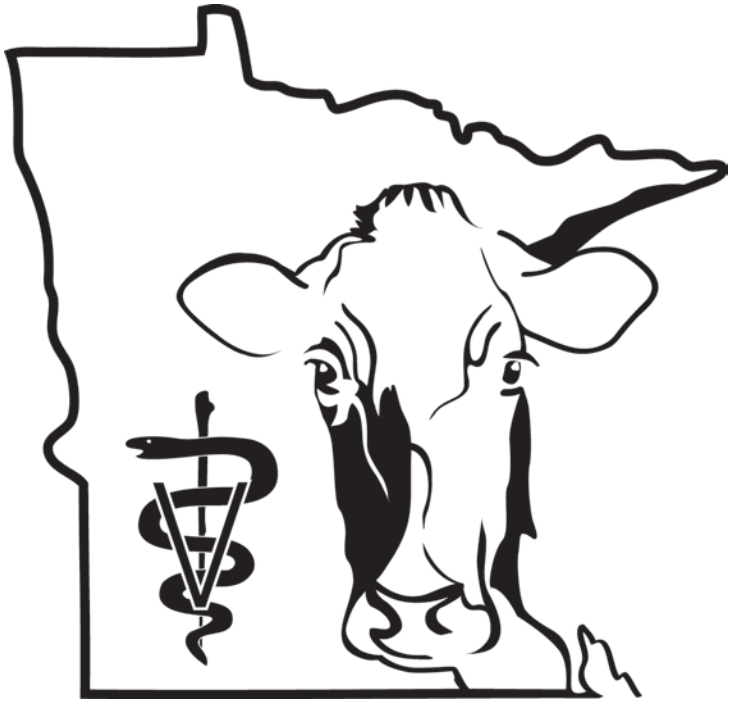
THIS ARTICLE IS SPONSORED BY THE
MINNESOTA DAIRY HEALTH CONFERENCE.

UNIVERSITY OF MINNESOTA

College of Veterinary Medicine

VETERINARY CONTINUING EDUCATION



ST. PAUL, MINNESOTA
UNITED STATES OF MINNESOTA

THE FUNCTION OF VACCINES IN THE DETERRENCE AND MODERATION OF CLINICAL MASTITIS

James S. Cullor DVM, PhD.
Department of Pathology
School of Veterinary Medicine
University of California
Davis, CA 95616-8739

Bovine mastitis: A recently completed survey documented that mastitis is the single most common disease syndrome in adult dairy cows, accounting for 38% of all morbidity (2). On an annual basis 3 of every 10 dairy cows will have clinically apparent inflammation of the mammary gland. Seven per cent of affected cattle will be culled and 1% will die as a consequence of the disease. The same survey presented data suggesting that in excess of 25% of all disease related economic losses can be directly attributed to mastitis.

Mastitis may result following the introduction of microorganisms through the teat sphincter, either as a result of the daily milking routine or occurring during the treatment of a clinical case of mastitis (Table 4). The clinical course will vary with the ability of bacteria to colonize and thrive in the mammary gland, their inherent virulence, and the host response. The inflammation of the mammary gland that follows will present with a wide variety of clinical signs. However, common pathogenic mechanisms may permit the development of systematic treatment, control, and preventative measures. In general mastitis can be subdivided into two broad and overlapping categories on the basis of the source of the infectious inoculum.

I. Major causes of contagious mastitis in the bovine

1. *Streptococcus agalactia*
2. *Streptococcus dysgalactia*
3. *Staphylococcus aureus*
4. *Staphylococci-* coagulase negative
5. *Mycoplasma bovis*

II. Major causes of bovine mastitis associated with the farm environment

1. *Escherichia coli*
2. *Klebsiella pneumoniae*
3. *Enterobacter aerogenes*
4. *Streptococcus faecalis*
5. *Streptococcus faecium*

Antibiotic residues: Antibiotic therapy for bovine mastitis is not without its risks for economic loss to the dairy industry. Antibiotic contamination of dairy and meat products poses a potential health risk to a small percentage of the human population. Residue avoidance in foods eliminates this health risk and the economic losses of product not reaching the consumer. Antibiotic contamination of the milk supply is of additional concern to creameries. Residues are capable of inhibiting the growth and activity of bacterial cultures used in the processing of many dairy products. Mastitis therapy should be administered in accordance with label instructions and the latest policies and recommendations of state and federal governing bodies. Every consideration concerning dosage and withholding periods for milk and meat of treated animals or neonates consuming milk or colostrum containing antibiotics must be followed. Rational administration of antibiotics is further complicated by limited numbers of agents approved for use in the lactating dairy cattle. When careful and considered appraisal of a clinical situation suggests extra-label (non-approved host species, dose, or route of administration) use of antibiotics, the veterinarian assumes direct responsibility for safety of the prescribed treatment and potential contamination of the human food chain. Such extra-label antibiotic usage presumes a current and active practitioner-client relationship, a knowledge of pharmacokinetics and drug clearance, adequate patient identification, and permanent treatment records. An important source of information concerning antibiotic residues is the: Food Animal Drug Residue Data Bank (FARAD:916-752-7507).

Basic mastitis control program: Remember, there is no effective method of "treating" your way out of problems created by the numerous organisms associated with bovine mastitis (Table 1). Mastitis control on the dairy is first designed around sound management techniques (Table 2, Table 3). Every producer, veterinarian, and those associated with allied industries that serve the dairy industry should be aware of the importance of good milking hygiene and proper milking machine function, as well as the other time-proven techniques mentioned in the tables. The purpose of this paper is not to go through all of these details again. However, there is absolutely no substitute for good management on the dairy production unit. Remember, poor management can overcome good immunology at any time in the production scheme.

The role of vaccines in the deterrence and moderation of clinical mastitis: The concept of immunization (vaccination) to substitute for lapses in proper management is absolutely the wrong path to journey for any length of time. *Vaccines cannot prevent the bacteria from colonizing the end of the teat where they remain poised for entry into the mammary gland. The vaccine can aid in aborting the new infection earlier in the disease process.* One obstacle encountered when employing "vaccinating for mastitis", is that those involved will tend to place too much confidence in the vaccine preparation and will neglect the important use of hygienic milking and proper milking-machine use and care. The purpose of vaccines for the producer is one of a **"supplemental role"** to other effective nutritional and management practices. In both experimental as well as herd-wide immunization trials on dairy production units, workers have prepared various kinds of bacterins, toxoids, or mixed bacterins-toxoids. These have included organisms and their toxins or other cellular or extracellular components. Organisms included have been those isolated from the affected herd in question (autogenous vaccines), or were multivalent vaccines which included more than one organism.

Regardless of the type of immunogen, dose, and schedule, there is a wide variation in animal response to the antigen. It seems that there is a strong likelihood that each herd will contain three categories of animals: 1) "non-responders" to the vaccine, 2) animals that respond "moderately", and 3) the animals that will be classed as "high responders"(Table 5). Some factors involved in this varied response include:

1) *Age:* The neonatal bovine does not respond to vaccine antigens as well as the adult.

2) *Stage of lactation:* Those subjects in early lactation (<45 days) may not respond as well as later in lactation.

3) *Type of antigen:* Humoral immunity to bacterial antigens is does not last as it does against viral antigens

4) *Identifying the important region of the mastitis pathogen:* If the vaccine produces an immune response to an unimportant portion of the pathogen, it will not result in helping the subject fight the infection.

5) Heavily parasitized or malnourished animals may be immunosuppressed (i.e. inadequate protein, micronutrients, Vit. E/Selenium in the ration)

The literature contains both reported successes and failures for various vaccines; therefore, a few comments seem appropriate. The successes must be accompanied with more than "testimonial" data. Sound experimental methodologies and statistical evaluations must be appropriately applied and evaluated in the efficacy studies (4).

Vaccine failures: The first scenario is quite simple when one encounters an **"Unsatisfactory vaccine"**. In this case, either the wrong strain of the organism was used to produce the immunogen or the vaccine is considered inadequate because of an inappropriate dose or mixture of antigen in the preparation.

The second scenario considers apparent vaccine failures with a **"Satisfactory vaccine"**. In this case, the situation breaks down into two pathways.

A. Satisfactory administration of the immunogen: When an apparent failure occurs in this system, the decision tree has two categories:

1. The animal is already incubating the disease and the immunogen is thus administered too late into the disease process

2. The subject fails to mount an immune response. This may be the result of the following:

   a. Biological/Genetic variation of the host species
   b. The host is immunosuppressed due to stress, viral infections, poor nutrition, etc.
   c. Prior passive immunization that results in interference with the immune response

The category of "stress" may include pregnancy, extremes of heat and cold, sorting into groups, fatigue and malnourishment. Any one, or a combination of these categories may reduce the magnitude of the normal immune response by the host, perhaps because of an increased production of corticosteroids.

B. **Unsatisfactory administration of the immunogen:** This breaks down to either an inadequate dose of the vaccine or perhaps death of a reportedly "live" antigen preparation.

Coliform mastitis: What's new in the area of vaccines?

J5 E. coli immunizations reduce the incidence of clinical coliform mastitis: Following initial observations that cattle with low naturally occurring serum titers recognizing the J5 *E.coli* experienced a 5-fold increase in the risk of clinical coliform mastitis (5), researchers at UC Davis and the VMTRC at Tulare, CA. conducted a series of experiments investigating the efficacy of R-mutant bacterins in reducing the incidence of coliform mastitis. These studies confirmed that immunization with J5 *E.coli* reduced the incidence of clinical coliform mastitis.

Study 1 (Safety Testing the J5 E. coli Antigen Preparation): 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 employed to: 1) determine endotoxin levels at various growth stages of the antigen preparation, 2) evaluate a procedure directed towards reducing the amount of endotoxin present in the antigen preparation of many different gram negative bacteria, and 3) determine the amount of endotoxin present in the final vaccine preparation. This assay demonstrated that the J5 strain of *E. coli* produced significantly lower amounts endotoxin on a CFU/ml basis than *Salmonella dublin*. We were able to determine that the strategy of implementing multiple washing procedures will significantly reduce the amount of endotoxin present in the antigen preparation. Therefore, when multiple washes of the vaccinal antigen were employed, the amount of free endotoxin activity present in the UCD immunogen remained below a total dose of 30 nanograms.

In contrast, commercial gram-negative immunogens contain >100 μg to milligram quantities of free endotoxin as measured by the LAL. This presents a potential problem when employing multiple vaccines that contain gram-negative antigens. If these preparations contain microgram to milligram amounts of free endotoxin, the total dose of free endotoxin may be enough to create a situation for "mediator shock" to occur.

This 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 1.7 million doses of immunogens containing the J5 antigen have been either administered or

purchased to this 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.

Study 2: UCD J5 Vaccine Field Trial: The field trial was implemented in two commercial California dairies (1). The treatment group, 246 cows, received three doses of a whole cell bacterin of J5 *E. coli* plus Freund's incomplete adjuvant (two in the dry period and one after calving) while 240 unvaccinated cows served as controls. A total of 35 cases of clinical coliform mastitis were diagnosed, six in J5 immunized cows and 29 in the unvaccinated group. Four control cows were culled, three of them because of chronic coliform mastitis, and one because of post-coliform mastitis agalactia. The incidence rate of clinical gram-negative mastitis was **2.57%** in J5 vaccinated cows and **12.77%** in the unvaccinated control cows. The results of this field trial indicate that the administration of the J5 *E. coli* antigen preparation is protective against natural challenge to gram-negative bacteria, and reduces the incidence of clinical gram-negative mastitis in dairy cows during the first three months of lactation[14].

Study 3: VMTRC J5 Vaccine Field Trial: This study employed a different immunization schedule than the UCD study described above. The protocol implemented a placebo vaccine and subcutaneous injection of the J5 *E. coli* immunogen: **First injection:** between 182-195 days gestation, **Second injection:** 210-223 days gestation, and **Third injection:** administered between 238-251 days of gestation. Four hundred and forty-one Holstein dairy cows on one dairy in the San Joaquin Valley of central California were randomly assigned to treatment (n=212) and placebo (n=229) groups. Milk samples from the quarters observed with clinical mastitis were collected aseptically for microbiological examination before treatment and for 4 consecutive days following the initiation of the clinical event. Clinical cases were considered positive for coliform mastitis if two of the five samples were positive for coliform organisms. The results were as follows: **J5 immunized group= 7 cases of clinical coliform mastitis; Placebo group= 25 cases of clinical coliform mastitis.** Once again, there was a statistically superior performance of the J5 immunized group over the control subjects.

Study 4: UCD Commercial J5 Vaccine Field Trial: A proprietary version of the UCD J5 *E. coli* research vaccine has been produced by Poultry Health Laboratory (Davis, CA.). Their **"J5-TC"** immunogen is available for sale to veterinarians only in California with current label claims directed at reducing the incidence of clinical coliform mastitis in dairy cattle. It is currently a three dose regiment, usually administered at drying off, again 30 days later, and the last dose at calving. Our preliminary results indicate excellent safety (i.e. no abortions, adverse reactions, etc.) with this immunogen. The treatment group (424 animals) has statistically fewer cases than the placebo group (421 animals). The incidence of clinical coliform mastitis is 65%% less in the J5-TC group.

It is my opinion that this series of investigations has demonstrated that this antigen preparation is a *safe* and *efficacious* immunogen. This immunogen is not a miracle

potion. Coliform mastitis can occur in immunized animals and this immunogen will not reduce the rate of *Strep.* or *Staph.* mastitis. However, the administration of an *E. coli* J5 vaccine is protective against natural challenges of the bovine mammary gland by gram-negative bacteria and significantly reduces the incidence of clinical coliform mastitis. 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 dairy products.

Clinical disease is the end-product of pathogen, environmental and host factors. Environmental contamination, meteorologic stressors and impaired host defenses may all tip the balance between health and disease. Traditional definitions of health and disease may not be adequately descriptive in livestock species, where the unstated goal is optimal productivity, rather than clinical normalcy. Three basic requirements exist for cross-reactive immunity to gram-negative disease: 1) the existence of common or shared structure, 2) the ability of this shared antigen to induce an immune response, and 3) this immune response must confer protection. From a practical viewpoint, the development of vaccinal reagents for all possible pathogens and opportunists cannot be considered a reasonable or productive strategy. The use of R-mutant bacteria as vaccinal antigens holds the greatest promise in the disease syndromes lacking a single, distinct etiologic agent or alternatively, as an adjunct to specific antimicrobial therapy.

Staphylococcus mastitis: What's new in the area of vaccines?

Bovine mastitis caused by *Staphylococcus aureus* continues to be responsible for major economic losses to the dairy industry. Autogenous bacterins have been employed extensively over the years in a relatively unsuccessful battle against Staph mastitis. However, scientists have gone back to the basics and re-evaluated methods to identify the important portions of the mastitis pathogen that establishes its ability to cause disease or escape the immune response[3,7]. It is now known that many *Staph. aureus* isolates from mammary gland secretions express a capsular material that is an important virulence factor. Encapsulation of the bacteria becomes a basic defense mechanism by inhibiting the complement-mediated response for phagocytosis by blocking the C3b molecule attachment to the bacteria.

A paper was presented at the International Symposium on Bovine Mastitis (Indianapolis, IN) that described a field trial investigating a new type of antigen preparation[6]. The vaccine contains a *Staph. aureus* antigen which was grown under circumstances designed to simulate conditions encountered in the live animal, staphylococcal toxoids, and an adjuvant. The immunogen was used in 5 commercial dairies in Australia. The study subjects were given intramuscular injections of either the test antigen or the placebo at 8 and 4 weeks before parturition. The authors reported: 1) clinical mastitis caused by *Staph. aureus* was reduced by 45-52% in those subjects receiving the test antigen, 2) numbers of subclinical mastitis cases were reduced by 18%, and 3) new subclinical infections with *Staph. aureus* were reduced by 25% in vaccinates. It appears that this antigen preparation is being tested in the United States and the efforts to bring this to the dairy industry are continuing at this time.

Similar new approaches are being employed at research institutions in the United States. For example, Dr. Norcross (Cornell University) has been conducting numerous investigations in this area for the past 10 years. Heightened resistance to bovine mastitis caused by *Staph. aureus* has been achieved in recent experiments.

It appears that all of the efforts of past research endeavors are beginning to come to fruition for the dairy industry. Significant advances are being made in new vaccine preparations for both coliform and staphylococcus mastitis. Additional research and testing will be required for commercialization; however, the re-examination of old vaccine technologies and subsequent improvements will bring economic benefit to the dairy industry in the near future. These products, as well as others, will serve as useful tools in the overall management scheme of the commercial dairy.

• All too often, cattle receive the vaccine when it is convenient to the management scheme rather than according to the schedule that may most benefit the cow's immune system. Remember that the reported mastitis vaccine successes must be accompanied with more than testimonial data. Sound experimental methodologies and statistical evaluations must be appropriately applied and evaluated in the efficacy studies.[10-13]

Three fundamental issues are essential when attempting to grant cross-reactive immunity to gram-negative disease: (1) the existence of common or shared structure between various gram-negative pathogens, (2) the ability of this shared antigen to induce the appropriate immune response, and (3) the ability of this immune response to confer protection. From a practical viewpoint, the development of immunogens for all possible pathogens and opportunists cannot be considered a reasonable or productive strategy. The use of R-mutant bacteria as immunogens holds great promise in the control of disease syndromes that do not possess a single, well-defined etiologic agent or, alternatively, as an adjunct to specific antimicrobial therapy[4,8,9,12-17]. Several products employing diverse applications of immunizing with "common core antigens" of gram-negative bacteria are likely to appear in the marketplace in the near future. Currently, there are several "look-a-like" vaccines that use testimonial claims for efficacy against coliform mastitis. When statistical data sets are examined, however, the J5 *E. coli* vaccine is the only one on the market with a mastitis claim that has over 3 years of continuous study in experimental and field trial conditions.

THE ECONOMICS ASSOCIATED WITH USING THE J5 E. COLI VACCINE

An article by DeGraves and Fetrow[13] describing the budget analysis of immunizing dairy cattle against coliform mastitis provides an excellent framework for deciding whether a mastitis vaccine is an economically viable tool to employ on the dairy. The spreadsheet layout includes the following parameters: (a) herd inputs, (b) clinical manifestations of the mastitis entity involved, (c) intervention description (e.g., the ability of the vaccine to alter the clinical case rate), (d) disease costs, (e) intervention costs, (f) profit analysis, (g) investment summary and break even analysis, and (h) sensitivity analysis.

The dairy industry is made up of a set of extremely diverse dairy farm management systems, and many factors are involved in the range of profitability for each of these

operations. Therefore, to determine the profitability of a vaccination program in a particular dairy, the herd inputs must be gathered and analyzed. The most important variable in the model is the incidence of mastitis caused by the target organism on the farm in question. It is imperative that accurate records of laboratory diagnostic results be available to aid in making this determination. Another critical point in the analysis is the estimated vaccinated-to-unvaccinated risk ratio for cows with clinical mastitis caused by the target organism. The final decision for determining the relative risk of not using the vaccine in the herd is heavily dependent on the capability of the vaccine to favorably alter the clinical case rate of the mastitis problem in question. Veterinary clinicians and scientists, must be able to critically evaluate the efficacy of products and not just take at face value what they may hear. Where's the data? If the product does not contain a label claim for the mastitis etiology of interest, how can a client's profit or loss margin be accurately determined? The bottom line is how accurately can the clinician document the magnitude of the mastitis problem and the etiological agent responsible for this dilemma. With this information in hand, the clinician must next decide if the data presented truly supports the efficacy claim of the vaccine manufacturer.

The advantage of having the scientific data to support the ability of the vaccine to reduce the clinical case rate of the mastitis pathogen of interest is shown by DeGraves and Fetrow.[13] In partial budget analysis supported the immunization of dairy cattle with the J5 *E. coli* immunogen. Increased profits of $57 per cow lactation were predicted when appropriate information was used in their model. This analysis indicated that herd immunization programs with this vaccine would be profitable when more than 1% of cow lactations resulted in clinical coliform mastitis, and the program was predicted to be profitable at all herd milk production levels.

Dairy producers cannot manage mastitis problems with vaccines and antibiotics alone. Proper milking procedures and hygiene, clean bedding, and good nutrition are prerequisites to successful mastitis control. Optimal milking machine function must be maintained on a daily basis, and herd health programs that reduce and focus the use of antibiotics should also be implemented. The true economic impact of using mastitis vaccines can be evaluated when each of these areas have been addressed.

Acknowledgment: The mastitis research pertaining to the *E. coli* J5 vaccine was supported by the California Milk Advisory Board, the USDA Formula Fund, and the Livestock Disease Research Laboratory (LDRL).

References

1. Gonzalez RN, JS Cullor, DE Jasper, et al.: Prevention of clinical coliform mastitis in dairy cows by a mutant *Escherichia coli* vaccine. Can J Vet Res 53:301-305, 1989.

2. Hird, D.W., B.J. Weigler. 1989. National Animal Health Monitoring System Technical Report. Submitted to the Veterinary Services, Animal and Plant Health Inspection Service.

3. Norcross, N.L. and J.P. Opdebeeck. 1983. Encapsulation of *Staphylococcus aureus* isolation from bovine milk. Vet. Microbiol. 8:397.

4. Sears, P.M., P.B. English and R.N. Gonzalez. 1989. Historical controls versus randomized controls in veterinary clinical trials. J Dairy Sci. 72(1):261.

5. 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 1988;49:1950-1954.

6. Watson, D.L. and C.L. Schwartzkoff. Sept. 13-16, 1990. A field trial to test the efficacy of a staphylococcal mastitis vaccine in commercial dairies in Australia. International Symposium On Bovine Mastitis: Indianapolis, IN., pg. 73.

7. Watson, D.L. and N.A. Watson. 1989. Expression of a pseudocapsule by *Staphylococcus aureus*: influence of cultural condition and relevance to mastitis. Res. Vet. Sci. 47:152.

8. Smith BP: Understanding the role of endotoxins in gram negative disease. Vet Med 12:1148-1160, 1986.

9. Smith BP: Bovine salmonellosis. Calif Vet 4:27-30, 1980.

10. Hogan JS, Weiss WP, Todhunter DA, et al: Efficacy of an *Escherichia coli* J5 mastitis vaccine in an experimental challenge trial. J Dairy Sci 75(2):415-422, 1992.

11. 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.

12. Cullor JS: The *Escherichia coli* J5 vaccine: Investigating a new tool to combat coliform mastitis. Vet Med, pp 836-844, Aug 1991.

13. DeGraves FJ, Fetrow J: Partial budget analysis of vaccinating dairy cattle against coliform mastitis with an Escherichia coli J5 vaccine. JAVMA 199(4):451-455, 1991.

14. Howell D: Survey on mastitis caused by environmental bacteria. Vet Rec 90:654-657, 1972.

15. Dupont HC, Spink WW: Infections due to gram-negative organisms. An analysis of 860 patients with bacteremia at the University of Minnesota Medical Center 1958-1966. Medicine 48:307-312, 1969.

16. Cullor JS, Fenwick BW, Williams MR, et al: Active immunization with *E. coli* J5 and its protective effects from endotoxic shock in calves. In Immunobiology and immunopharmacology of bacterial endotoxins. New York, Plenum Publishing, 1986, pp 265-268.

17. Cullor JS, Fenwick BW, Smith BP, et al: Decreased mortality and severity of infection from salmonellosis in calves immunized with *E. coli* (strain J5). Proceedings of the sixty-sixth Annual Conference of Research Workers in Animal Disease, Chicago, November 1985 (abstract 352).

Table 1 (Bovine milk isolates): The wide variety of bacteria reported to be isolated from "mastitic cows"

a.*Staphylococcus spp.*
b. *Streptococcus agalactia*
c. *Streptococcus dysgalactia*
d. *Streptococcus uberis*
e. *Escherichia coli*
f. *Mycoplasma spp.*
g. *Pseudomonas aeruginosa*
h. *Serratia marcesens*
i. *Pasteurella multocida*
j. *Aerobacter aerogenes*
k. *Spheropherous necrophorous*
l. *Klebsiella pneumoniae*
m. *Nocardia asteroides*
n. *Actionmyces bovis*
o. *Mycobacterium tuberculosis*
p. *Streptococcus pyogenes*
q. *Clostridium perfringens*
r. *Coxiella burneti*
s. *Brucellae*
t. *Leptospirae*
u. *Alcaligenes faecalis*

Fungi: *Candida spp.*, *Cryptococcus neoformans*, *Trichosporon*

Some viruses have been considered, but no specific virus isolated

Table 2: Basic Mastitis Control Program

1. Correct milking management and properly functioning milking machines
2. Use an approved teat dip on all cows immediately after milking
3. Implement "dry-cow treatment" as a standard herd health practice
4. Medical management of all clinical cases of mastitis during lactation
5. If *Streptococcus agalactia* is in high prevalence, initiate therapy during lactation
6. Segregate cows with Staphylococcal mammary gland infections away from the "normal" herd
7. Cull cows with chronic mastitis
8. Control populations of environmental organisms
9. Be certain replacement animals are "free" of detectable mammary gland infections

Table 3: Ways of Reducing New Mammary Gland Infection Rates

1. Good milking machine design and proper function

2. Good milking management and hygiene practices

3. Dip teats in sanitizer after milking and allow to dry (Reduces new infection rate by about 50% in most cases)

4. To reduce coliform infections, dip teats before milking: be certain to wipe dry before milking the cow. Final concentration of 0.25 to 0.05% iodine if using iodophor dip.

Table 4: Major causes of bovine mastitis acquired during therapeutic intervention

Product or Water Contamination

a. *Pseudomonas aeruginosa*
b. *Yeasts* or *Fungi*
c. *Nocardia asteroides*

Poor sanitation: (Equipment, Drug inventory, etc.)

a. *Bacillus cereus*
b. *Mycobacterium fortuitum*
c. *Coliforms*
d. *Prototheca*
e. *Mycoplasma*

• Tables 5 and 6 introduce the consideration of vaccine safety as it relates to gram-negative immunogens. Although no pyrogenic thresholds have been established for cattle, such limits have been established for pharmaceutical agents. This upper limit of endotoxin has been set at 5 Endotoxin Units (EU) per Kg of body weight. A 700 Kg dairy cow should receive no more than a total dose of 3,500 EU's if this limit was applied to vaccines. As you can clearly see, our common immunization protocols can surpass this limitation, and we do not know all of the adverse consequences that may result from exceeding this limit. This issue will be discussed further in the presentation.

Table 5 Endotoxin Production (EU/ml): [J5 E. coli versus Salmonella dublin]

SAMPLE SET	J5 ESCHERICHIA COLI	SALMONELLA DUBLIN
BROTH	1,000	8,000
WASH #1	200	16,000
WASH #2	10	170
WASH #3	5	100
CELL PELLET	3	2,000

• 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 *S. 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 detectable 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 J5 *E. coli* vaccine.

Table 6 Comparison of Endotoxin Units (EU) in Some Commercially Available Vaccines

Product•	Endotoxin Content (EU/ml)[a]
UCD J5 experimental immunogen	100
J5-TC (*E. coli* core bacterin)[b]	100-1,825
Immvac (*Salmonella* core antigen)[c]	2,000-10,000
Piliguard® *E. coli*-1 [d]	1,465,000
Lepto-5[e]	52,500
Somna Tech® (*H. somnus* bacterin)[f]	117,000
Bovishield(IBR-PI-3-BVD-Vibrio-Lepto-5) [g]	143,000
Scour Guard 3 (K)/C[h]	38,800
Salmo Shield T® (*S. typhimurium* bacterin)[i]	2,975
S. dublin/typhimurium bacterin[j]	33,875
TriVib-5L[k]	155,000

• The pyrogenic threshold for pharmaceutical compounds is 5 EU/kg body weight
[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] Fermenta Animal Health, Omaha, Neb. (Biocor)
[g] Norden Laboratories, Lincoln, Neb. (SKB)
[h] SmithKline Beecham Animal Health, Exton, PA.
i Grand Laboratories, Larchwood, IA
[j] Colorado Serum Co., Denver, CO.