

ACTIVE IMMUNIZATION OF COWS WITH A *SALMONELLA TYPHIMURIUM* MUTANT BACTERIN-TOXOID AND THE PASSIVE TRANSFER OF ANTI-CORE-ANTIGEN ANTIBODIES IN COLOSTRUM.

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INTRODUCTION

Gram-negative endotoxins have been implicated in the pathogenesis of equine CHO laminitis¹, bovine coliform mastitis², and adult and neonatal septicemias.^{3,4,5,6} Fortunately, the demonstration that cattle^{2,5,6} and horses^{3,4} could be protected from various Gram-negative endotoxins via anti-core-antigen antibodies offers viable possibilities for immunologically protecting animals from the deadly effects of endotoxemia.

The anti-core-antigen antibody research work conducted prior to investigations in cattle and horses was centered around laboratory animal studies utilizing a mutant *Salmonella typhimurium*⁸ or the J5 mutant of *E. coli* utilizing laboratory animal and human clinical endotoxemia cases.⁹ This foundation of knowledge has recently been applied to problems in cattle^{2,5,6} and horses^{3,4} and has added a new immunological dimension to the possibilities for controlling Gram-negative endotoxin mediated diseases.

The specific source or sources of endotoxin involved in a particular case of endotoxemia may be one or more members of the large Gram-negative family Enterobacteriaceae. Because there are hundreds of serotypes, it is impractical to combine sufficient autogenous vaccines to provide broad-spectrum protection. Thus, there is a paramount need for a single source bacterin that provides cross-protection against virtually all Gram-negative endotoxins.

The R-mutants of Gram-negative bacteria 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 small case letters "a" through "e" with Re mutant of *S. typhimurium* completely lacking "O" side chains. In contrast, the J5 *E. coli* mutant is characterized as Rc and possesses some "O" side chains. Removal of these "O" side chains via mutation allows the core antigen of the cell wall to be presented to the immune system for the subsequent production of cross-protective antibodies.

A mutant *S. typhimurium* bacterin-toxoid was administered to cows late in gestation, first) to learn if seroconversion could be achieved in cows, second) to learn if the anti-core-antigen antibody titer in the colostrums was higher in the vaccinated cows than in control cows, and third) to determine whether or serum anti-core-antigen antibody titers in calves that suckled vaccinated cows were higher than in calves that suckled unvaccinated cows.

MATERIALS AND METHODS

Vaccine

The vaccine (ENDOVAC-Bovi™: IMMVAC, Inc., Columbia, MO, USA) used in these experiments contained a killed bacterial Re-mutant of *Salmonella typhimurium* (bacterin), and immune modulator (endotoxoid), and oil adjuvant and a protein/lipid binding carrier/adjuvant (dialuminum trioxide).⁵ Each cow was vaccinated and boosted within 2 weeks with either the vaccine or a dialuminum trioxide/saline placebo.

Animals

Thirty-seven normal Holstein and Guernsey cows ranging from 2 – 7 years of age in the last six weeks of gestation were used in this study.

Serum Analysis

Serum samples collected from each cow prior to and 4 weeks after the first injection of vaccine or placebo, as well as those collected from the calves at birth and 24 hours after suckling, were analyzed by an ELISA assay adapted from a previously developed radioimmunoassay for measuring specific IgG(t) anti-endotoxin antibody levels.¹⁰

Statistical Analysis

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

Results

The post-vaccination mean Log₂ 12.15 (1:4545 dilution) anti-core-antigen antibody titer of the 19 cows that received *S. typhimurium* bacterin-toxoid was significantly (P=0.0003) higher than the mean prevaccinated titer of Log₂ 10.73 (1:1722 dilution). The post vaccination Log₂ 10.75 (1:1722 dilution) titer of the placebo treated cows was not significantly (P=0.8976) different than the mean pre-vaccination Log₂ 10.80 (1:1783 dilution) titer (Table 1).

There were no untoward effects such as abortions or other logical or systematic illnesses that arose from vaccinating cows with *S. typhimurium* bacterin-toxoid.

The mean Log₂ 13.18 (1:9281 dilution) core-antigen-antibody titer of the colostrums from 9 vaccinated cows was significantly (P=0.04) higher than the mean Log₂ 12.22 (1:4771 dilution) titer of colostrums from placebo treated cows, mean Log₂ 10.64 (1:1596 dilution) vs. Log₂ 9.20 (1:588 dilution) respectively (Table 2).

Six calves of vaccinated mothers increased their mean anti-core-antigen serum antibody titers from Log₂ 6.7 (1:104 dilution) to Log₂ 10.64 (1:1596 dilution). Eight calves from placebo treated cows increased their mean anti-core-antigen antibody titers from Log₂ 6.19 (1:73 dilution) to Log₂ 9.2 (1:588 dilution) (Table 2).

A significant increase in serum anti-core-antigen antibodies was produced in both the cows and calves in this study. The increase in mean serum antibody titer of the cows was due to active immunization and in the calves due to passive immunization via colostrums absorption.

DISCUSSION

The adult pregnant cows responded to an anti-core-antigen vaccination regime by significantly seroconverting. Core-antigen-antibodies have been shown to significantly lower the incidence of coliform mastitis in lactating dairy cows.² Whether or not cows in this study possessed increased resistance to Gram-negative induced mastitis awaits completion of ongoing epidemiological studies. However, the results of previously conducted studies of *S. typhimurium* bacterin-toxoid vaccinated calves challenged with *E. coli* and *Pasteurella* endotoxins suggested that significant cross-protection can be provided by the anti-core-antigen antibodies produced in response to *S. typhimurium* bacterin-toxoid vaccination.⁵

Whether or not the increases in serum anti-core-antigen antibodies in both cows and calves significantly increased their resistance to Gram-negative endotoxins can be answered either by long term epidemiologic or direct endotoxin challenge studies. Epidemiological studies are presently in progress. The results of the study and the investigative work of others 2,6,7 suggest that the application of core-antigen-antibody immune strategies could translate into significant reduction of economic losses in both the dairy and beef cattle industries. The many Gram-negative endotoxin mediated diseases in calves and adult cattle provide a wide potential spectrum of maladies that may be very responsive to this new immunological approach to controlling Gram-negative diseases.

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SUMMARY

Thirty-seven normal Holstein or Guernsey cows in their last six weeks of gestation were injected with either a placebo or *Salmonella typhimurium* mutant bacterin-toxoid. The vaccine stimulated a significant ($P \leq 0.05$) increase in the mean Log_2 anti-core-antigen serum antibody titers from 10.73 to 12.15 while there was no significant increase in serum antibody titers in placebo treated cows.

There was a significant ($P \leq 0.05$) difference between the colostral mean anti-core-antigen antibody Log_2 titers of the placebo, 12.22, and vaccinated, 13.18, cows. The mean Log_2 serum antibody titers of suckled calves at 24 hours of age from the placebo and vaccinated groups were 9.20 and 10.64 respectively and significantly ($P \leq 0.05$) different.

It was concluded that cows vaccinated with bacterin-toxoid in the last six weeks of gestation seroconverted in terms of anti-core-antigen antibodies and that they passively transferred significantly ($P \leq 0.05$) higher levels of these antibodies through colostrums to their calves.

	Common Slope	Least square estimates				Statistical tests Pre-vac vs. Post-vac
		Placebo-treated		Vaccine-treated		
		Pre-vac	Post-vac	Pre-vac	Post-vac	
LS mean	-7.08	10.75	10.80	10.73	12.15	Placebo.. $P \leq 0.8976$ Vaccine.. $P \leq 0.0003$
Std. Error	± 0.09	± 0.27	± 0.27	± 0.27	± 0.27	
		Simple group statistics				Pre-treatment Placebo vs. Vaccines $P \leq 0.9572$
N		18	18	19	19	
Average		10.75	10.80	10.73	12.15	
Std. dev		± 1.23	± 1.31	± 0.97	± 1.62	Post-treatment Placebo vs. Vaccines $P \leq 0.0006$
Std. err		± 0.30	± 0.32	± 0.23	± 0.38	

	Common Slope	Least square estimates					
		Placebo treated			Vaccine treated		
		Colostrum	Calf suckle		Colostrum	Calf suckle	
		Pre-	Post-	Pre-	Post-		
LS mean	-8.77	12.22	6.19	9.20	13.18	6.70	10.64
Std err	± 0.20	± 0.33	± 0.48	± 0.43	± 0.33	± 0.51	± 0.47
		Simple group statistics					
N		11	8	8	11	6	6
Average		12.22	6.19	9.20	13.18	6.70	10.64
Std dev		± 1.41	± 0.92	± 1.43	± 0.68	± 0.85	± 0.81
Std err		± 0.45	± 0.35	± 0.54	± 0.22	± 0.38	± 0.36
		Statistical tests					
Within placebo		Presuckle vs. postsuckle			$P \leq 0.0001$		
Within vaccine		Presuckle vs. postsuckle			$P \leq 0.0001$		
Placebo vs. vaccine		Presuckle			$P \leq 0.4501$		
		Postsuckle			$P \leq 0.0269$		
		Colostrum			$P \leq 0.0397$		