2005, Rapid City, South Dakota

FOOD-BORNE PATHOGENS — CONTROL THROUGH MANAGEMENT

Terry J. Klopfenstein
Department of Animal Sciences

David R. Smith
Rodney A. Moxley
Department of Veterinary and Biomedical Sciences

Robert Peterson
Galen Erickson
Department of Animal Sciences
University of Nebraska-Lincoln

INTRODUCTION

We likely have the safest food in history. So why the concern about food safety? Why did NCBA have an E. coli Summit in 2003 and a Beef Safety Summit in 2005? The obvious answer is that it is an important issue for the Beef Cattle Industry. Meat and Poultry magazine estimated that *E. coli* O157:H7 cost the Cattle Industry about $2.7 billion from 1993 to 2003. From a local standpoint, we witnessed the bankruptcies of Hudson Foods and Beef America, two important beef processors in Nebraska. *E. coli* O157:H7 causes illness in less than .00002% of the meals eaten in the U. S. and fewer than 61 deaths annually. Statistically those numbers are very small, but if it is my grandchild, one is too many (every child has a grandparent like me). We want to share our experiences with *E. coli* O157:H7 over the past seven years because this is the primary food-borne pathogen concerning us at the present time.

ECOLOGY OF *E. coli* O157:H7 IN BEEF CATTLE.

It appears that feedlot cattle are the primary reservoir of *E coli* O157:H7. Feedlot cattle also represent most of the slaughter cattle although cull cows (20% of slaughter) are certainly important. We (Smith et al., 2001) conducted a study in five commercial feeds to estimate prevalence of cattle shedding E. coli in feces. Cattle from 29 pens were sampled at reimplant time during the summer of 1999. *E. coli* O157:H7 was isolated from 719 cattle of the 3,162 tested (23%) including at least one animal from each of the 29 pens. The percentage of cattle shedding E. coli did not vary between feedlots but varied widely between pens within a feedlot (0.27% to 79.8%). Pen prevalence was not associated with temperature, pH or cleanliness of water from water tanks or with pH of feed, number of cattle in pen, body weight or number of days in feedlot. E. coli prevalence was higher in pens of cattle if pen condition was muddy (22.4%) or dusty (17.9%) compared to normal (6.5%).

The study of Smith et al. (2001) was also designed to develop a technique for sampling cattle while in the pen without disturbance. We used the natural curiosity of feedlot cattle to develop this very simple technique. Seven ropes are hung on the rails above the feedbunks. The ropes are hung in the evening and the cattle chew on the ropes which are then retrieved early the next morning. The ropes are then cultured for *E. coli* O157:H7 and the pen is considered positive if any of the ropes are positive. Irwin et al. (2002) determined that about 50% of the cattle in the pen made contact with the ropes. Concurrent sampling of feces and ropes allowed us to determine that the ropes discriminate between low and high prevalence pens (Smith et al., 2004).

We then used the rope technique to study *E. coli* O157:H7 prevalence in commercial feedlot pens over time (Smith et al., 2005). During two summers 55 pens of cattle were followed with 1052 pen-week observations. *E. coli* O157:H7 was recovered from ropes at least once for each pen of cattle. About 43% of the pen tests were positive. Feedyards differed in the proportion of time that summer-fed pens of cattle were positive.

Over two winters, 20 pens of cattle were monitored for about 18 weeks for 348 pen-week observations. Less than 16% of the pen measurements with ropes were positive. Certainly *E. coli* O157:H7 prevalence is lower in the winter than in the summer. Other factors associated with E. coli prevalence were prior seven-day mean air temperature, recovery of E. coli from a composite fecal sample (from pen floor) and E. coli in the water tanks. The condition of the pen surface explained in part the probability for pens of cattle to be rope positive in the winter but not summer. In the winters, 67% of the observations were muddy pens while only 28% of summer pens were muddy.

Interestingly, 41% of the pens of cattle were rope positive the week they were marketed. A positive pen would generally have more than 16% of the cattle positive for *E. coli* O157:H7 in the feces. This suggests many of the cattle going to slaughter are carrying E. coli.

In the summer of 2004, we conducted a study in 19 commercial feedlots (Peterson et al., 2006b). Cattle were evaluated for *E. coli* O157:H7 prevalence using ropes plus a newly developed technique at slaughter. There were 485 pen observations using ropes throughout the feeding period and the pens of control cattle were determined to be about 46% positive which is in agreement with the previous commercial study (43%).

A newly developed technique was used at the slaughter plant. Terminal rectal mucosal (TRM) cells were collected by scraping the mucosa of the rectum one to two inches proximal to the retoanal juncture. The samples were collected at the “gut table” after evisceration. Samples were collected from a subsample of the cattle (720) and 19.5% of the control cattle were positive for *E. coli* O157:H7.

These studies in commercial feedlots suggest the following:

1) Feedlot cattle are a reservoir of *E. coli* O157:H7 and probably 15 to 20% of the feedlot cattle going to market carry E. coli.

2) It is not clear how the cattle become inoculated.
3) Prevalence is higher in summer than winter.
4) Muddy pens and probably dry, dusty pens increase prevalence — pen cleaning did not reduce prevalence.
5) All feedlots have *E. coli* O157:H7.
6) It is not clear if E. coli in feed or water is a route of inoculation or a result of positive cattle contaminating feed or water.
7) It is not clear that any Best Management Practices can be recommended.
8) Prevalence varies GREATLY over time and location without clearly understood reasons why it varies.
9) A few samples may be very misleading — it takes a BUNCH!

Feedlot cattle seem to be the primary reservoir of *E. coli* O157:H7. What is the prevalence in cow herds and how important is this to the Beef Industry? Lagreid et al. (1999) sampled calves in 15 herds in Kansas, Missouri, Montana, Nebraska and South Dakota. They analyzed fecal samples for E. coli and blood samples for antibody titres. Thirteen of the 15 herds had at least one positive fecal sample but overall prevalence was low (7.4%). Positive antibody titres were high. They concluded that infection before weaning is widespread and that most calves (83%) and all herds have been exposed to *E. coli* O157:H7.

Sargeant et al. (2000) sampled ten commercial cow-calf farms in Kansas throughout the year. Only 1.3% of fecal samples were positive. Fecal shedding by specific cattle was transient. Dunn et al. (2004) sampled 408 calves before and after weaning. The calves came from 29 southeastern farms and were preconditioned at three sites. There was only 2.5% level of prevalence at weaning and none after preconditioning. We have studied calves from three Montana ranches and have found very low prevalence in the calves at weaning (Peterson et al., 2005a). We conclude that *E. coli* O157:H7 is widely distributed throughout the Cattle Industry but that prevalence is quite low as cattle enter the feedlot.

INTERVENTIONS

We have worked with two interventions — vaccination and direct fed microbials (DFM). Clinical trials were conducted during the summers of 2002 and 2003 to determine the effect of DFM on prevalence of *E. coli* O157:H7 in feces of feedlot steers. The DFM consisted of Lactobacillus acidophilus (NPC 747) fed at the rate of 1×10^9 colony forming units per day. The DFM was reconstituted with water and added to the diet daily. Care was taken not to cross-contaminate the cattle fed the control diet. Feedlot steers fed DFM were 35% less likely to shed *E. coli* O157:H7 in the feces compared to control cattle not fed DFM (Figure 1). The DFM gave a small (2%) numerical increase in feed efficiency. Similar reductions in E. coli prevalence has been shown by Texas Tech researchers (Brashears et al; 2003; Elam et al.; 2003; Younts-Dahl et al., 2004; 2005).

E. coli O157:H7 colonize the terminal rectum of health cattle and have no apparent affect on cattle health. Colonization likely enhances the ability of the microorganisms to "hang around" and proliferate causing significant shedding in the feces. E. coli colonize by secreting proteins that allow attachment to intestinal cells. We therefore theorized that cattle

could be vaccinated against these proteins and thereby prevent colonization which would lead to reduced fecal shedding and lower overall prevalence.

Canadian researchers, Finaly and Potter, were working on the same hypothesis in 2001 and we developed a collaboration with them. Dr. Finaly prepared vaccine in his laboratory in British Columbia for research in 2002 and then Bioniche obtained rights to the technology and produced vaccine for use in 2003 and 2004.

In 2002 we conducted a clinical trial in our UNL research feedlot (Potter et al., 2004). We used 192 steers in 24 pens. One half the pens of cattle were vaccinated with three vaccinations, each three weeks apart. Fecal samples were collected from individual cattle at three-week intervals. Prevalence of control cattle varied from 10 to 25% over the 12 week sampling period. Vaccination reduced shedding of *E. coli* O157:H7 in feces from 21.3% in controls to 8.8% in vaccinated cattle (Figure 2).

Most cattle feeders do not process cattle more than two times during the feeding period. The average feeding period is 150 to 155 days and cattle are typically reprocessed and reimplanted 80 to 100 days prior to slaughter. Vaccination against E. coli attaching proteins at these two times would be most logical. In 2003 we conducted a second clinical trial in our research feedlot using 480 steers in 60 pens (eight/pen; Peterson et al., 2005b). Two steers in each pen were unvaccinated or vaccinated one, two or three times. An additional 128 steers were assigned to 12 pens to serve as unvaccinated controls.

Prevalence varied from 18 to 42% for controls in four test periods, three weeks apart (Figure 3). However, vaccinated cattle varied in a similar pattern to the controls. Vaccine efficacy of one, two or three doses was 68, 67 and 63% respectively (Figure 4). Unvaccinated cattle in pens receiving vaccine treatments were less likely to shed *E. coli* O157:H7 than cattle in pens not receiving vaccine. This suggests “herd immunity” occurred. Herd immunity results when some or many of the cattle in a herd are immunized creating an environment where there is less exposure for non-immunized cattle. This suggests that with large scale vaccination of a feedlot, overall prevalence should decline markedly over time.

In the summer of 2004, we conducted studies in our research feedlot (Peterson et al., 2006a) and in commercial feedlots (Peterson et al., 2006b). Overall prevalence of E. coli was low in our research feedlot (6.1%) and likely because prevalence was so low there was not a significant reduction in fecal shedding of E. coli. TRM samples were collected at the slaughter plant from all 288 steers in this study. The probability for steers to be colonized by *E. coli* O157:H7 was greatly reduced for vaccinated (0.3%) compared with non-vaccinated controls (20.0%) (Figure 5). This clearly demonstrates that the vaccine is effective in reducing colonization.

The large-scale commercial feedlot study was conducted with the cooperation of 19 Nebraska feedlots and involved 20,566 cattle in 140 matched pens. The pens were unvaccinated or vaccinated twice at initial processing and at reprocessing (reimplant). Ropes were used to determine pen-level *E. coli* O157:H7 prevalence. At least four rope tests were conducted per pen at three-week intervals during the feeding period. The probability for pens

of vaccinated cattle to test positive for E. coli (34%) was less than unvaccinated controls (46%; Figure 6).

TRM samples were obtained in the slaughter plant for a subset of the cattle (720) used in the commercial study. Probability for *E. coli* O157:H7 colonization of the mucosal cells of the terminal rectum among vaccinated cattle was lower (4.7%) compared with non-vaccinated cattle (19.5%). Vaccine efficacy was 76% (Figure 7).

Elder et al. (2000) and Arthur et al. (2004) demonstrated that carcasses are contaminated in the packing plant from contact with hides. From a practical standpoint, hide contamination is very important and may be more important than the measures (fecal samples, ropes, TRM samples) we have used to evaluate intervention strategies. Hide positive samples have been as high as 88% (Nou et al., 2003) and 76% (Arthur et al., 2004). In addition Collis et al. (2004) have shown that pathogenic bacteria are extensively spread on hides during transport from the feedlot and holding at the slaughter facility.

We conducted a study to evaluate the effect of vaccination against E. coli on the occurrence of *E. coli* O157:H7 on hides of cattle. Additionally, the effect of transport and lairage on hide contamination was evaluated by collecting hide samples at the feedlot of origin on the day of shipment to the processing facility and collecting hide samples again at the processing facility during the harvest process. A total of 332 steers housed in 42 research pens were studied. A hide sample was collected from steers at the feedlot on the day of shipment for harvest and a second hide sample was collected from the same steers at the processing facility during the harvest process (post-harvest). Vaccination resulted in a 44% reduction in *E. coli* O157:H7 hide contamination. Vaccination was equally effect at reducing the occurrence of *E. coli* O157:H7 on hides at the feedlot and at the processing facility. However, cattle hides were more likely to test positive at the processing facility than at the feedlot. These results suggest that pre-harvest intervention strategies can reduce hide contamination at harvest and that the probability for hide contamination my increase during transport and lairage.

This series of experiments clearly demonstrates that vaccination is effective in reducing prevalence of *E. coli* O157:H7 in feedlot cattle. DFM are also effective in reducing E. coli shedding. These two strategies offer good promise to reduce this problem in feedlot cattle. We theorize that the use of one or both of these interventions in feedlots will have a "herd immunity" effect and overall E. coli prevalence will decline. Combined with multiple hurdles employed in slaughter plants, *E. coli* O157:H7 adulteration of ground beef and other beef products should be markedly reduced. The DFM is on the market and being used by some feedlots. The vaccine is in the approval process. Other interventions are being researched elsewhere. These include another vaccine, feeding of sodium chlorate just prior to slaughter, feeding antibiotics (Neomycin sulfate or ceftiofur) prior to slaughter, and feeding bacteria phage (virus that kills E. coli). None of these strategies are approved for use.

LITERATURE CITED

Arthur, T. M., J. M. Bosilevac, X. Nou, S. D., Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Panling, D. W. Allen and M. Koohmaire. 2004. *E. coli* O157:H7 prevalence and enumeration of aerobic bacteria, Enterobacteriacae, and *E. coli* O157:H7 at various steps in commercial beef processing plants. J. Food Prot. 67:658-665.

Brashears, M. M., M. L. Galyean, G. H. Lonergan, J. E. Mann, K. Killinger-Mann. 2003. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. J. Food Prot. 66:748-754.

Collis, U. J., C. A. Reid, M. L. Hutchinson, M. H. Davies, K.P.A. Wheeler, A. Small, and S. Buncic. 2004. Spread of marker bacteria from the hides of cattle in a simulated livestock market and abattoir. J. Food. Prot. 67:2397-2402.

Elam, N. A., J. F. Gleghorn, J. D. Rivera, M. L. Galyean, P. J. Defoor, M. M. Brashears, S. M. Younts-Dahl. 2003. Effects of live cultures of *lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium fruendenreichii* on performance, cacass, and intestinal characteristics, and *Escherichia coli* strain O157 shedding of finishing beef steers. J. Anim. Sci. 81:2686-2698.

Elder, R. O., J. E. Keen, G. R. Siraqusa, G. A. Barkocy-Gallagher, M. Koohmarie, and W. W. Lagried. 2000. Correlation of enterohemorrhagic *E. coli* O157:H7 prevalence in feces, hides and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. 97(2) 2999-3003.

Dunn, J. R., J. E. Keen, R. Del Vecchio, T. E. Wittum, and R. Alex Thompson. 2004. *E. coli* O157:H7 in a cohort of weaned preconditioned range beef calves. J. Food Prot. 66:2391-2396.

Irwin, K. E., D. R. Smith, J. T. Gray, et al., 2002. Behavior of cattle towards devices to detect food safety pathogens. Bovine Practitioner. 36:5-9.

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.

Nou, X., M. Rivera-Betancourt, J. M. Bosilevac, T. L. Wheeler, S. D. Shackelford, B. L. Gwartney, J. O. Reagan, and M. Koohmarie. 2003. Effect of chemical dehairing on the prevalence of *E. coli* O157:H7 and the levels of aerobic bacteria and enterobacteriaceae on carcasses in a commercial beef processing plant. J. Food Prot. 66:1978-1986.

Peterson, R., J. Paterson, D. Smith, R. Moxley, T. Klopfenstein, G. Erickson, W. Choat and S. Hinkley. 2005a. Vaccination on the ranch as an intervention strategy to reduce the probability of detecting *E. coli* O157:H7 associated with commercial feedlot cattle. J. Anim. 83(Suppl 2):120.

Peterson, R. E., D. R. Smith, R. A. Moxley, T. J. Klopfenstein, S. Hinkley, and G. E. Erickson. 2005b. Vaccination for *E. coli* O157:H7 in market ready feedlot cattle. Nebraska Beef Cattle Report MP 83-A:61.

Peterson, R. E., D. R. Smith, R. A. Moxley, T. J. Klopfenstein, S. Hinkley, and G. E. Erickson. 2006a. Vaccination for *E. coli O157:H7* in market ready feedlot cattle. Nebraska Beef Cattle Report MP 88-A:68.

Peterson, R. E., D. R. Smith, R. A. Moxley, T. J. Klopfenstein, S. Hinkley, and G. E. Erickson. 2006b. Large scale clinical trial to evaluate an experimental E. coli vaccine. Nebraska Beef Cattle Report MP 88-A:70.

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. Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. Vaccine 22:362-369.

Sargeant, J. M., J. R. Gillespie, R. D. Oberst, R. K. Phebus, Dr. Hyatt, L. K. Bohra and J. C. Galland. 2000. Results of a longitudinal study of the prevalence of *Escherichia coli* O157:H7 on cow-calf farms. Am. J. Vet. Res. 61:1375-1379.

Smith, D. R., M. P. Blackford, S. M. Younts, R. A. Moxley, J. T. Gray, L. L. Hungerford, C. T. Milton, and T. J. 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., J. T. Gray, R. A. Moxley, et al. 2004. A diagnostic strategy to determine the Shigatoxin producing *Escherichia coli* O157 status of pens of feedlot cattle. Epidemio. Infect. 132:297-302.

Smith, D. R., R. A. Moxley, S. L. Clowser, J. D. Folmer, S. Hinkley, G. E. Erickson, 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 2:50-60.

Younts-Dahl, S. M., M. L. Galyean, G. H. Lonergan, N. A. Elam, M. M. Brashears. 2004. Dietary supplemntation with *Lactobacillus*- and *Propionibacterium*-based direct-fed microbials and prevalence of *Escherichia coli* O157 in beef feedlot cattle and on hides at harvest. J. Food Prot. 67:889-893.

Younts-Dahl, S. M., G. D. Osborn, M. L. Galyean, J. D. Rivera, G. H. Lonergan, M. M. Brashears. 2005. Reduction of *Escherichia coli* O 157 in finishing beef cattle by various doses of *Lactobacillus acidophilus* in direct-fed microbials. J. Food Prot. 68:6-10.

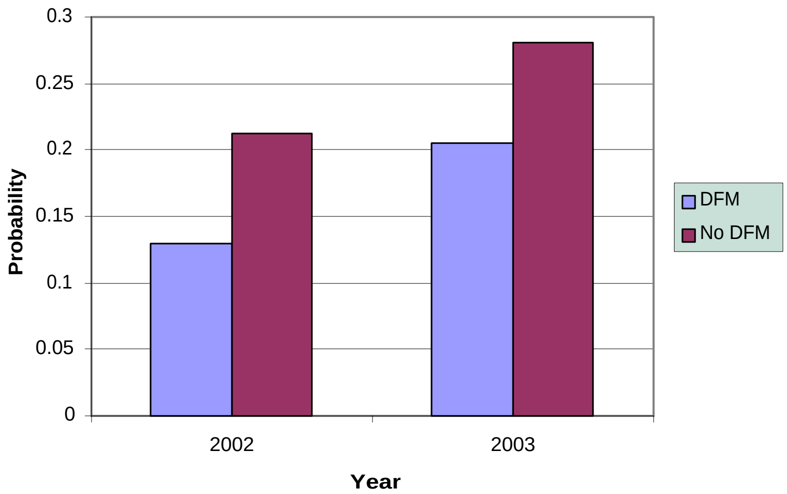

Figure 1. Probability of *E. coli* O157:H7 shedding, by direct-fed microbial treatment and year.

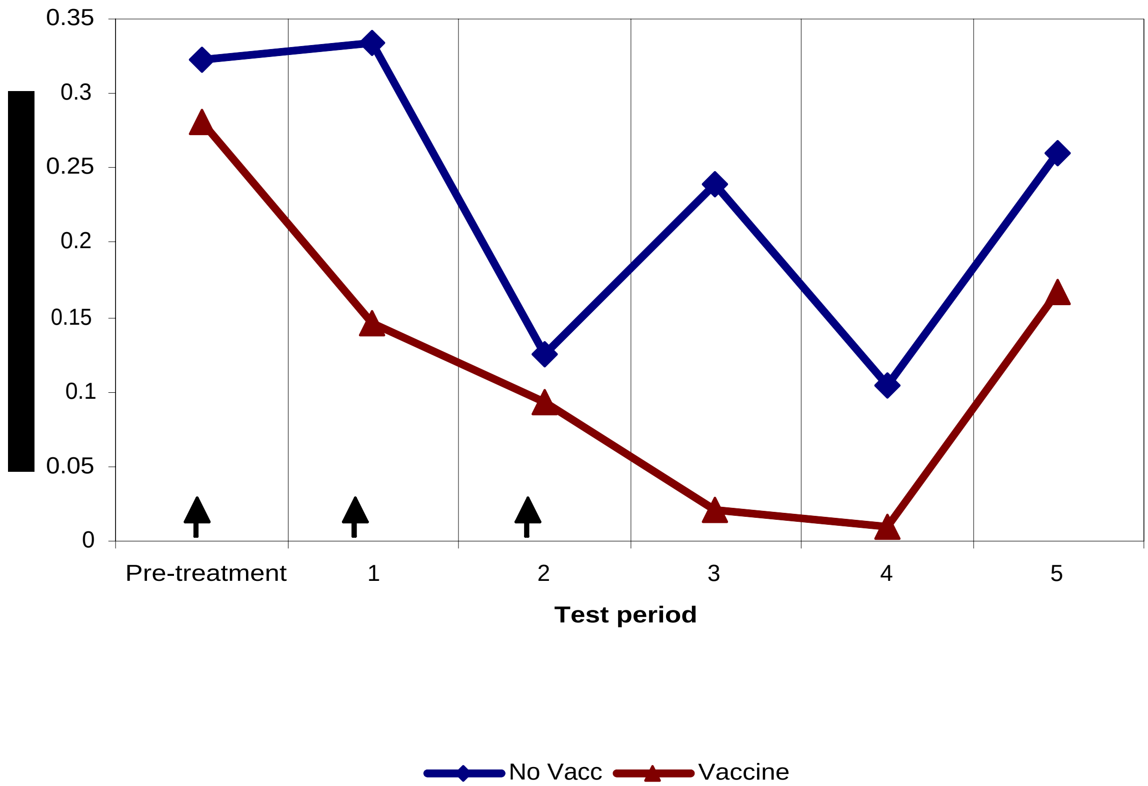

Figure 2. 2002 Clinical Trial: Results.

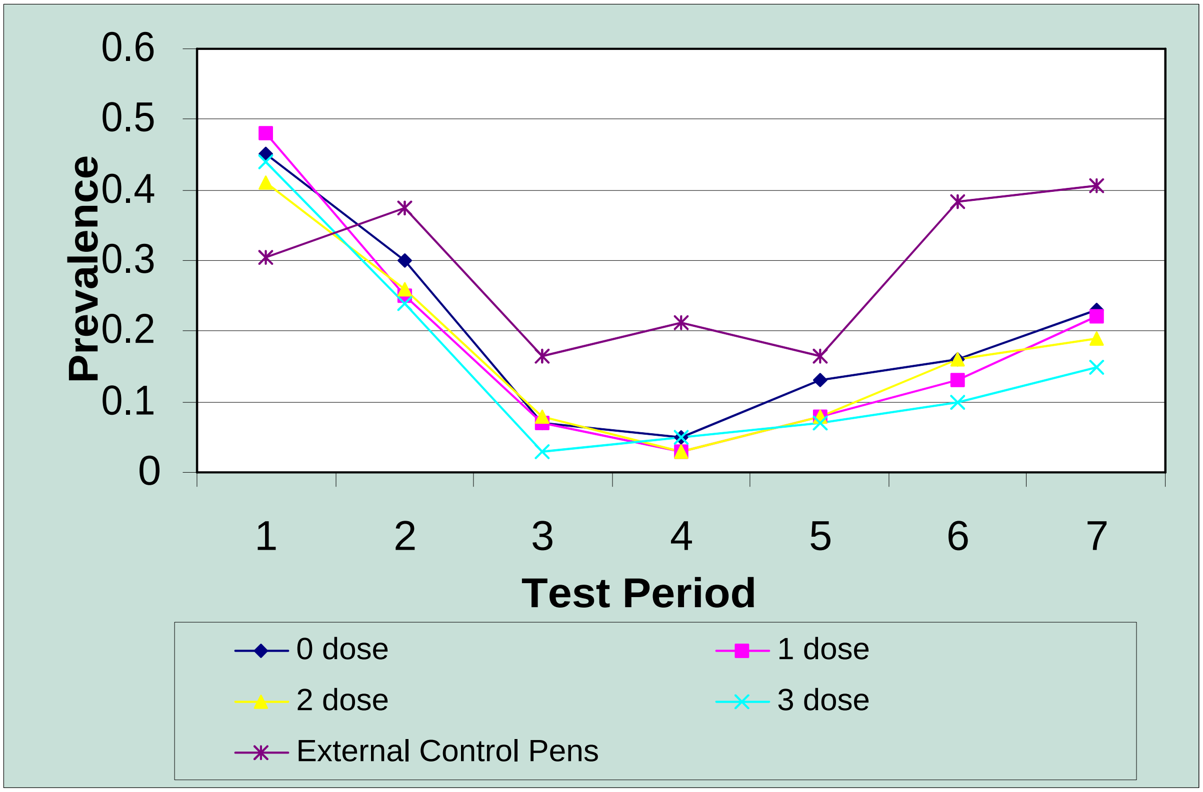

Figure 3. The proportion of steers shedding *E. coli* O157:H7 by test period and treatment (0, 1, 2, or 3 doses of vaccine) within treated pens compared to untreated pens.

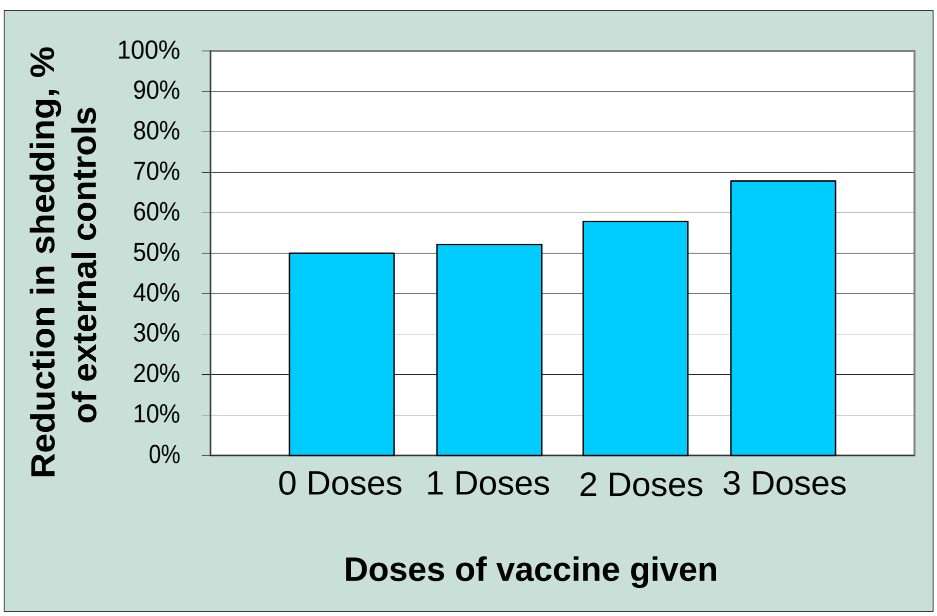

Figure 4. Percentage reduction in *E. coli* O157:H7 shedding (vaccine efficacy) by steers receiving 1, 2, or 3 doses of vaccine within treated pens compared to pens receiving no vaccine (21-84 days post treatment).

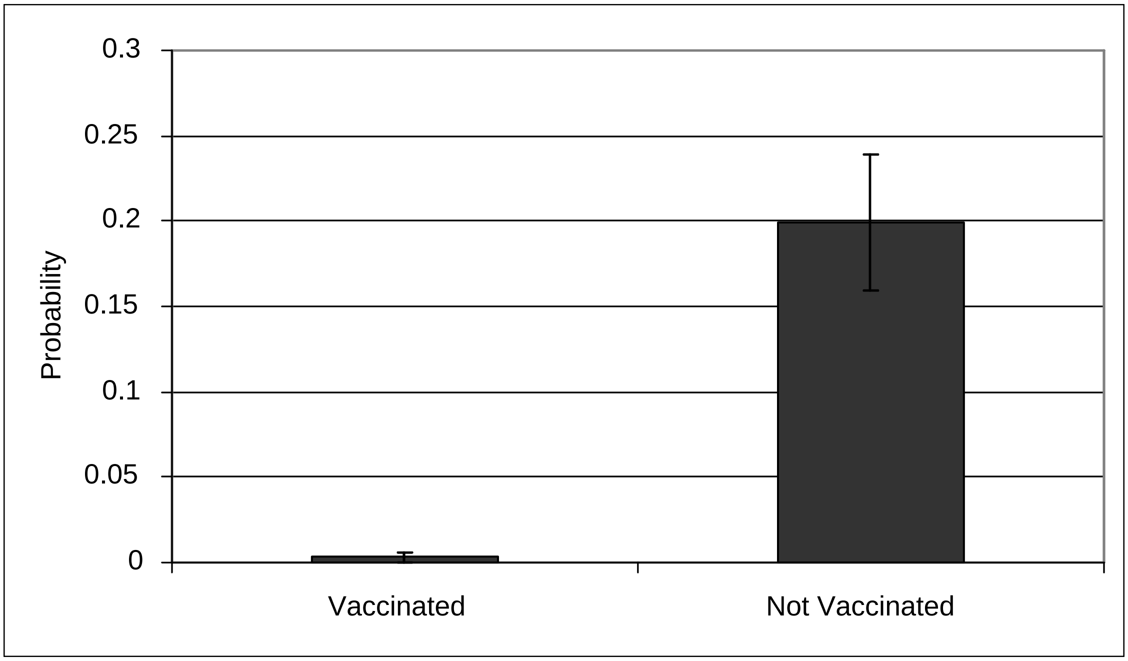

Figure 5. Probability of steers to be colonized by *E. coli* O157:H7 in TRM at harvest by vaccination treatment adjusted for dietary treatment.

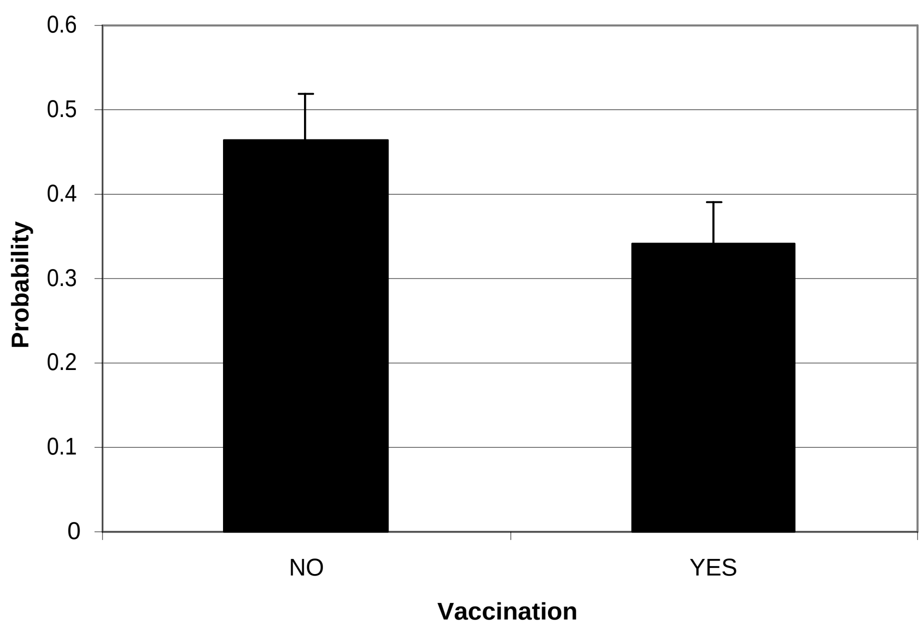

Figure 6. Adjusted probabilities for vaccinated and unvaccinated pens to test ROPES-positive for *E. coli* O157:H7.

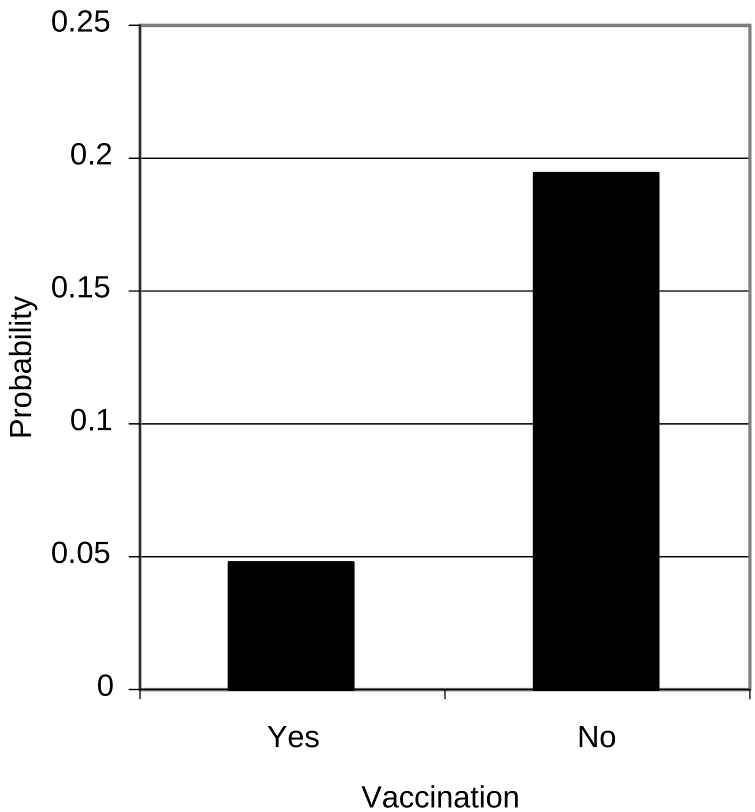

Figure 7. Probabilities for *E. coli* O157:H7 colonization of the rectoanal junction at slaughter