

Safety and Efficacy of Gram-Negative Bacterial Vaccines

J.S. Cullor, DVM, Ph.D.

*Department of Pathology, Microbiology and Immunology
School of Veterinary Medicine
University of California
Davis, CA 95616-8739*

Abstract

Many of the bacterial pathogens that threaten our farm animal species are Gram negative organisms. It is equally notable that many of the foodborne human pathogens of animal origin are also Gram negative bacterial contaminants. This report will discuss the new approach of immunizing against common core antigens of Gram negative bacterial pathogens. In fighting against this diverse group of pathogens, we must also address the historical problem of adverse vaccine reactions associated with Gram negative vaccines. Methods that were employed at the University of California in developing a safe, efficacious, and cost effective immunogen for coliform mastitis will be presented. Several commercial preparations with gram negative antigens are on the market today, while others will soon be approved for sale to the public. Salient discussions regarding what questions should be asked concerning vaccine safety and efficacy are in order.

Introduction

In the Code of Federal Regulations (9 CFR) the testing terminology for safety in vaccine approval is defined as the "freedom from properties causing undue local or systemic reactions when used as recommended or suggested by the manufacturer." Unfavorable reactions are defined as "overt adverse changes which occur in healthy test animals subsequent to initiation of a test and manifested during the observation period prescribed in the test protocol which are attributable either to the biological product being tested or to factors unrelated to such product as determined by the responsible individual conducting the test." Even though USDA:APHIS attempts to evaluate the safety of veterinary biologicals prior to their release for sale to animal agriculture, deaths, illness, and adverse reactions due to vaccine administration continue to be a daily event in the United States and elsewhere. This same section of the 9 CFR defines **vaccine efficacy** as the "specific ability or capacity of the biological product to effect the result for which it is offered when used under the conditions recommended by the manufacturer."

The veterinary profession is coming under closer scrutiny in the area of recommendations for maintaining animal health and well-being on the farm. Some areas of emphasis of health professional recommenda-

tions that are being reviewed are those that pertain to residue avoidance of antibiotics, hormones, pesticides, herbicides, and now, potential human pathogen contamination of the animal on the farm. In the context of Preharvest Food Safety issues, vaccines will no longer be purchased simply as inexpensive "insurance policies" against animal diseases. The manufacturer of the vaccine will be encouraged by producers, veterinarians, consumers, and perhaps required by USDA, to demonstrate that their product does not temporarily suppress immune defenses and open a window of opportunity for infection with either animal infectious disease entities or human pathogens. The day may come when the efficacy data sets for veterinary food animal biologicals will be divided into label claims for animal pathogens and label claims for immunizing farm animals against human pathogens. The market potential for both of these approaches already exist today.

Prerequisites for determining vaccine safety and efficacy

The most important first step in this regard is the absolute identification of the causal organism for the disease and the verification of relevant epitopes for eliminating the challenge organism. Next, one must establish that an immune response can protect against the disease in question. The data sets generated for this determination may need to include aspects of both humoral and cellular immune responses. Lastly, the manufacturer must be certain that the risks of immunization do not exceed those associated with the chance of contracting the disease.

Basic principles of immunoprophylaxis

1. Immunization--practiced for the purpose of providing the host defense an enhanced capability to reduce the challenge dose of the infectious agent before it can create a disease state that adversely affects the health of the animal.

Safety Testing the UC Davis Experimental J5 *E. coli* Antigen Preparation

2. Targeted immunity--the biological must induce immunity against relevant epitopes of specific infectious agents.
3. Awareness among those administering the biological that animals with an impaired immune response exist in each population of animals, and this can affect the level of protection against the pathogen.
4. Storage, handling, proper administration are all important components in affecting efficacy of the immunogen.
5. Poor management can overcome good immunology anytime.

Example: process for evaluating the safety, and potency of a potential Gram-negative vaccine.

Gram-negative vaccines (immunogens) must be evaluated for animal safety requirements before entering a test herd. Initial safety parameters to be determined by the manufacturer that should be available to the practitioner are the following: a) the amount of free endotoxin present in the vaccine preparation, b) the immune response and clinical reaction of the test subjects to the adjuvant and vaccine antigen being employed in the study, c) the host serological and clinical response to the dose of immunogen to be injected, and d) determining the most efficacious route and frequency of vaccine administration. Part D is important because the market place may want a 1- or 2-shot regiment, but the bovine immune system may require a 3-shot system in order to provide the best protection for the animal. Therefore, as the primary health advisor, which do you recommend: a) the more sought after 1-shot or 2-shot system that offers some protection, or b) the 3-shot system that provides the best opportunity for optimal protection? The vaccine manufacturer should be able to provide the data sets necessary to support their claims and thus, make this decision.

Further understanding of the subclinical and clinical effects of endotoxin present in vaccine preparations necessitates continued investigation. Safety and tolerance levels for endotoxins in biologicals have not been established by any U.S. agency. The variability in endotoxin potency among Gram-negative lipopolysaccharides will become a major consideration in producing such guidelines. Additionally, the ability of manufacturers to "mask" the presence of endotoxin in their preparations will be of concern. This may be done by adding polymyxin B as a preservative, increasing the oil adjuvant above 50% in the antigen mixture, or employing alhydrogel in the vaccine preparation. Simply because an assay such as the LAL indicates one level of endotoxin in the preparation does not mean that there will not be adverse consequences when the vaccine is administered in various biological systems.

Traditional Gram-negative vaccine preparations have been plagued by problems of adverse reactions in the host species, thus earning the distrust of many veterinarians and producers. The objective of this series of investigations was to determine the safety of an alternative *Escherichia coli* immunogen, *E. coli* (strain J5), in food animal species.

The limulus lysate test (LAL) was used to (1) determine endotoxin levels at various growth stages of the antigen preparation, (2) evaluate a procedure directed towards reducing the amount of endotoxin units (EU's) present in the antigen preparation of many different Gram-negative bacteria, and (3) determine the amount of endotoxin present in the final vaccine preparation. The usual conversion of 5 EU's per nanogram of endotoxin applies to all of the figures in this report. This assay demonstrated that the J5 strain of *E. coli* produced significantly lower amounts of endotoxin than does *Salmonella dublin* when both were grown under identical conditions (Table 1). Next, we were able to determine that multiple washing procedures significantly reduce the amount of endotoxin present in the antigen preparation. Following multiple washes of the vaccinal antigen, the amount of free endotoxin activity present in the UCD immunogen remained below a dose of 150 EU's/ml of vaccine. In contrast, commercial Gram-negative immunogens contain thousands of EU's/ml of vaccine, and may contain up to millions of EU's/ml of free endotoxin as measured by the LAL (Table 2). As producers become more aware of the possible adverse reactions that can result from immunization protocols, the veterinarian in charge of herd health programs must be aware of the endotoxin levels present in the vaccines being administered to the animals. For example, veterinarians may need the products tested and then weigh safety and efficacy considerations in selecting which immunogen is to be administered (Table 2).

The UCD J5 *E. coli* antigen preparation did not produce adverse reactions in bovine or porcine neonates, adults, or study subjects in advanced stages of pregnancy. Over 1.5 million doses of immunogens containing the J5 *E. coli* antigen have been administered to dairy cattle in California to date. This antigen presents a low risk, efficacious tool for animal agriculture in an arena that has been troubled with reports of adverse reactions in the host.

This table presents data that depict the substantial difference between the production of free endotoxin by the J5 *E. coli* vaccine antigen and by a *Salmonella dublin* vaccine antigen when both were grown under identical conditions (a 24-hour culture in trypticase soy broth). It also shows the dramatic reduction in detect-

able endotoxin levels after subsequent washings of the antigen preparations. Note that even after the multiple washings, the cell pellet of the *S. dublin* product still contained a substantial level of free endotoxin compared with that of the UC Davis experimental J5 *E. coli* vaccine.

Table 1. Endotoxin Production (Endotoxin Units/ml): [J5 *E. coli* versus *Salmonella dublin*]

SAMPLE SET	J5 <i>Escherichia coli</i>	<i>Salmonella dublin</i>
BROTH	5,000	40,000
WASH #1	1,000	80,000
WASH #2	50	850
WASH #3	25	500
CELL PELLETT	15	10,000

A Coliform Mastitis Vaccine: The Common Core Antigen Approach

Bacteria can evade attack by the immune system in many ways. The most common approach is to change the way they look. This is often termed "antigenic variation". With this strategy, the bacteria take advantage of the immune system by using the fact that antibodies are generally quite specific in what they can recognize and then attach (bind) to on the bacteria's cell surface. The bacteria constantly change their outer structure appearance; thus, putting the immune system continuously behind in producing a new type of antibody. This is one of the reasons why a mastitis vaccine may work one year quite well on a dairy, and fail miserably the next year.

A solution to this clever tactic is to enhance the immune system's capability to: a) recognize a part of the bacteria that it cannot easily change, and b) choose a structure that is common to all coliform bacteria (i.e. *E. coli*, *Salmonella*, *Klebsiella*, etc.). This has been accomplished by identifying a region on the interior of the bacterial cell wall that is common to all coliform bacteria. This region contains "Common Core Antigens" that the immune system can recognize and produce antibodies that will bind to the interior of the cell wall of coliform bacteria. This region is exposed to the immune system when the bacteria are in their most rapid phase of growth, called "log phase".¹ The "original" common core vaccine developed to take advantage of this approach in fighting coliform mastitis is the J5 *E. coli* bacterin.²

Once again, the goal of immunization against coliform mastitis is to abort the infection early in the disease process so the patient is not faced with the situation of combating the affects of prolonged endotoxin release. The most efficient way to accomplish this goal

Table 2. Comparison of Endotoxin Units (EU) in Some Commercially Available Vaccines

Product*	Endotoxin Content (EU/ml) ^a
UCD J5 experimental immunogen	≤100
J5-TC (<i>E. coli</i> core bacterin) ^b	≤100-1,825
Immvac (<i>Salmonella</i> core antigen) ^c	2,000-10,000
Piliguadr [®] <i>E. coli</i> -1 (Lot 261068) ^d	2,930,000
Lepto-5 ^e	52,500
Somna Tech [®] (<i>H. somnus</i> bacterin) ^e	117,000
Bovishield (IBR-PI-3-BVD-Vibrio-Lepto-5) ^f	143,000
Scour Guard 3 (K)/C ^g	38,800
Salmo Shield T [®] (<i>S. typhimurium</i> bacterin) ^h	2,975
<i>S. dublin</i> / <i>typhimurium</i> bacterin ⁱ	33,875
TriVib-5L ^j	155,000
One-Shot ^k	97,200
Somnugen-2P ^l	226,500
Bar Sommnus/Lepto 5 ^m	414,250
Clostri Shield 8 ^j	10.1
Fermicon-CD ^o	0.51
BRSV Vac 4 ⁿ	2.4
Elite 4 ^o	3.9
Premier 4 ^o	11,500

* The pyrogenic threshold for pharmaceutical compounds is 5 EU/kg body weight

* At this level, a 700 Kg cow would have 3,500 EU as the maximum target amount

^a Endotoxin levels determined via LAL methodology by Associates of Cape Cod, Inc., Woods Hole, MA.

^b The Upjohn Co., Kalamazoo, MI and Poultry Health Laboratory Associates, Davis, CA.

^c IMMVAC, Columbia, MO.

^d Schering-Plough Animal Health, Kenilworth, NJ.

^e Fermenta Animal Health, Omaha, Neb. (Biocor)

^f Norden Laboratories, Lincoln, Neb. (SKB)

^g SmithKline Beecham Animal Health, Exton, PA.

^h Grand Laboratories, Larchwood, IA

ⁱ Colorado Serum Co., Denver, CO.

^j Fort Dodge Labs, Ft. Dodge, IA.

^k SmithKline Beecham Animal Health, Exton, PA.

^l Bioceutic, St. Joseph, MO.

^m Anchor Labs, St. Joseph, MO.

ⁿ Bioceutic, St. Joseph, MO.

^o Diamond Sci., Des Moines, IA.

is to help the cow kill the bacteria early in its most rapid growth phase; thus, reducing the challenge dose of bacteria available to release endotoxin. This is exactly the approach employed by the J5 common core antigen concept. The vaccine helps the immune system build antibodies that recognize common core antigens present in hundreds of different serotypes of Gram-negative bacteria. These antibodies bind (opsonize) to the bacte-

ria, this promotes enhanced phagocytosis of the bacteria and results in reducing the challenge dose of the pathogen. Bacterial growth has been inhibited, thus decreasing the exposure of the quarter and the cow to the release of endotoxin. Therefore, the vaccine has addressed a primary requirement in host defense.

The second requirement is to diminish the exposure of the quarter and the cow to the effects of endotoxin release. This can be accomplished by providing antibody that will bind to the endotoxin. This objective is also met by the J5 antigen because it contains Lipid A in its structure, and this is the endotoxic moiety in Gram-negative bacteria. This is an advantage because no additional endotoxin is required to be added to the vaccine preparation.

• **To decrease the severity of acute coliform mastitis:**

1. **Bacterial growth must be inhibited to reduce the exposure of the quarter and the cow to the release of endotoxin**
2. **The effects of the endotoxin release must be neutralized**

The common core antigen approach of the J5 *E. coli* vaccine helps the cow meet these important host defense criteria.

J5 *E. coli*. The "Original" Core Antigen Vaccine for Coliform Mastitis

Researchers in the School of Veterinary Medicine at the University of California at Davis conducted a field study designed to ascertain if this vaccine approach might be beneficial. They observed that cattle with naturally occurring low antibody titers to the UCD J5 *E. coli* vaccine experienced a five-fold increase in risk for clinical coliform mastitis. This led to the development, safety testing, and efficacy trials conducted by faculty and staff at the UC Davis School of Veterinary Medicine and the UC Veterinary Medical Teaching and Research Center, Tulare, California.³⁻⁶ The experimental vaccine that was employed in many of these studies possessed the lowest endotoxin unit/ml of vaccine (≤ 100 EU/ml) of any bacterial immunogen on the market today. This series of investigations has demonstrated that this vaccine is a safe, efficacious, and cost effective immunogen. The studies have been performed at different sites and the same animals have been given the vaccine for at least three consecutive years with no adverse reactions. There have been approximately 1.5 million doses of this immunogen used in California to this date. DeGraves and Fetrow determined in a partial budget analysis that the J5 *E. coli* vaccine could produce an economic benefit

of \$57.00 per cow receiving the vaccine.⁷ However, this immunogen is not a miracle potion. Coliform mastitis can occur in vaccinated animals, and this vaccine will not reduce the rate of *Streptococcal* species or *Staphylococcal* species mastitis. Nevertheless, the administration of the *E. coli* J5 vaccine in these studies did reduce the incidence and severity of natural challenges to the bovine mammary gland by coliform bacteria. The subsequent reduction in clinical cases directly translates into reduced utilization of antibiotics in therapeutic regimens and this in turn, converts into a decreased risk for antibiotic residues in milk and meat.

A Field Trial of the J5 *E. coli* Vaccine in Ohio Dairies

The efficacy of a J5 *E. coli* bacterin was examined as to its capability to prevent naturally occurring intramammary infection and clinical mastitis in a 225 cow commercial herd over a 2.5-year period.⁸ Study subjects with odd-numbered identification were immunized with the J5 bacterin, and cows with even-numbered identification served as unvaccinated controls for each lactation during the study. Immunizations were given via subcutaneous injection on the upper part of the rib cage just posterior to the scapula; cows were immunized at drying off, 30 days after drying off, and at calving. The percentage of quarters infected at calving with Gram-negative bacteria did not differ between treatment groups. During the first 90 days of lactation however, a total of 67% of the Gram-negative bacterial intramammary infections present at calving in control cows developed into clinical disease compared with only 20% of the infections developing into disease in the J5 *E. coli*-immunized cows. It is clear in this report that immunization with the J5 *E. coli* bacterin did not reduce the rate of Gram-negative bacterial intramammary infection at calving but did reduce incidence of clinical mastitis.

Discussion

The series of investigations presented in this discussion has demonstrated that the J5 *E. coli* antigen preparation is a safe and efficacious immunogen. It is not a miracle potion, however. Coliform mastitis can occur in vaccinated animals, and this immunogen does not reduce the rate of streptococcal or staphylococcal mastitis present in the herd. Nevertheless, the administration of the J5 *E. coli* vaccine in these studies was protective against natural challenges of the bovine mammary gland by Gram-negative bacteria and significantly reduced the incidence of clinical coliform mastitis. A reduction in clinical cases should directly translate into reduced utilization of antibiotics in therapeutic regi-

mens which; in turn, decreases the risk of antibiotic residues in dairy products.

• Vaccine Safety: A Dairy Perspective

If the immunogen creates a situation where the dairy cow is more susceptible to mastitis, a drop in milk production occurs, and the SCC goes up --- Have we really helped the cow or the dairyman? (Figure 1)

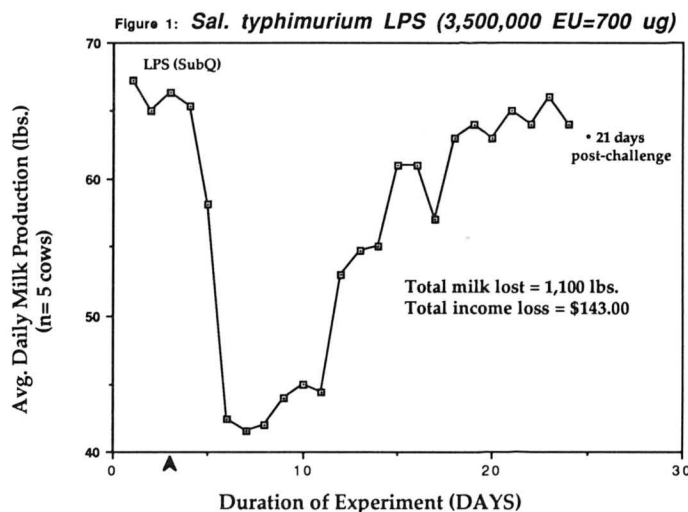
1. USDA safety requirements are in place, yet illness and deaths due to vaccine administration continue to occur
2. Safe or Tolerance levels for endotoxin(s) in vaccines are not known for any species
3. No pyrogenic threshold has been established for cattle

It is my opinion that we must step back and consider points 1-3 and perform the appropriate experiments to correct this situation in the approval process for veterinary biologicals. We will never eliminate adverse reactions, but when considering the well-being of a farm animal, we must stop and ponder whether the immunization either creates a window of opportunity for infectious disease to take hold, increases milk somatic cell counts and adversely affects milk quality, or suppresses milk production to the point that the economic benefit of immunizing for any disease is really in question. I know this may be a bit of a stretch, but dairyman can measure milk production losses after vaccinating cows during lactation. In addition, if the biological product makes a lactating cow sick enough to reduce milk production, the dry cow will experience the same insult to homeostasis. Therefore, animal health and well-being when vaccinating the dry cow is an important data set to be provided for any veterinary biological.

Although endotoxin content of a vaccine is only one of several risk factors in causing adverse reactions, attempts to reduce the amount of endotoxin in vaccine preparations (Table 2) is an issue that can be addressed by currently available production technologies. The experimental vaccine produced and tested by the University of California College of Veterinary Medicine is an example that this is an important point to be evaluated. The advantage of low endotoxin content in the vaccine preparation has been proven to be an advantageous vaccine safety consideration in California dairies for the past our years.

Additional parameters that should be evaluated for each vaccine with a label claim for vaccinating lactating cows are as follows:

Figure 1. The resulting milk and economic loss after administering a total dose of 3,500,000 Endotoxin Units subcutaneously in a saline vehicle to lactating dairy cattle.



1. Monitor milk production
 - At least 2x/day each day of the trial
 - 7 days prior to first immunization through 7 days after immunization
 - Study subjects must be grouped by stage of lactation and level of milk production
2. Additional Parameters to be monitored
 - Rectal temperature each morning and evening for entire trial
 - Somatic Cell Count (quarter composite); am/pm milking for the entire trial
 - Milk microbiology- each Tuesday and Thursday

The necessity of feeding the world's population will increase the importance of animal agriculture. The demand for safe foods of animal origin, with particular attention paid to preventing animals leaving the farm with potential food-borne human pathogens, is going to re-emphasize our responsibility to animal health and well-being. Therefore, prevention of animal disease by employing safe, efficacious, cost effective immunogens will once again emerge as an important tool in addressing Preharvest Food Safety concerns on the farm.

References

1. Tyler J.W., Spears H., Cullor J.S., et al. Antigenic homology among gram-negative organisms isolated from cattle with clinical mastitis. *J Dairy Sci* 74:1235, 1991.
2. Cullor J.S. The *Escherichia coli* J5 vaccine: Investigating a new tool to combat coliform mastitis. *Vet Med*,

August, pp.836, 1991. 3. Gonzalez R.N., Cullor J.S., Jasper D.E., Bushnell R.B., *et al.*: Prevention of clinical coliform mastitis in dairy cows by amutant *Escherichia coli* vaccine. *Can J Vet Res* 53:301-305, 1989. 4. Borelli C.L., L.D. Weaver and J.S. Cullor. Effect of *E. coli* J5 vaccination on incidence of clinical coliform mastitis and milk production loss in dairy cows. (Abstr. #12) Eleventh Annual Food Animal Disease Research Conference, Reno, NV, March 7-8, 1990. 5. Cullor J.S. Mastitis in dairy cows: Does it hinder reproductive performance? *Vet Med* August, pp. 830, 1991. 6. Tyler JW,

Cullor JS, Osburn BI, *et al.*: Relationship between serologic recognition of *Escherichia coli* O111:B4 (J5) and clinical coliform mastitis in cattle. *Am J Vet Res* 49:1950-1954, 1988. 7. DeGraves F.J. and Fetrow J. Partial budget analysis of vaccinating dairy cattle against coliform mastitis with an *Escherichia coli* J5 vaccine. *JAVMA* 199(4):451, 1991. 8. Hogan JS, Smith KL, Todhunter DA, Schoenberger PS: Field trial to determine efficacy of an *Escherichia coli* J5 mastitis vaccine. *J Dairy Sci* 75(1):78-84, 1992.

© Copyright American Association of Bovine Practitioners; open access distribution.

