

AGRI-PRACTICE – COW/CALF – DISEASE CONTROL

At 14-day intervals, 12 calves were vaccinated twice with a *S. typhimurium* bacterin-toxoid, and 12 control calves each received two injections of dialuminum trioxide/saline placebo. Two weeks following the vaccination booster, or the second placebo injection, ten calves – five vaccinated and five treated with placebo – were challenged with 100 ng/kg of *E. coli* 055:B5 endotoxin. Similarly, another 14 calves – seven vaccinated and seven placebo-treated – were challenged with 50 ng/kg of *P. multocida* endotoxin. There was a significant difference ($P < 0.05$) between the clinical responses of the vaccinated and placebo-treated group challenged with either *E. coli* 055:B5 or *P. multocida* endotoxin as measured by the endotoxin colic index, mean anorexia time intervals, and IgG(t) serum antibody titers.

Cross-Protection of Calves from *E. coli* and *P. multocida* Endotoxin Challenges Via *S. typhimurium* Mutant Bacterin-Toxoid*

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Introduction

Some of the most common and devastating diseases encountered by the bovine practitioner are those associated with endotoxins. Gram-negative diarrheas and pneumonias are often complicated by endotoxins.^{1,2} Failure of passive transfer of uniformity is the primary predisposing factor to neonatal septicemia, which is caused most frequently by gram-negative bacteria.¹ The host's biological responses to endotoxins result in many of the recognizable clinical signs exhibited, and often culminate in death.³⁻⁶ An active immunization strategy aimed at host inactivation of gram-negative endotoxins represents a rational approach for preventing the devastating effects of endotoxemia.

Immune strategies that would aid cattle by providing cross-protection from the overwhelming effects of various gram-negative endotoxemias have been difficult to develop.⁷ In a case of endotoxemia, the specific serotype sources of endotoxin involved may be from one or more members of the large gram-negative family, *Enterobacteriaceae*. Because there are hundreds of gram-negative serotypes, it would be impractical to combine sufficient autogenous vaccines to provide broad-spectrum protection. Thus, a single source bacterin that provides cross-protection against virtually all gram-negative endotoxins is needed.

The fact that almost all species of gram-negative bacteria possess analogous cell wall characteristics has provided the basis for many immunological studies conducted over the past 20 years.⁷⁻¹⁵ R-mutants of *Salmonella* sp. and *Escherichia coli* have been the focus of many of these studies.^{8,10}

R-mutants are "rough"-appearing cell colonies of mutant gram-negative bacteria. These mutants are biochemically characterized by their relative absence of oligosaccharides ("O") side chain attachments. The relative degree of "O" side chain absence is designated by the capital letter "R" accompanied by the lowercase letters "a" through "e" with Re completely lacking "O" side chains.^{8,10,11} The J5 *E. coli* mutant previously studied by us and others is characterized as Rc and thus possesses "O" side chains.

Removal of these "O" side chains via mutation allowed the core antigen of the cell wall to be presented to the immune system for the subsequent production of cross-protective antibodies,^{15,16} thereby, circumventing problems associated with specific serotype characteristics. Antibodies formed in response to these core antigens devoid of the "O" side chains can cross-protect an animal from many, and possibly all, gram-negative endotoxins.

An Re-type mutant bacterial strain from a parent *Salmonella typhimurium* was engineered to form an Re-type mutant that possesses no "O" side chains. This naked core Re-mutant was combined with a toxoid and dialuminum trioxide to make a cross-protective vaccine.¹⁶ The results of Heterologous efficacy testing in calves immunized with this vaccine are presented.

Materials and Methods

The vaccine used in these experiments contained a killed bacterial Re mutant of *S. typhimurium* (bacterin), and immune modulator (endotoxin), a protein/lipid binding carrier/adjuvant (dialuminum trioxide), and oil. Each calf was vaccinated and boosted within 2 weeks either with the vaccine or a dialuminum trioxide/saline placebo. Each calf was intravenously challenged with endotoxin 2 weeks post-booster injection.

Twenty-four healthy calves ranging from 3 to 4 months in age and 79 kg to 200 kg in body weight were used in this study. The 22 bulls and two heifers were divided as evenly as possible into two groups of 14 and 10, respectively, on the basis of sex and then randomized into two groups of seven and two groups of five. One group of seven and one group of five were administered two 1.6 ml doses of the vaccine into the cervical musculature 14 days apart. The United States Department of Agriculture required 80%, or 1.6 ml doses of a 50% dialuminum trioxide/50% saline placebo intramuscularly 14 days apart. This experimental design allowed each group that received the vaccine to be compared with a group that received placebo when all were challenged with endotoxin.

Ten calves, five vaccinated with the bacterin-toxoid and five injected with the placebo, were challenged with an intravenous bolus of 100 ng/kg of *E. coli* 055:B5 endotoxin. The other 14 calves, seven vaccinated with the bacterin-toxoid and seven injected with the placebo, were challenged with an intravenous bolus of 50 ng/kg of *Pasteurella multocida* endotoxin. Each calf was fasted for 12 hours prior to endotoxin challenges but was allowed free access to water until tied in a box stall for observation. Each calf was observed 60 minutes prior to endotoxin injection to establish control behavior and was then allowed free choice of alfalfa and observed for 1 hour following endotoxin injection to observe clinical responses. Responses were continuously recorded. In addition, during the second hour following endotoxin administration, each calf was turned loose in a box stall and allowed free choice of alfalfa, hay, and water and closely observed to determine whether or not it was anorexic.

The endotoxin colic index scoring method used to generate the data in Figures 1 and 2 was established prior to the present study by statistically analyzing the observations of three individuals recording the clinical signs exhibited by 30 head of tied calves for 1 hour prior to and 1 hour following intravenous bolus administration of varying dosage levels of either *Pasteurella* or *E. coli* endotoxin.¹² Kicking, leg flexing, stretching, bowing-stretches, looking at flank, hyperpnea, and dyspnea along with CNS depression progressing to comatosis were all included as signs used to describe the progression of behavior, which ranged from Level 1.0 to Level 6.0 of the endotoxic colic index. During efficacy studies, the assessment of the observations was accomplished via a blinded scorer. All of the calves, whether they possessed protective levels of anticore-antigen antibodies or not exhibited signs that approached Level 2.0 when they were scored. The unprotected animals developed sufficient clinical signs to progress through level 2.0 and higher, while those that were protected exhibited colic index score levels of less than 2.0.

Serum samples collected from each calf before and 4 weeks following the first injection of vaccine or placebo were analyzed for the present study by an ELISA assay adapted from a previously developed radioimmunoassay (RIA) for specific IgG(t) antiendotoxin antibody levels.^{17,18} The technician that analyzed the pre- and post-vaccination serum samples for anticore-antigen antibody levels was not aware of any animal's category.

Data were analyzed via analysis of variance statistical techniques. The predetermined acceptable probability level was 0.05 or less.

Results

When challenged with endotoxin, calves vaccinated with the *S. typhimurium* bacterin-toxoid compared with those injected with the placebo were significantly ($P < 0.05$) different in terms of the mean endotoxin colic index scores reflecting colicky pain, dyspnea and somnolence, mean IgG(t) antibody levels, and anorexia time intervals (Tables 1 & 2; Figs. 1, 2, 3, & 4). The line 2.0 represents the previously established threshold that divided those with protective levels of anticore-antigen antibodies from those without.¹² The mean endotoxin colic respiratory index scores of immunized vs. placebo-injected groups heterologously challenged with *E. coli* 055:B5 endotoxin were significantly ($P < 0.001$) different (Table 1; Figs. 1 & 2).

The differences between these groups (Table 2; Fig. 4) in terms of either mean IgG(t) antibody titers ($P < 0.001$) or mean anorexia time intervals ($P < 0.05$) were significant. Similarly, the mean endotoxin colic index scores of immunized vs. placebo-injected groups heterologously challenged with *P. multocida* endotoxin were significantly ($P < 0.001$) different (Table 1; Fig. 1). The differences between these groups in terms of either mean IgG(t) antibody tiers ($P < 0.05$) (Table 2; Figs. 3 & 4) or mean anorexia time intervals ($P < 0.05$) (Table

3) were also significant. In this study, 90% of the calves that received the vaccine exhibited a transient palpable 1-cm diameter swelling in the cervical musculature injection site 2 to 4 days postinjection, which was nonpalpable 2 weeks following injection. None required treatment or went off feed.

Discussion

The increase in serum IgG(t) antibody levels in the vaccinated calf groups apparently provided the active immunity responsible for protection against the outward clinical effects of the heterologous endotoxin challenges. It is interesting that these results confirmed the results of other laboratories when various species were vaccinated with similar gram-negative mutant bacterins and challenged with Heterologous endotoxins.^{9-11,19} It is also important to note that the protection provided by the antibodies produced in response to the core antigen of the Re-mutant *S. typhimurium* bacterin-toxoid cross-protected the calves from the heterologous *E. coli* O55:B5 endotoxin challenge as well as from the heterologous *P. multocida* endotoxin challenge.

The dialuminum trioxide adjuvant in this vaccine stimulated the localization of macrophages in the muscular tissue at the injection site. The macrophage-processed antigen then slowly leaked out of the localized macrophages providing a prolonged antigenic stimulus.²⁰ Therefore, a local response was expected following injection of the vaccine and was indicative of a viable hose immunization. Dialuminum trioxide influenced the primary immune response and helped maintain the other two vaccine components in suspension.

The toxoid portion of the combination cross-protective vaccine stimulate the B-lymphocytes to divide and produce antibodies directed against the naked core determinant while the killed Re-mutant bacterial cells (bacterin) provided the naked core determinant to serve as antigen for antibody production.

Since conducting these efficacy studies, results of field study observations, including a rise in body temperature and/or generalized muscular soreness, were not detected in any calves or cows. In accordance with USDA recommendations, any animal that suffers an allergic response following vaccination should be treated immediately with epinephrine or its equivalent. No allergic responses were discerned during the efficacy and subsequent field studies.

Conclusion

The anticore-antigen antibody efficacy demonstrated in this study offers new possibilities for aiding in the control of end-stage consequences of such gram-negative diseases as *E. coli* sp. diarrhea, *Salmonella* sp. diarrhea, and *Pasteurella* sp. pneumonia. Because of the cross-protectiveness of the antibodies demonstrated in this study, it is suspected that cattle can also be protected from endotoxins arising from other gram-negative bacteria such as *Klebsiella* sp., *Enterobacteriaceae* sp. *Proteus* sp., and others. Application of this technology may add a new dimension to immunologic control of economically important gram-negative bovine diseases.

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TABLE 1
Comparison of Mean Endotoxin Respiratory Colic Index Scores and Serum IgG Antibody Titers of *E. coli* Endotoxin-Challenged, Placebo-Treated, and Vaccinated Calves

Parameter	Endotoxin-Challenged Calves			
	Placebo (control); N = 5		Vaccinate; N = 5	
Mean Endotoxin Colic Respiratory Index Score^a				
Mean	2.31		0.32 ^c	
SD	± 2.40		± 0.64	
SEM	± 1.20		± 0.30	
Range	0.60-3.6		0.0-1.0	
Mean Serum IgG Titer (Log 2)^b				
	Pre-	Post-	Pre-	Post
Mean	8.60	9.00 ^d	9.80	12.20 ^c
SD	± 1.20	± 1.55	± 1.47	± 1.17
SEM	± 0.60	± 0.77	± 0.74	± 0.59
Range	8-11	8-12	8-11	11-14

^a Endotoxin respiratory colic index scores were analyzed via three-factor analysis of variance with repeated measures on one factor.

^b Serum IgG antibody measurements were analyzed via two-factor analysis of variance techniques with repeated measures on one factor.

^c Mean value significantly ($p < 0.05$) different from control or pretreatment values.

^d Mean value not significantly ($p > 0.05$) different from pretreatment values.

SE = Standard deviation; SEM = Standard error of the mean.

TABLE 2
Comparison of Mean Endotoxin Respiratory Colic Index Scores and Serum IgG Antibody Titers of *Pasteurella* Endotoxin-Challenged, Placebo-Treated, and Vaccinated Calves

Parameter	Endotoxin-Challenged Calves			
	Placebo (control); N = 7		Vaccinate; N = 7	
Mean Endotoxin Colic Respiratory Index Score^a				
Mean	2.35		0.64 ^c	
SD	± 2.26		± 1.17	
SEM	± 0.92		± 0.48	
Range	1.0-3.7		0.10-2.1	
Mean Serum IgG Titer (Log2)^b				
	Pre-	Post-	Pre-	Post
Mean	9.29	9.71 ^d	8.29	13.10 ^c
SD	± 1.28	± 1.03	± 0.45	± 1.36
SEM	± 0.52	± 0.42	± 0.18	± 0.56
Range	8-11	8-11	8-9	11-15

^a Endotoxin respiratory colic index scores were analyzed via three-factor analysis of variance with repeated measures on one factor.

^b Serum IgG antibody measurements were analyzed via two-factor analysis of variance techniques with repeated measures on one factor.

^c Mean value significantly ($p < 0.05$) different from control or pretreatment values.

^d Mean value not significantly ($p > 0.05$) different from pretreatment values.

SE = Standard deviation; SEM = Standard error of the mean.

TABLE 3
Comparison of Combined Anorexia Time Intervals of *E. coli* and
***Pasteurella* Endotoxin-Challenged, Placebo-Treated, and Vaccinated Calves**

Parameter	Endotoxin-Challenged Calves	
	Placebo (control); N = 12	Vaccinate; N = 12
Mean Anorexia Time Interval (minutes)^a		
Mean	98.1	63.0 ^b
SD	± 22.2	28.8
SEM	± 6.4	± 8.31
Range	48-112	29-102

^a Anorexia time interval measurements were analyzed via two-factor analysis of variance techniques with repeated measures on one factor.

^b Mean value significantly ($p < 0.05$) different from control values.

SE = Standard deviation; SEM = Standard error of the mean.