Figure 8. Number of days of abnormal stool scores during oocyst shedding in Deccox® treated and control calves.

CONTROLS	875mg	1750mg	3500mg
5/7	0/6	0/1	2/4
3/6	2/9	0/3	2/9
3/10	1/1	1/1	4/9
3/5	3/9	3/7	7/7
	0/4	0/5	0/1
		0/2	
Mean 3.5	1.2	.67	3

(SIGNIFICANT DIFFERENCE)

Figure 9. Average daily gain between Deccox® treated and control calves challenged with *Cryptosporidium* oocyst

CONTROLS	875mg	1750mg	3500mg
.31	.55	.55	-.24
.21	.38	.66	.62
.38	.48	.38	.62
.59	.45	.83	.07
	.55	.41	.59
		.83	
Mean .3725	.482	.61	.33

The result of the trial, although not conclusive, suggests there may be some beneficial clinical response using Deccox® with experimental cryptosporidial infections. It would be seen beneficial to investigate this in a larger experimental trial or actual field study.

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The Use of J-5 *E. Coli* Common Core Antigens in Controlling Bovine Endotoxemic Disease

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Abstract

Intramammary infections with coliform organisms can lead to endotoxemic disease in the dairy cow, as well as localized disease in the mammary gland itself. Gram negative bacterial core antigen technology is now being used to help prevent both localized and systemic effects of endotoxins. Information concerning the use of an Escherichia Coli Bacterin Toxoid J-5 Mutant Vaccine, J-Vac™ produced by Sanofi Animal Health, in combating an experimental coliform infection in lactating dairy cows will be presented.

Introduction

The endotoxins produced by gram negative bacteria can be devastating to various classes of livestock. Endotoxins produced in cases of coliform mastitis can be especially dangerous and troubling in the dairy cow. Coliform mastitis has become a very prevalent form of mastitis, especially as contagious mastitis appears to be better controlled over the last few years. In addition, some of the most severe cases of clinical mastitis are caused by coliform organisms.

Recent technology has developed a new weapon against the endotoxins formed by the coliforms, the core antigen vaccine. Endotoxins are made up of three main portions: 1) the overlying 0-specific polysaccharide, 2) underlying core antigen and 3) lipid A fraction. It has been found that antibody formed against the core antigen, which has a similar configuration across all gram negative bacteria, is protective against the endotoxins from the coliform group of bacteria. The core antigen technology is based on rough mutations from some gram negative bacteria (*E. coli* and Salmonella) with defects in the overlying O-specific chain, exposing the underlying, cross protective core antigen, allowing for the creation of a truly multivalent anti-endotoxin vaccine.

Objective

The purpose of this study was to determine if an *E. coli* Bacterin Toxoid J-5 Mutant Vaccine (J-Vac™, Sanofi Animal Health) would protect lactating dairy cows against an intramammary challenge with *E. coli* and it's associated endotoxins. An earlier study by Sanofi[1] has shown protection against heterologous endotoxin challenge by the subcutaneous route utilizing this same vaccine.

The study was conducted by Dr. James Cullor and staff from the University of California at Davis.

Methods

The vaccine used in the study is a bacterin-toxoid containing a J-5 rough mutant *E. coli* and Sanofi's proprietary adjuvant (J-Vac™).

Fifteen pregnant dairy cows from a large California dairy herd were used in the study. Ten of the animals were vaccinated at dry-off (50-60 days precalving) and again about 4 weeks later (2-3 weeks pre-calving). The dose was 2.0 ml injected subcutaneously. Following the two vaccinations, all of the cows remained in good health and calved normally.

Five animals at about the same stage of pregnancy were retained without vaccination to serve as controls.

At 30-60 days after freshening, the challenge was administered by canula into the left rear quarter. The challenge was a fresh, 18-20 hour culture of *E. coli* strain 0 1 1 1:B4 diluted to contain approximately 4 X 10^4 (40,000) bacteria per 1.0 ml of challenge.

Following challenge, milk samples were examined for bacterial counts at 0 (pre-challenge), 12, 24, 36, 60, 84, 108, and 132 hours. Unclotted blood samples at the same time periods were evaluated for total white blood cell counts (WBC) and absolute neutrophil counts (PMN). Clinical signs to be observed were primarily those of quarter inflammation (heat, swelling and firmness).

Milk production was monitored for 132 hours following challenge from both the vaccinated and control cows.

Serum samples were also taken on day 0 (pre-vaccination) and on days 7, 14 and 24 to examine the Elisa titers obtained from vaccinated cows after one (1) injection as compared to the control cows. The antigen made from whole cell J-5 rough mutant *E. coli* bacteria.

Results

Bacteriological Analysis (Plate counts on blood agar)

None of the prechallenged samples contained bacteria. Eight of the 10 challenged vaccinates had either no CFUs (3 animals) or very low counts (less than 200 CFU/ml). Two challenged vaccinates had moderate counts at 12 and 24 hours (2,000-20,000 CFU/ml). In contrast, all 5 challenged control cows shed moderate to large numbers of bacteria (20,000->5,000,000 CFU/ml) for longer periods of time than any of the vaccinates (more than 60 hours).

Hematology

WBC - The total WBC of 8 of 10 challenge vaccinates did not vary from normal counts for any of the blood samples taken during the post-challenge period. The other 2 vaccinates had a greater than 50% drop in total counts at only 1 sample period (12 or 24 hours). In contrast 4 of 5 challenged controls demonstrated a greater than 50% depression of WBC at the 12 and 24 hour sample periods.

Table 1. Experimental Coliform Mastitis Study
WBC Counts (x 1000)

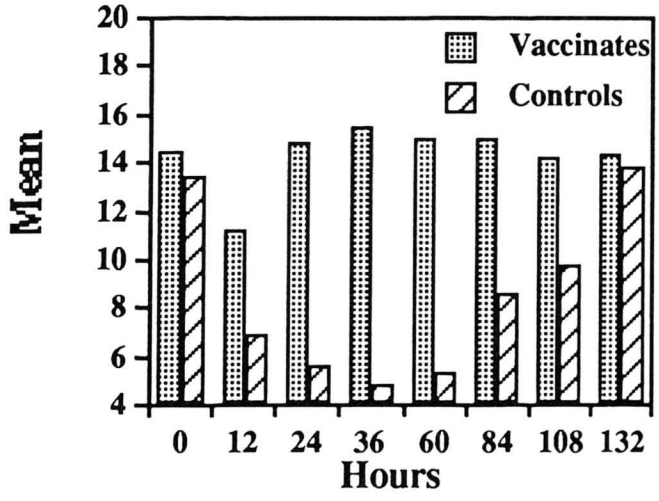
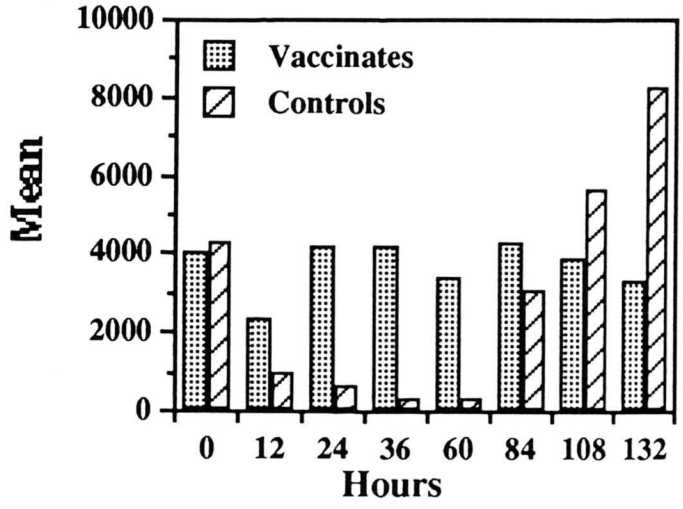


Table 2. Experimental Coliform Mastitis Study
Absolute PMN Counts



Absolute PMNs - Two challenged vaccinates had a PMN count of <1,000 at only one sample time (12 or 24 hours post-challenge). The PMN counts of all others were in the normal range for all of the post-challenge period. The 5 challenged controls, however, showed marked depression (<1,000) of PMNs at the 12, 24, 36 and 60 hour sampling times.

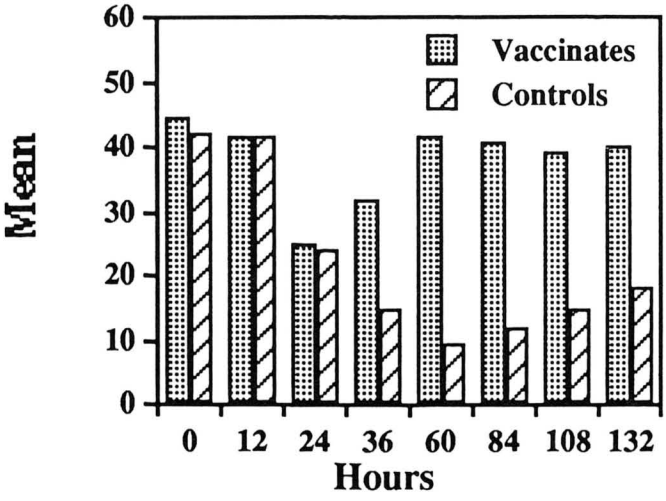
Milk Production

Nine of the 10 vaccinates had only a slight (<20%) drop in milk production at 12 or 24 hours post-challenge. One vaccinate had a >50% drop in milk volume at the 12 and 24 hour sampling time. All 5 challenged controls experienced a greater than 50% reduction in milk production and 2 of these almost completely lost milk volume for the balance of the post-challenge test period (>90% drop from pre-challenge volume).

Clinical Quarter Observations

Six of 10 challenged vaccinates showed little, if any, quarter inflammation. Four vaccinates had moderate inflammation for only one or two observation periods (12, 24 or 36 hours). All controls demonstrated moderate inflammation of the challenged quarter starting at 12 hours, persisting throughout 72 hours.

Table 3. Experimental Coliform Mastitis Study
Milk Production (lbs)



Serological Responses

The table below shows the results of the post-vaccination titers:

Table 4. Geometric Means

		Post-Vaccination		
Group	**Pre-Vac**	**7 days**	**14 days**	**21 days**
Vaccinates	<246	<1131	1970	6400
Controls	<200	<200	230	264

Although this serology test is not a specific indicator of protection against endotoxin, it suggests antibody formation against the core antigen, which would be protective against endotoxin.

Conclusions

This study demonstrates the protection provided by a gram negative core antigen vaccine, derived from a rough mutant *E. coli* adjuvanted vaccine. Marked differences were noted by a decreased amount of quarter inflammation with minimal milk volume loss in vaccinated cows; only slight depressions of the total WBC and absolute PMN in the vaccinated animals, contrasted to the control cows; as well as a large difference in bacterial shedding noted in the challenged vaccinated cows compared to the control animals. In addition, the serology results suggests that the J-Vac™ vaccine can elicit a strong antibody response to an *E. coli* core antigen.

References

1. Safety, Immunogenicity and Protection of Cattle Against Heterologous Endotoxin Challenge Following Vaccination With An *E. coli* J-5 Bacterin-Toxoid. Field, M.F.; Sheddrick, K. E.; Cullor, J.S.; Anderson, G.A. Sanofi Animal Health, Lenexa, KS 66285 and Dept. Vet.Pathology, School of Vet. Med., U. of CA, Davis 95616. Poster presented at the Conf. of Research Workers in Animal Diseases. Chicago, IL, Nov. 1992.

"The sun never sets on the Bovine Practitioner."