

## AGRI-PRACTICE – BACTERIOLOGY

In this study, 149 lightweight feeder steers were randomly divided into three groups. All steers were administered appropriate viral vaccines upon arrival and 10 days following arrival. Each steer in Group 1 was administered a cross-protective Re mutant *Salmonella typhimurium* bacterin-toxoid while each steer in Group 2 was vaccinated with an autogenous gram-negative bacterin combination. The steers in Group 3 were not vaccinated. The difference in the number of sick steers pulled from Group 1 was significantly less than the number pulled from Group 3. The difference in the number of sick steers pulled from Group 2 was not significantly different from Group 3. The significant reduction in the number of ill steers exhibiting clinical signs of respiratory disease in Group 1 compared to the number of sick pulls in Group 3 suggests that the cross-protective Re mutant *S. typhimurium* bacterin-toxoid is cross-protective against *Pasteurella*-induced endotoxemia.

### Cross-Protection of Feedlot Calves Against *Pasteurella* Endotoxemia With an Re Mutant *Salmonella typhimurium* Bacterin-toxoid

Leon Mills, DVM

RR #1

Herrington, Kansas 67449

#### Introduction

Feedlot calves suffering from the consequences of gram-negative endotoxic pneumonia have historically been treated with antimicrobials, electrolytes, and other support modes of therapy and or prophylactically vaccinated with gram-negative vaccines. Results of such rational therapies are often frustrating because they do not neutralize the precise problem.<sup>1</sup> The control of *Pasteurella* sp. pneumonias via prophylactic administration of a live *Pasteurella* vaccine<sup>2</sup> or bacterin<sup>3</sup> often results in less than satisfactory results. Recent studies have confirmed that endotoxins from various sources such as *Pasteurella* sp. and *Hemophilus* sp. can deliver the death blow to young calves and pigs, respectively, suffering from respiratory disease.<sup>4-7</sup> Gram-negative endotoxins are the common components of gram-negative bacterial cell walls that are released during rapid growth and upon bacterial cell death. The common denominator of all gram-negative bacteria is the core lipopolysaccharide cell wall structure (endotoxin) or core-antigen.

Historically, homologous antibodies produced in response to gram-negative bacterins made from organisms with complete "O" side chains have been effective in blocking specific endotoxins. These homologous vaccines, however, have not provided cross-protective antibodies because they have contained antibodies that were effective against only one serotype and it would have required the combination of many homologous bacterins to make a cross-protective vaccine that would have provided broad-spectrum protection against various endotoxins. The reality that there can be more than one gram-negative organism involved in a given illness and, therefore, more than one source of endotoxin can be involved in any given clinical illness, along with the fact there are various serotypes of gram-negative organisms in the environment, contribute to the essential need for development of cross-protective immune strategies. The search for cross-protective immune strategies that could be used to actively immunize or passively immunize individuals against endotoxemia has been the object of several investigative groups.

The recent development of a mutant bacterin has resulted in two cross-protective anti-endotoxin vaccines (Endovac-Bovi® and Endovac-Equi®: IMMVAC, Inc., Columbia, MO.), and an antiserum (Endoserum®; IMMVAC, Inc., Columbia, MO.) being made available to veterinarians for the first time.<sup>8-10</sup> Removal of the "O" side chains (which provide the many gram-negative bacteria for their individual characteristics [serotype]), was accomplished through mutation, thus, exposing the core antigen of the cell wall so that it could be presented to the immune system. Such naked core antigens common to most gram-negative organisms when injected as bacterins stimulate the host's immune system to produce antibodies against many gram-negative endotoxins. Classification of the "O" side chains to describe the relative absence of oligosaccharides is accomplished by assigning the letters Ra through Re with Re designating complete removal. The *S. typhimurium* used in the study reported here is an Re mutant ("O" side chains completely removed) while the J-5 mutant of *Escherichia coli*, also cited in this report, is an Rc mutant ("O" side chains partially removed).<sup>8,10,11</sup>

Vaccine containing such mutant bacterins used to hyperimmunize donor animals produces serum containing high levels of cross-protective anti-endotoxin antibodies. These same antibodies in antiserum from hyperimmunized donors passively immunize the recipient against many gram-negative endotoxins.

Successful cross-protective anti-endotoxin passive immunity has been demonstrated in calves<sup>8</sup> and horses<sup>10</sup> in experiments reported recently. These studies have confirmed that calves passively immunized with plasma derived from hyperimmunized donors vaccinated with mutant gram-negative bacterins were protected from endotoxin challenges.<sup>8,11</sup> Equids passively immunized with anti-core-antigen antibody antiserum and challenged under experimental conditions with a specific dose of heterologous endotoxin were also protected.<sup>9</sup> Furthermore, the commercially available antiserum (Endoserum) has been successfully used as an immunotherapeutic agent to treat and/or prevent endotoxemia in foals and horses for more than 2 years.

Recent studies have confirmed that *P. hemolytica*<sup>4</sup> and *P. multocida*<sup>9</sup> endotoxins produce severe respiratory signs. These studies also demonstrated that *P. hemolytica* endotoxin caused release of arachidonic acid from pulmonary artery endothelial cells,<sup>5</sup> development of lung lesions,<sup>1</sup> and development of severe clinical signs<sup>4,8</sup> in calves. Results of these studies strongly suggest that *Pasteurella* sp. endotoxins may result in serious respiratory disease and death in calves.

The purpose of this study was to test the effectiveness of vaccinating lightweight Holstein feeder steers with an Re mutant *S. typhimurium* bacterin-toxoid upon arrival and 10 days after arrival on the morbidity, mortality, and treatment costs due to respiratory disease during the first 30 days in a feedyard.

## Materials and Methods

The study was initiated by randomly dividing 149 lightweight feeder steers into three groups consisting of 50, 50, and 49 head, respectively. Each of these 149 calves was identified with an individual ear tag and randomly assigned to one of the three groups concomitantly placed in the same pen in a feedyard with a recent history of pasteurellosis. Each steer in Group 1 was administered 2 ml of the Re mutant *S. typhimurium* bacterin-toxoid IM upon arrival and 10 days following arrival; each steer in Group 2 was vaccinated with an autogenous combination vaccine containing autogenous *P. hemolytica*, *P. multocida*, and *Hemophilus somnus* bacterins (autogenous vaccine consisting of aluminum hydroxide precipitated *P. hemolytica*, *P. multocida*, and *H. somnus* bacterins) upon arrival and 10 days following arrival; and each of the steers in Group 3 were not vaccinated with any gram-negative vaccine. All 149 steers were injected with a vaccine consisting of killed BVD virus combined with modified live IBR, PI3, and BRSV viruses (Horizon™ I + VAC 3™: Haver/Diamond Scientific, Shawnee, Kansas) and another 7-way clostridial bacterin-toxoid (Site-guard® MLG: Cooper's Animal Health, Inc., A Pitman-Moore Co., Mundelein, Illinois) upon arrival and 10 days following arrival.

The reason cattle introduced into this feedyard were studied was because *P. multocida* organisms were consistently cultured from lung lesions at necropsy as well as from swabs taken from the nasal cavities of contemporarily ill calves from adjacent pens, previous occupants of adjacent pens, and immediately previous occupants of the same pen. Neither *Salmonella*, *E. coli*, or gram-negative organisms other than *Pasteurella* spp. were cultured from these cattle.

The number of ill calves removed (sick pulls) from the pen on the basis of exhibiting respiratory clinical signs was compared between the groups using Fisher's exact test in chi-square analysis of the data. The treatment costs were calculated on a per head basis for each group.

## Results

There were 5, 8, and 15 head pulled from Groups 1, 2, and 3, respectively, because they exhibited clinical signs of respiratory disease. The difference in the number of steers pulled from the Re mutant *S. typhimurium* bacterin-toxoid vaccinated group (5, 10% in Group 1) was significantly ( $P < 0.025$ ) less than the number pulled from the non-vaccinated group (15, 30% in Group 3). The difference in the number of sick steers pulled from the group that received the autogenous combination vaccine (8, 16% in Group 2) was not significantly ( $P > 0.05$ ) different from Group 3 nor significantly ( $P > 0.05$ ) different from Group 1. The per head cost of medicine for treating the sick pulls was \$1.80, \$2.42 and \$5.05 for Groups 1, 2, and 3, respectively. Results are summarized in Table 1.

## Discussion

The significant reduction in the number of sick pulls in Group 1 suggests that the mutant *S. typhimurium* bacterin-toxoid vaccine was cross-protective against gram-negative respiratory endotoxemia. Although rate of gain and feed efficiency were not compared in the three groups, one would suspect the rate of gain and best feed efficiency of the group with the lowest number of sick pulls would have had the highest rate of gain and best feed efficiency due to less overall stress. The \$3.25 difference in cost of medicine between Group 3 and Group 1 would have paid for the vaccine. Although the rate of gain was not calculated in any of the three groups, one would suspect that the group with fewer sick pulls would have gained more weight and exhibited a higher rate of gain than the others.

Additional studies on larger numbers of cattle measuring the same parameters as those quantitated in this study along with rate of gain, feed efficiency, and labor costs for treatment are indicated and will be the object of future studies.

## REFERENCES

1. Nash D: A Comparison of Three Therapeutic Programs for the Treatment of Shipping Fever in Weaned Calves. Bovine Respiratory Disease: A Symposium. Ed by R Loan, College Station, Texas A&M University Press, 1984, pp 471-472.
2. Purdy CW, Livingston CW, Frank GH, et al: A Profile of Lightweight Feeder Calves Vaccinated With Modified Live *Pasteurella hemolytica*. Bovine Respiratory Disease: A Symposium. Ed by R Loan, College Station, Texas A&M University Press, 1984, pp 464-467.
3. Shewen PE, Wilkie BN: Immunity to *Pasteurella haemolytica* Serotype 1. Bovine Respiratory Disease: A Symposium. Ed by R Loan, College Station, Texas A&M University Press, 1984, pp 480-481.
4. Slocombe RF, Mulks M, Killingsworth CR, et al: Effect of *Pasteurella haemolytica*-derived Endotoxin on Pulmonary Structure and Function in Calves. Am J Vet Res 51:433-438, 1990.
5. Paulsen DB, Confer AW, Clinkenbeard KD, et al: *Pasteurella hemolytica* Lipopolysaccharide-induced Arachidonic Acid Release from and Neutrophil Adherence to Bovine Pulmonary Artery Endothelial Cells. Am J Vet Res 51:1635-1644, 1990.
6. Cullor JS, Fenwick BW, Williams MR, Smith MP, 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, 1986, pp 265-268.
7. Fenwick BW, Cullor JS, Osburn BI, Olander HJ: Mechanisms Involved in Protection Provided by Immunization Against Core Lipopolysaccharides of *Escherichia coli* J5 from Lethal *Hemophilus pleuropneumoniae* in Swine. Infects Immun, 53:298-302, 1986.
8. Sprouse RF, Garner HE, Lager K: Cross-protection of Calves From *E. coli* and *P. multocida* Endotoxin Challenges Via *Salmonella typhimurium* Mutant Bacterin-toxoid. Agri Pract 11:29-37, 1990.
9. Garner HE, Sprouse RF, Lager K: Cross-protection of Ponies From Sublethal *E. coli* Endotoxemia by *Salmonella typhimurium* Antiserum. Eq Pract 10:10-17, 1988.
10. Sprouse RF, Garner HE, Lager K: Protection of Ponies From Heterologous and Homologous Endotoxin Challenges Via *Salmonella typhimurium* Bacterin-toxoid. Eq Pract 11:45-49, 1989.
11. Tyler JW, Cullor JS, Spier SJ: Immunity Targeting Common Core Antigens of Gram-negative Bacteria. J Vet Int Med 4:17-25, 1990.

**TABLE 1**  
**Feedlot Field Study<sup>a</sup>**

	Endovac-Bovi™ Vaccine (Group 1)	Autogenous Vaccine (Group 2)	No Vaccine (Group 3)
Total steers	50	50	49
Steers pulled	5	8	15
Steers repulled <sup>b</sup>	1	3	3
Dead	0	0	1
	1 <sup>c</sup>		
Treatment days	26	34	71
			(66) <sup>d</sup>
Treatment days/pull	5.2	4.25	4.4
			(4.4) <sup>d</sup>
Treatment costs/head	\$1.80	\$2.42	\$5.05
			(\$4.76) <sup>d</sup>

<sup>a</sup> Study sponsored by IMMVAC, Inc., Columbia, MO.

<sup>b</sup> Repulled = pulled out 1 or more times after the initial pull.

<sup>c</sup> One animal died at 1 month and 2 days.

<sup>d</sup> For respiratory disease only.