

ENDO VAC-Bovi[®]

with **IMMUNE[®]**
Plus



A Better Method for Managing Clinical Mastitis

- + Increases Lymphocytes and Antibody Levels
- + Offers Earlier Protection
- + Reduces Annual Losses Due to Clinical Mastitis



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What is gram-negative ENDOTOXEMIA?

Gram-negative endotoxemia contributes to the signs associated with coliform mastitis,² diarrhea septicemias,⁵ and pneumonias⁹ in cattle.

The signs of endotoxemia can be alleviated by anti-endotoxin antibodies.⁵ An enzyme-linked immunosorbent assay of sera from control and vaccinated calves showed that antibodies produced in response to a mutant Salmonella typhimurium bacterin-toxoid reduced the clinical responses to both Escherichia coli and Pasteurella endotoxins,⁵ thus achieving cross-protection via core antigen technology.

How does core antigen technology work?

Lipid A, the toxic moiety of endotoxin, appears to be identical in all gram-negative bacteria, although endotoxins from different gram-negative bacteria exhibit slightly different toxicities. Differences are related to the biochemical arrangement and complexity of the sugars and side chains that compose the mucous capsule of different gram-negative bacteria. These sugars and side chains are covalently bonded to Lipid A through keto-deoxyoctanoic acid.

What is ENDOTOXIN?

Endotoxin is the key component of the cell walls of all aerobic and anaerobic gram-negative bacteria (Figure 1). When the cell dies, the cell wall is disrupted and endotoxin is released. Most endotoxin entering the body is rapidly eliminated by the liver; however, when the cow's clearance mechanisms are overwhelmed, endotoxin enters the blood stream. There it rapidly affects the diseased cow's immune system, heart function, blood pressure, and ability to control body temperature. The classic symptoms of endotoxemia are depression, fever, labored breathing, staggering, and finally, collapse.

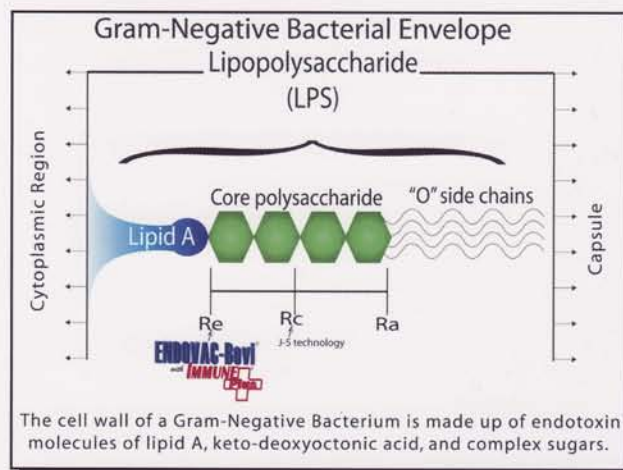
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The current serotyping system automatically associates *E. coli* serotype K99 with calf diarrhea, *Pasteurella haemolytica* 1 with shipping fever complex, and a variety of *E. coli* serotypes with coliform mastitis. These bacteria, which cause endotoxin-associated diseases, are classified by sero-agglutination of their capsular materials ("O" side-chains). However, these and most other gram-negative bacteria have a common antigenic core (Lipid A and keto-deoxyoctanoic acid) in their cell walls. This common core offers the opportunity to genetically engineer workable cross-protective vaccines.⁶

In the search for a "universal" vaccine, various laboratories have isolated or genetically engineered rough mutants of gram-negative bacteria that have temporarily or permanently lost the ability to produce part (*E. coli* J-5)^{10,13} or all (*S. typhimurium* R/17)¹⁷ of their capsular or "O" side-chain carbohydrates. Vaccines prepared with these rough mutants expose the bacterium's usually protected naked core to the cow's immune system. Because this core is common to all Enterobacteriaceae and to most other gram-negative bacteria, humoral (B-lymphocyte stem cells) and cell-mediated (T-lymphocyte stem cells) immunity elicited by these genetically engineered vaccines is cross-protective for essentially all gram-negative bacterial diseases. These vaccines are used in conjunction with some of the new immune modulators (e.g., IMMUNEPlus[®] and muramyl dipeptide), which selectively potentiate the B- and T-lymphocyte stem cells.¹⁷ It appears that these vaccines offer a solution to the problems caused by endotoxin.

How can this technology aid in the fight against mastitis?

Historically, coliform mastitis, which is often accompanied by



The cell wall of a Gram-Negative Bacterium is made up of endotoxin molecules of lipid A, keto-deoxyoctonic acid, and complex sugars.

Figure 1 Core Antigen Technology

endotoxemia, has been treated with antimicrobials. Unfortunately, antimicrobial administration in lactating cows requires the disposal of milk during the administration period and during the withdrawal time. Often, milk must be disposed of for two weeks. Treatment expenses and the loss of income due to milk disposal may cost the dairy owner more than \$1,500 per case. What's more, antimicrobials may facilitate the persistence of antimicrobial-resistant gram-negative serotypes, and thereby increase the pool of resistant pathogens on a given dairy farm.¹⁴ Vaccination, therefore, would seem to offer a better method for managing Coliform mastitis.

In dairy cows, Coliform mastitis is most commonly associated with *E. coli* bacteria and endotoxins.¹⁰ Because there is no way of knowing in advance which specific serotype of a particular species of gram-negative bacteria is responsible for any given case of Coliform mastitis, it is impossible to formulate effective broad-spectrum homologous vaccines. Such vaccines would need to contain numerous gram-negative bacterins to provide any degree of cross-protection.

A logical approach, then, to formulating an efficacious vaccine would be to use a single antigen that induces the immune system to produce antibodies that cross-protect against gram-negative organisms and their endotoxins. Specific R-mutants of a *Salmonella* and *E. coli* have been found to provide such cross-protection against septicemias and endotoxemias arising from various gram-negative infections. The antibodies produced by these bacterins, which are coupled with a potent immune stimulant, have provided cross-protection to cows and horses either naturally-challenged or arbitrarily-challenged in the laboratory. Independent studies of California and Arizona dairy cows, for example, have shown that mutant gram-negative bacterins lowered the incidence and severity of Coliform mastitis.^{10,8}

What is endotoxin's role?

Diarrhea invariably alters the balance of the intestinal microflora. Impaired movement of luminal contents and increased water content of evacuated feces decrease the numbers of lactobacilli and homofermentative streptococci. Accompanying this change is a concomitant increase in the numbers of Enterobacteriaceae. [The normal death of these increased numbers of Enterobacteriaceae increases endotoxin in the gut lumen.] Endotoxin, aided by the damaged mucosal barrier and greater vascular permeability, can then enter the circulation. When endotoxemia complicates the diarrhea syndrome, it creates a potential for life-threatening, irreversible hemorrhagic shock, disseminated intravascular coagulation, and acute oliguric renal failure.

Increased calving intervals. Increased calving intervals have been associated with low levels of anti-endotoxin antibody in dairy cows.¹⁹ Although increased calving intervals are a more subtle manifestation of gram-negative endotoxemia, they do suggest that sublethal endotoxemia may cause early embryonic death and aberrant cycling in cows. Therefore, reducing the incidence of endotoxemia may help eliminate these problems.

Better colostrum is key (B1). Colostrums can be enhanced with good cow vaccination. More antibody (higher titer) in the colostrums means more antibody passed on to the calf, thus creating longer duration of immunity in the calf.²¹

Endotoxemia's role. Endotoxemia and endotoxic shock are serious complications of Coliform mastitis. Endotoxemia results from the release of endotoxins through the death of gram-negative bacteria, such as *E. coli* during Phagocytosis by udder leukocytes^{3,7} or by the action of antimicrobials used in treatment.¹¹ The clinical signs of coliform mastitis include serous secretion in the affected quarter or quarters, pyrexia, depression, anorexia, swelling and firmness of the affected quarter or quarters, ruminal hypo-motility, muscle fasciculation, cold skin temperature, and diarrhea - all signs of endotoxemia as well.

Treating mastitis. Traditionally, treatment of Coliform mastitis has been initiated only after the development of clinical illness. Therapy has been limited to the use of anti-inflammatory agents, fluid therapy, and combinations of antimicrobials such as oxytetracycline, chloramphenicol, gentamicin, kanamycin, and polymyxin B.

The chief disadvantage of initiating treatment after clinical illness has developed is that the disease has frequently advanced to an irreversible state. Moreover, this treatment requires withholding the cow's milk from market for several days, depending on the type and amount of drug used to counter the infection. Even with successful treatment, only 20% of mastitis cows ever return to normal production. Most are culled for agalactia.¹⁵ An additional concern is the development of drug-resistant salmonellae with the potential for entry into the food chain.

Preventing occurrence. Two methods are currently available for decreasing the prevalence of Coliform mastitis. First, better management of bedding and teat sanitation techniques decrease the exposure of teat ends to bacteria. Second, vaccination enhances the cow's immunologic resistance to environmental bacteria.

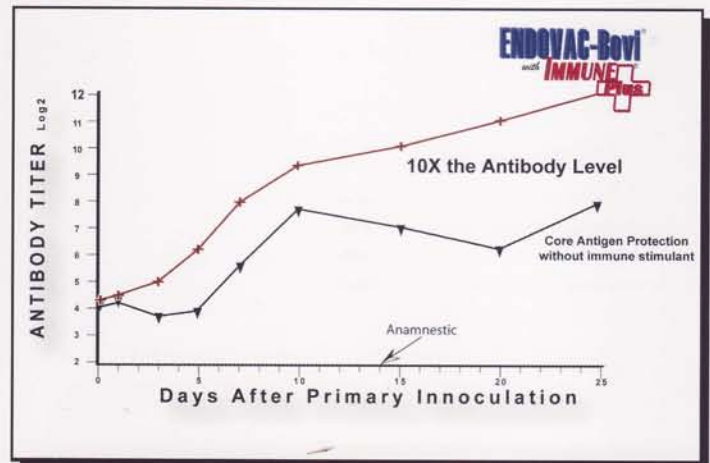


Table 1 Antibody Titer

Previously, vaccines were limited to three types: autogenous bacterial isolates expressing various specific antigens (K antigens or O-carbohydrate side-chains), live vaccines composed of attenuated or deletion-modified bacteria, and polyvalent vaccines composed of serotypes sometimes associated with mastitis.

Often, the use of autogenous vaccines is neither timely nor cost- or production-efficient because such vaccines are manufactured after the disease has developed. This is too late for the affected herd to develop active immunity. Live vaccines may revert to the wild-type parental strain and thereby become pathogenic for vaccinated animals. The primary disadvantage of polyvalent vaccines composed of multiple bacterial isolates expressing various antigenic epitopes (K antigens or O-carbohydrate side-chains) is that the bacterial isolates causing disease are subject to epidemiologic shifts and drifts in antigenic epitopes. If a shift or drift occurs, the vaccine is no longer efficacious.

Moreover, the K and O-carbohydrate (LPS/endotoxin) antigens are potent stimulators of inflammation. O-carbohydrate antigens are released on bacteriolysis in *E. coli* mastitis, increasing mammary blood flow and contributing to marked swelling of the gland.²⁶ Absorption into the blood stream can cause high fever, depression, and leukopenia, followed by leukocytosis, prolonged hypoglycemia, and, in severe cases, irreversible shock and death of the mastitic cow.²⁷

Better vaccines. Cross-protective vaccines have been manufactured using genetically engineered mutants such as the patented R/17 strain of *Salmonella typhimurium* and the J-5 strain of *E. coli*. A combination vaccine, ENDOVAC-Bovi® (IMMVAC), manufactured with the R/17 mutant and combined with an immune-potentiating adjuvant, significantly reduces the

devastating diseases caused by gram-negative bacteria producing various endotoxins.¹⁸ An 80% reduction in Coliform mastitis in cows vaccinated with a vaccine (J-5 TC) made of the J-5 mutant has been demonstrated.¹⁰ However, the J-5 *E. coli* mutant is characterized as an Rc mutant; thus, it possesses some of the sugars composing the O-carbohydrate side-chains.

The patented R/17 mutant, conversely, is an Re mutant totally devoid of O-carbohydrate side-chains and is referred to as a "naked-core" mutant.²² Vaccines composed of Re mutant, or naked core mutants, expose the core antigens of the bacterial cell wall to the cow's immune system for the subsequent production of cross-protection antibodies. This circumvents undesirable anaphylaxis and the specific problems associated with the polyvalent vaccines. These cross-protection antibodies aid the cow's liver in neutralizing *E. coli*, *Salmonella typhimurium*, *Pasteurella multocida* and *Pasteurella hemolytica* endotoxins.²⁶ What's more, the naked-core vaccines stimulate opsonizing antibodies that enhance phagocytosis of the *E. coli* bacteria causing Coliform mastitis.²³

Gram-negative bacterial endotoxins are also known to mediate *in-vivo* production of tumor necrosis factor (TNF)²⁴, which undoubtedly is involved in the cascade of events leading to Coliform mastitis. Other studies have shown that when antibodies of the immunoglobulin G (IgG) class are injected intravenously into rabbits, they suppress some of the undesirable effects of TNE.²⁵ Thus, one would expect that E3, the patented immune potentiator incorporated in ENDOVAC-Bovi®, also may stimulate the production of antibodies against TNE.

What is the effect of IMMUNEPlus®?

- Increases T-lymphocytes, thereby enhancing cellular immunity, which is important to protection against intracellular bacteria and viruses.^{12,16,20}
- Increases B-lymphocytes, thereby enhancing humoral immunity.^{4,12,16,20}
- Increases serum antibody levels that neutralize endotoxins and enhance bacterial killing.^{1,4,8}
- Decreases time required to develop antibodies, thereby affording earlier protection.^{4,12,16}

Calves vaccinated with ENDOVAC-Bovi® containing core antigen bacterin with IMMUNEPlus® were compared with calves receiving core antigen bacterin only. The graphs show antibody and lymphocyte levels in the blood of both groups. T- and B-lymphocytes were significantly increased in the calves receiving the core antigen with IMMUNEPlus®. (Table 2) Correspondingly, the 25-day anti-endotoxin antibody levels were over 10 times greater in calves receiving the vaccine containing IMMUNEPlus® than in calves receiving core antigen bacterin alone.^{4,8} (Table 1)

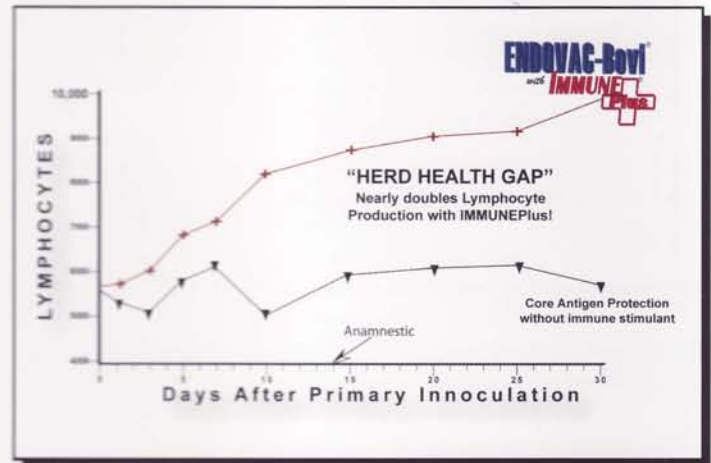


Table 2 Increase in Lymphocytes

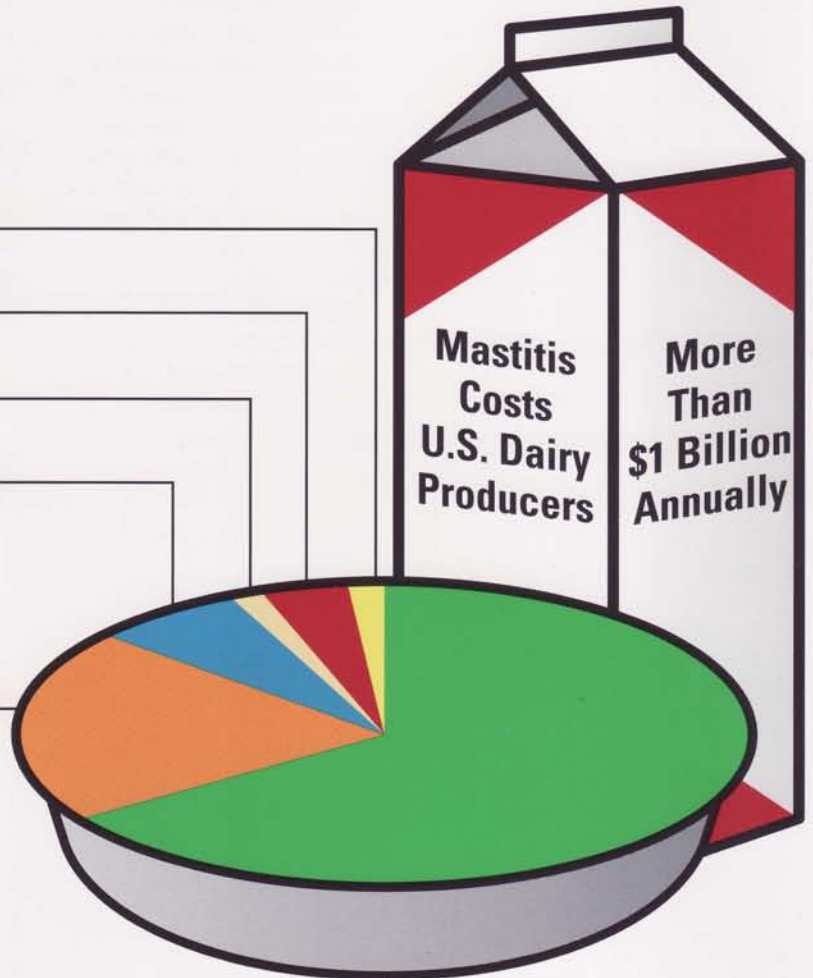
Why is protecting dairy cattle against environmental *E. coli* mastitis so important?

Ten percent to 12% of all lactating cows in the United States have mastitis. In 30% to 40% of these cattle, inflammation is due to *Escherichia coli*. Mastitis costs U.S. dairy producers more than \$1 billion annually. Diminished milk production, discarded milk, the need for replacement cows, the decreased sale value of cows, and the cost of drugs, veterinary services, and additional labor all contribute to the economic loss.

Cost Per Cow

Estimated Annual Losses Due to Clinical Mastitis

| Source of Loss | Loss Per Cow | % of Total |
|---------------------|--------------|------------|
| Veterinary Services | \$2.72 | 1.48% |
| Treatment | \$7.36 | 3.99% |
| Extra Labor | \$1.14 | 0.62% |
| Discarded Milk | \$10.45 | 5.67% |
| Replacement Cost | \$41.73 | 22.63% |
| Reduced Production | \$121.00 | 65.62% |



= \$184.40 Per Cow

Assumptions: One-third of cows infected in an average of 1.5 quarters; Milk loss 856 lbs/infected quarter; Milk price \$12.07/cwt

Source: National Mastitis Council, Current Concepts of Bovine Mastitis.

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