PEER REVIEWED

Efficacy of an *Arcanobacterium pyogenes-Fusobacterium necrophorum* Bacterin-Toxoid as an Aid in the Prevention of Liver Abscesses in Feedlot Cattle

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Abstract

Two studies were done to test the efficacy of a single-dose, bivalent *Arcanobacterium pyogenes - Fusobacterium necrophorum* bacterin-toxoid to reduce liver abscess prevalence and severity when given to cattle entering a feedlot. In each study, steers (~300/ treatment group) were randomized to pens and the pens randomly assigned to treatment groups. Treatment groups included 1) steers given a high-antigen dose bacterin-toxoid, 2) steers given a lower-antigen dose bacterin-toxoid, 3) steers fed tylosin-medicated feed, 4) control steers fed feed without tylosin, and in Study 2 only, 5) vaccinated steers fed tylosin-medicated feed. Steers were followed to slaughter and the liver abscesses scored, the USDA carcass and yield grades recorded, and the pen average daily gain and feed conversion calculated. Pen effects were not detected in the analysis of liver abscess prevalence data. A single vaccination with the high-antigen dose bacterin-toxoid reduced the prevalence of liver abscesses 48.4% (16% vaccinates vs 31% controls, P = 0.0001) in Study 1, and 37.5% (30% vaccinates vs 48% controls, P = 0.001) in Study 2.

Résumé

Deux études ont été menées pour évaluer l'efficacité d'une simple dose de bactérine-toxoïde bivalente obtenue d'*Arcanobaterium pyogenes* et de *Fusobacterium necrophorum* pour réduire la prévalence et la sévérité des abcès du foie lorsque administrée aux bovins à l'entrée dans le parc d'engraissement. Dans chaque étude, des bouvillons (près de 300 par traitement) ont été alloués aléatoirement à des enclos qui pour leur part furent aléatoirement alloués à différents traitements. Les traitements incluaient 1) des bouvillons recevant une forte dose d'un antigène avec la bactérine-toxoïde, 2) des bouvillons recevant une moindre dose d'un antigène avec la bactérine-toxoïde, 3) des bouvillons recevant de la moulée médicamentée avec la tylosine et enfin 4) des bouvillons témoins recevant de la moulée sans addition de tylosine. Dans la seconde étude, il y avait en plus un traitement 5) comprenant des bouvillons vaccinés recevant de la moulée médicamentée avec la tylosine. Les bouvillons ont été suivis jusqu'à l'abattage et les abcès au foie ont été scorés, les grades de rendement et des carcasses selon les normes de l'USDA ont été obtenus de même que le gain moyen quotidien et le taux de conversion par enclos. Il n'y avait pas d'effet de l'enclos au niveau de la prévalence des abcès du foie. L'injection simple d'une forte dose d'un antigène avec la bactérine-toxoïde a réduit la prévalence des abcès au foie de 48.4% (16% au niveau des individus vaccinés et 31% au niveau des individus témoins, P = 0.0001) dans la première étude et de 37.5% (30% au niveau des individus vaccinés et 48% au niveau des individus témoins, P = 0.001) dans la seconde étude.

Introduction

Fusobacterium necrophorum has been shown to cause liver abscess in cattle,[5] and *Arcanobacterium pyogenes* has frequently been associated with it.[2,4] Liver abscesses in cattle are part of a disease complex in which

abscessation is secondary to the development of primary foci of infection in the ruminal epithelium.[4] Lesions in the rumen are primarily induced by acidosis that follows a rapid change in diet from high roughage to high grain, or prolonged feeding of a high grain diet. *Fusobacterium necrophorum* and/or *A. pyogenes* invade the epithelium and form focal abscesses in the rumen wall. The bacteria enter the portal circulation, are carried to the liver, localize in the parenchyma and subsequently produce abscesses.

The concentration of *F. necrophorum* in rumen fluid can increase 10-fold during ruminal acidosis.[8] It is postulated that *F. necrophorum* and *A. pyogenes* act synergistically; *A. pyogenes*, a facultative anaerobe, may create the anaerobic conditions necessary for growth of *F. necrophorum*, an obligate anaerobe.[4] A leukotoxin of *F. necrophorum* and a hemolysin (pyolysin) of *A. pyogenes* are thought to be the principal virulence factors, respectively.[1,3,5,7] The pyolysin of *A. pyogenes* increases the iron available to the bacteria, and the leukotoxin of *F. necrophorum* impairs the host's normal defensive mechanisms. The lysis of neutrophils and macrophages by the leukotoxin is thought to lead to the release of reactive oxygen radicals and cytolytic enzymes that damage the hepatic cells.

Toxoided *F. necrophorum* culture supernatants have been shown to protect steers from experimentally induced hepatic abscesses.[5] The studies reported herein were done to test the efficacy of a single dose, bivalent *A. pyogenes-F. necrophorum* bacterin-toxoid to aid in the reduction of liver abscess prevalence and severity under field conditions and natural challenge.

Materials and Methods

Bacterins and vaccination

Bacterin-toxoids were formulated to contain varying amounts of inactivated *Fusobacterium necrophorum* leukotoxin and *Arcanobacterium pyogenes* pyolysin with a proprietary water-in-oil adjuvant. High (A1 and A2)- and low (B1 and B2)- antigen/dose bacterin-toxoids were formulated independently for each study. The antigenic mass of the low-antigen dose product (B2) formulated for the second study approximated that of the high-antigen dose product (A1) used in the first study. A saline placebo was used in the second study only. All vaccinations with the experimental bivalent bacterin-toxoids were given as a single subcutaneous injection at the beginning of the feeding period.

Study design

Steers entering a Texas feedlot were randomly allotted to treatment groups and pens within six days of arrival. Approximately 300 steers were allotted to each treatment group in each of the studies. Treatment groups in Study 1 were 1) steers vaccinated with a high-antigen dose bacterin-toxoid (A1), 2) steers vaccinated with a low-antigen dose bacterin-toxoid (B1), 3) non-vaccinates, and 4) non-vaccinates fed a ration containing tylosin phosphate[a] (~ 90mg/hd/d). Treatment groups in Study 2 were fed rations without tylosin or rations with tylosin (~ 90mg/head/day). Groups that were not fed tylosin-medicated feed were 1) placebo-vaccinated, 2) vaccinated with a high-antigen dose bacterin-toxoid (A2), or 3) vaccinated with a low-antigen dose bacterin-toxoid (B2). Groups fed tylosin-medicated feed were 1) placebo vaccinated, or 2) vaccinated with the bacterin-toxoid A2. Steers were housed under outdoor commercial feedlot conditions (dirt lots with in-line feed bunks and automatic waterers) without windbreaks or shelters. Treatment groups were not commingled.

Data from the steers were collected through slaughter in each study. The steers were weighed by pen at the start and end of the studies. The feed delivered to each pen and the percent dry matter of the feed were recorded daily. Livers were graded as normal, abscessed, or condemned (contamination, liver flukes, telangectasis, or other causes) by USDA-FSIS meat inspectors. Condemned livers were not examined for abscesses and were excluded from the data set. The livers were scored by West Texas A & M University, Canyon TX (WTAMU), Cattlemen's Carcass Data Service as normal, A-, A, or A+/A++ (Table 1). USDA quality and yield grade data were collected following slaughter.

Steers

The steers were cross-bred beef breeds in assembled lots in both Study 1 (n = 1,202) and Study 2 (n = 1,510). Average pen weights ranged from 738 to 757 lb (335 to 344 kg) at the beginning of Study 1, and from 718 to 726 lb (326 to 330 kg) in Study 2. Animals at extremes of the weight range for each shipment were excluded.

Randomization

Pens were blocked in groups of adjacent pens to control for pen effects, one pen of each treatment group per block, with buffer pens between the blocks. Steers, in groups of 10 or less in the first study, and individually in the second study, were randomly assigned to treatment groups and pens when sufficient steers were available to fill all the pens in a block. This assured that similar numbers of steers from each source were assigned to each treatment group. Stocking rates for pens were approximately 100/pen, adjusted so that bunk space per head was constant in all pens.

Veterinary care

Other treatments and vaccinations, medications in the feed (monensin[b]), and formulation of the rations

Table 1. Liver scoring system used to differentiate severity of abscesses.

Liver condition	Liver abscess score	
Normal	N/A	0
Abscessed		
1 or 2 small abscesses (≤ 1.0 inch [2.5 cm] in diameter) or abscess scars	A -	1
2 to 4 well-organized abscesses present, < 1.0 inch (2.5 cm) in diameter	A	2
1 or more large active abscesses present > 1.0 inch (2.5 cm) in diameter, with inflammation of the liver parenchyma	A+	3
Abscess with adhesions and/or an open abscess	A++	3

Livers were graded at slaughter as normal, abscessed, or condemned (flukes, contamination, etc.) by USDA inspectors. Abscessed livers were scored by representatives of the WTAMU Cattlemen's Carcass Data Service.

were as per the standard practices of the feedlot, and were standardized across all treatment groups, except as previously noted for tylosin. Metaphylactic treatments for respiratory disease were not given. Treatment for concurrent diseases were as per the standard practices of the feedlot, and were examined to assure that bias had not inadvertently been introduced by disproportionate administration of antimicrobial treatments to any treatment group.

Steers were removed from the study because of injury, chronic disease, poor weight gain (three standard deviations below average pen weight at re-implant), or irreparable loss of identification. Data were not included in the analysis from steers that did not complete the study or were rejected by the slaughterhouse before data were collected because of conditions unrelated to liver abscesses. Steers that died or were euthanized because of serious disease were necropsied. Euthansia was by an AVMA-approved method.

Bacterial culture

Liver abscesses and scars from randomly selected placebo-vaccinated steers from medicated and non-medicated treatment groups of Study 2 were selected for bacterial culture. Swabs from the abscesses and abscess scars (n = 15) were inoculated onto two sets of tryptic soy agar plates with 5% sheep blood for aerobic and anaerobic incubation. Commercially available kits were used to biochemically identify anaerobic[c] and aerobic[d] isolates.

Analysis

Statistical analysis was performed using SAS®(version 8.2, SAS Institute, Cary, NC). The primary variables were liver abscess prevalence and severity scores. Secondary variables were average daily gain (ADG), feed conversion (DMI:ADG), and USDA quality and yield grades. Statistical significance was declared at $P \leq 0.05$, two sided analysis. Comparisons in which $0.05 < P < 0.10$ were described as "approaching significance".

The pen was the experimental unit for comparisons between medicated and non-medicated groups, and for analysis of growth performance (ADG and DMI:ADG). The prevalence of liver abscesses was examined for pen effect by logistic regression using the generalized linear mixed models procedures (SAS® GLIMMIX macro) with pen as the random variable. The individual animal was accepted as the experimental unit for liver abscess prevalence and severity and USDA quality and yield grades if a significant pen effect was not found.

Liver abscess prevalence was analyzed using Fisher's exact test to test overall significance between groups fed non-medicated feed and between groups fed medicated feed if no significant pen effects were present. Odds ratios and confidence intervals for comparisons between groups fed similar feeds were calculated by logistic regression using the generalized estimating equations (GEE) for correlated data.

The severity of liver abscesses was analyzed by the Kruskal-Wallis Test for overall treatment group comparisons and the Wilcoxon Rank Sum Test for pairwise comparisons. Odds ratios and confidence intervals for severity were determined using the continuation ratio model of the GENMOD Procedure.

Pen mean ADG and DMI:ADG were compared by ANOVA procedures. Dead and rejected animals were excluded from the performance analysis. Pen mean body weights were adjusted for shrinkage (4%), and the pen mean ADG was adjusted to hot carcass weight (pen mean ADG x pen dressing %/study dressing %). The daily DMI was adjusted for animals removed from the pen for treatment (hospital pens), stress (buller pens), and for dead and rejected steers.

USDA carcass and yield grades were grouped into desirable (prime and choice, yield grades 1 and 2) and less desirable (select or lower, yield grades 3 to 5) as defined by the cattle feeding industry.[6] The data were analyzed by logistic regression, using the GENMOD procedure. Morbidity, mortality, number of rejected animals and number of animals per treatment group and block medicated for other diseases were tabulated and summarized but not statistically analyzed.

Results

Study 1

Steers (n = 1,202) enrolled in the study were received in four shipments from three sources in Oklahoma, Colorado and Texas. Mass medication for respiratory disease was not necessary during the study. Fewer than 5% of enrolled steers were pulled for treatment and no experimental group contained a disproportionate number of treated steers (Table 2).

Mortality during the ~160-day feeding period was low (seven steers, 0.6%). Thirteen steers were rejected from the study because of chronic respiratory disease, injury, or other debilitating illness. The livers of 1,182 steers were available for examination. The livers of two steers were lost during slaughter, and 59 were condemned without grading for liver abscesses because of visible contamination, liver flukes, or telangectasis (Table 3). A significant pen effect for liver abscess prevalence was not found. Steers vaccinated with the bacterin-toxoid A1 had a prevalence of liver abscesses (15.5%) that was significantly lower (P = 0.0001) than that of the non-vaccinated controls (31.2%) and that of the steers given bacterin-toxoid B1 (26.3%, P = 0.002). The liver abscess prevalence of steers vaccinated with the bacterin-toxoid B1 did not differ from the non-vaccinated steers (P < 0.19).

Table 2. Summary of treatment rates for concurrent disease for each treatment group.

	Steers treated for concurrent disease				
	Non-medicated feed			Medicated feed	
Study	Bacterin-toxoid A*	Bacterin-toxoid B*	Placebo	Bacterin-toxoid A*	Placebo
Study 1	4.4%	5.0%	4.4%	N/A	3.2%
Study 2†	9.5%	9.0%	10.0%	8.0%	7.6%

*For each study, bacterin-toxoid A was the vaccine with the higher antigen content and bacterin-toxoid B was the vaccine with the lower antigen content. The high-antigen dose bacterin-toxoid used in Study 1 (bacterin-toxoid A1) approximated the antigenic content of the low-antigen dose bacterin-toxoid in Study 2 (bacterin-toxoid B2).
†Based on steers for which liver abscess data were available.

Table 3. Study 1–Liver abscess prevalence and severity.

			Liver scores				
				Abscess present, No. (%)†			
Treatment group	Steers slaughtered	Livers scored*	Normal	Total	A-	A	A+ & A++
Bacterin-toxoid A1	289	271	229	42 (16)[a]	16 (6)	9 (3)	17 (6)
Non-Vaccinates	289	266	183	83 (31)[b]	30 (11)	10 (4)	43 (16)
Bacterin-toxoid B1	308	301	222	79 (26)[b]	20 (7)	24 (8)	35 (12)
Tylosin-medicated	296	283	258	25 (9)[a]	13 (5)	1 (0.4)	11 (4)
Study totals Number	1182	1121	892 (76)	229 (19)	79 (7)	44 (4)	106 (9)

*Livers condemned without scoring because of liver flukes, telangectasis, or distoma. (n = 59), and livers lost to scoring (n = 2) were excluded.
†Percentages calculated based on the number of livers scored after exclusion of condemned and lost livers.

The liver abscess prevalence of steers fed tylosin-medicated feed (8.8%) was significantly lower than that of the non-vaccinated controls and of the steers vaccinated with bacterin-toxoid B1, when compared using the SAS GLIMMIX macro, with the pen block as a random effect (Table 3). The results were the same when analyzed using the Bootstrap or Permutation adjusted Fisher's Exact test. Analyzed by the same methods, the liver abscess prevalence of steers vaccinated with bacterin-toxoid A1 was significantly lower than that of the non-vaccinated steers ($P = 0.044$), and did not differ from that of steers fed tylosin-medicated feed ($P = 0.17$).

The number of steers with the most severe and the most economically important liver lesions (A+ or A++) was greatest in the non-vaccinates and least in the tylosin-medicated group (Table 3). Steers given the bacterin-toxoid B1 had an A+ liver abscess prevalence similar to the non-vaccinates, while that of the steers vaccinated with bacterin-toxoid A1 approached that of the tylosin-medicated steers.

The mean liver abscess score of steers vaccinated with bacterin-toxoid A1 (0.31 ± 0.81) was lower than that of the non-vaccinated steers (0.67 ± 1.13) or steers given bacterin-toxoid B1 (0.57 ± 1.05). The median abscess score for each group was 0, as more than 50% of livers were normal in each group; however, the liver scores of steers vaccinated with bacterin-toxoid A1 were lower than those of non-vaccinates ($P = 0.0001$) or steers vaccinated with bacterin-toxoid B1 ($P = 0.001$). The mean score of steers fed medicated feed (0.17 ± 0.62) was significantly lower than the non-vaccinated steers ($P < 0.015$, ANOVA of pen means) or steers vaccinated with the bacterin-toxoid B1 ($P < 0.044$). The liver abscess scores of the steers vaccinated with the bacterin-toxoid A1 did not differ ($P > 0.35$) from those of the steers fed tylosin-medicated feed.

There were no overall significant differences in USDA carcass and yield grade, average daily gain or feed conversion (deads and rejects out, adjusted for dressing percentage, data not shown). With only three pens/treatment group, the retrospective statistical power of the study design to detect a significant difference in ADG or feed conversion was low (< 37% for each). The group carcass and yield grades were similar in all treatment groups (data not shown). Average daily gain (ADG) and feed conversion were most favorable in the steers fed tylosin-medicated feed (3.31 lb [1.50 kg] and 5.92, respectively). The non-vaccinated, non-medicated group had the lowest ADG (3.18; 1.44 kg) and least economical feed conversion (6.13), while the vaccinated groups were intermediate for both values (high-antigen dose, 3.21 [1.46 kg] and 6.05; low-antigen dose, 3.25 [1.48 kg] and 6.03, respectively).

Study 2

Head count

Steers (n = 1,510) assembled from 11 order buyers in Oklahoma, Texas, Kansas and Colorado were enrolled in the study, tagged and randomly assigned to pens. The pens were blocked in three blocks of adjacent pens, with one pen per treatment group in each block, as in the previous study.

The feeding period was 172-174 days. Metaphylactic treatments for respiratory disease were not used during the study. Antimicrobial treatments for concurrent diseases were given to 8.8% of the steers in the study, but were not disproportionately segregated in any treatment group (Table 2). Liver abscess data from 1,427 steers were available after exclusion of steers that died (n = 24), were rejected from the study because of conditions unrelated to liver abscesses (n = 36), escaped from their home pens (n = 1), were inadvertently not vaccinated or had inadequate documentation of vaccination (n = 21), or whose identity could not be determined (n = 1). The data available on USDA carcass and yield grades were further reduced to 1,424 steers because of condemnation or accidental loss at slaughter. Average daily gain was adjusted to hot carcass weight, and ADG and dry matter intake (DMI) were adjusted to exclude dead and rejected steers, as in the first study.

Liver abscess prevalence and severity

Of the 1,427 livers available for examination, 114 were condemned before scoring because of fluke infestation or visible contamination. Liver scores from 1,313 steers were available for analysis (Table 4). There was an overall significant difference in liver abscess prevalence among treatment groups ($P = 0.0001$, GLIMMIX), but a significant pen effect was not present when the data were tested with pen as a random variable ($P = 0.20$).

Non-medicated feed groups

The liver abscess prevalence of the placebo-vaccinated, non-medicated steers (48%) was greater than that of steers given either bacterin-toxoid A2 (30%, $P < 0.001$) or B2 (36%, $P < 0.004$, Fisher's Exact test, Table 4, Figure 1). The odds that a placebo-vaccinated steer would develop a liver abscess were 2.2 times greater (95% CI, $1.74<OR<2.78$, GEE) than that of a steer given bacterin-toxoid A2, and 1.67 times greater (95% CI, $1.05<OR<2.66$) than that of a steer given bacterin-toxoid B2 in the steers fed non-medicated feed. There was not a difference in the prevalence of liver abscesses in steers given bacterin-toxoids A2 and B2 ($P = 0.16$).

The number of steers treated for concurrent disease in Study 2 was greater than expected (Table 2).

Table 4. Study 2–Liver abscess prevalence and severity.

Treatment group	Slaughtered steers	Livers scored*	Normal livers	Abscess present, No. (%)			
				Total	A-	A	A+ & A++
Non-medicated feed							
Bacterin-toxoid A2	283	263	185 (70)	78[a] (30)	15 (6)	19 (7)	44 (17)
Placebo	295	279	144 (52)	135[b] (48)	34 (12)	37 (13)	64 (23)
Bacterin-toxoid B2	287	259	166 (64)	93[a] (36)	27 (10)	20 (8)	46 (18)
Tylosin-medicated feed							
Placebo	293[†]	275	242 (88)	33[c] (12)	10 (4)	11 (4)	12[†] (4)
Bacterin-toxoid A2	269	237	217 (92)	20[c] (8)	9 (4)	3 (1)	8 (3)
Study totals	1427	1313	954 (73)	359 (27)	95 (7)	90 (7)	174 (13)

Bacterin-toxoid A2 was the high-antigen dose vaccine used in Study 2, and bacterin-toxoid B2 was the low-antigen dose vaccine. Bacterin-toxoid B2 approximated the antigenic dose of bacterin-toxoid A1, the high-antigen dose vaccine used in Study 1.
*Livers condemned due to contamination or fluke infestation (n = 114) were excluded from analysis.
[†]A steer that died was found on necropsy to have a large abscess in the liver as the only lesion found. A difference in superscripts in a column indicates a significant difference in prevalence of liver abscesses ($P < 0.05$, Fisher's Exact test).

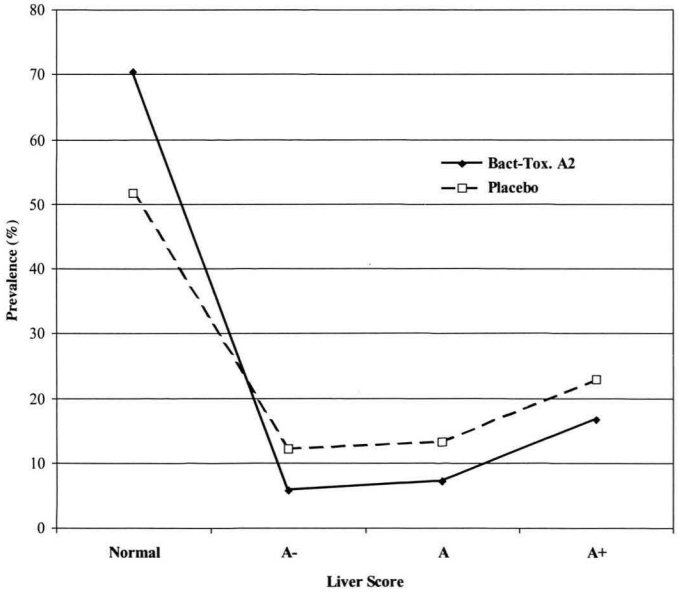

Figure 1. Study 2–Prevalence of liver abscesses by score in non-medicated steers vaccinated with a placebo or bacterin-toxoid A2.

The analysis of the liver abscess prevalence was repeated, after excluding the data of steers that had been treated with antimicrobial medication, to determine if the treatments had biased the study. Significance statements on the differences in liver abscess prevalence were unchanged.

The median abscess score for each group was 0, as more than 50% of livers were normal in each group (Table 5). Scores of liver abscesses were significantly greater in the placebo-vaccinated steers than in steers vaccinated with the bacterin-toxoids A2 (Group A, $P = 0.0001$, Figure 1) or B2 ($P = 0.0047$). Odds of a higher liver abscess score for placebo-vaccinated steers were 1.52 (95% CI, $1.15 < OR < 2.00$) times greater than for steers vaccinated with bacterin-toxoid A2 and 1.36 (95% CI, $1.03 < OR < 1.78$) times greater than for steers vaccinated with bacterin-toxoids B2, respectively.

Tylosin-medicated groups

The prevalence of liver abscesses of steers fed tylosin-medicated feed was less than that of any group fed non-medicated feed ($P < 0.0001$, GLIMMIX macro, Table 4). The prevalence and severity of liver abscesses in steers vaccinated with bacterin-toxoid A2 did not differ from the placebo-vaccinated steers in the groups fed the tylosin-medicated ration ($P = 0.108$, GEE, and $P = 0.214$, Wilcoxon Rank Sum Test, respectively, Tables 4 and 5).

Culture of livers

Fusobacterium necrophorum was isolated from lesions in five of nine livers from non-medicated, placebo-vaccinated steers, and from three of six livers from tylosin-medicated, placebo-vaccinated steers. *Arcanobacterium pyogenes* and *F. necrophorum* were

Table 5. Liver abscess severity in Study 2.

Feed	Group	Group mean Score	Group percentile score 50% (Median)	75%	90%	95%	99%
Non-medicated	Bacterin-toxoid A2	0.66 ± 1.14	0[a]	1	3	3	3
	Placebo	1.02 ± 1.24	0[b]	2	3	3	3
	Bacterin-toxoid B2	0.71 ± 1.15	0[a]	2	3	3	3
Tylosin-medicated	Placebo	0.23 ± 0.71	0[c]	0	1	2	3
	Bacterin-toxoid A2	0.15 ± 0.57	0[c]	0	0	1	3

Median scores for all groups were 0. The score of the upper 75%, 90%, 95% and 99% are also presented. For comparisons within ration type, different superscripts in a column are statistically different ($P < 0.05$, Wilcoxon Rank Sum test).

isolated from a lesion in a liver of a non-medicated, placebo-vaccinated steer.

Safety

No adverse events attributable to the experimental bacterin-toxoids were reported during the study.

Performance data

The pen (n = 3/treatment group) was the experimental unit for analysis of performance data. Vaccinated pens generally had slightly more favorable results than did the placebo vaccinated steers for ADG and feed conversion, but the differences were not significant (Table 6).

USDA carcass and yield grades

USDA carcass and yield grades were tabulated by grade and by desirable grades (Prime + Choice and Y1+Y2) vs less desirable grades (Select and lower and Y3 to Y5, Table 7). The USDA carcass grades were similar. In steers fed non-medicated feed, the proportion of carcasses graded Y1 and Y2 was significantly lower in the placebo-vaccinated group (55%) than in steers given bacterin-toxoid A2 (64%, P = 0.04). The difference in the proportion of steers fed non-medicated feed that graded Y1 and Y2 between the placebo and bacterin-toxoid B2 vaccinated steers (63%, P = 0.0503) approached significance. Steers fed tylosin-medicated feed and given bacterin-toxoid A2 also had a significantly greater proportion of carcasses graded Y1 and Y2 (64.4%, P = 0.009) than did the placebo-vaccinated steers on the tylosin-medicated ration.

Serum from ~10% of the steers in both studies was collected to test the response to vaccination and natural exposure to *F. necrophorum* leukotoxin and *A. pyogenes* pyolysin. Results are not yet available.

Discussion

Steers under field conditions, on rations without antimicrobial medication, and vaccinated with a single dose of a bivalent, high-antigenic-mass bacterin-toxoid had a significantly lower prevalence and severity of liver abscesses than non-vaccinated steers. This set of studies is the first reported use of a single dose *A. pyogenes-F. necrophorum* bivalent bacterin-toxoid in large numbers of cattle. The cattle were housed and managed under feedlot conditions which led to the development of a high prevalence of liver abscesses (31.2% and 48.4%) in the non-vaccinated, non-medicated steers. The

Table 6. Performance (ADG and DMI:ADG) of steers in Study 2 for the total feeding period.*

Treatments	ADG (lb)	DMI:ADG
Non-medicated feed		
Bacterin-toxoid A2	3.46 ± 0.12	5.41 ± 0.06
Placebo	3.43 ± 0.14	5.48 ± 0.15
Bacterin-toxoid B2	3.45 ± 0.14	5.28 ± 0.16
Medicated feed		
Placebo	3.50 ± 0.18	5.33 ± 0.15
Bacterin-toxoid A2	3.55 ± 0.11	5.25 ± 0.18
Overall Study	3.48 ± 0.13	5.35 ± 0.15

*Dead and rejected steers excluded, data adjusted to average hot yield.

All weights pen average, in pounds. The high- and low-antigen dose bacterin toxoids were designated A2 and B2, respectively. ADG = average daily gain. Overall differences in ADG and DMI:ADG were not significant (P = 0.83 and P = 0.33, ANOVA).

Table 7. Carcass yield and USDA grades of steers in Study 2.

Group	n	Carcass grade* (%)		Yield grade (%)	
		PR + CH	SE & Lower	Y1+Y2	Y3-Y5
Non-medicated					
Bacterin-toxoid A2	283	46.3	53.7	64.0[a]	36.0
Placebo	294	49.3	50.7	55.4[b,c]	44.6
Bacterin-toxoid B2	287	51.2	48.8	63.4[a,b]	36.6
Tylosin-medicated					
Placebo	292	52.1	47.9	53.4[c]	46.6
Bacterin-toxoid A2	268	54.9	45.1	64.4[a]	35.8
Study	1,424	50.8	49.2	60.0	40.0

*Prime (PR), Choice (CH), Select (SE), No roll (NR). A difference in superscripts in a column indicates a difference ($P < 0.05$, logistic regression) between treatment groups.

results of these studies demonstrated that bacterin-toxoids A1 and A2 reduced the prevalence and severity of liver abscess, and although the formulation of the bacterin-toxoids used in the studies differed somewhat, the results were repeatable.

The results of the liver cultures, although limited in scope, are in agreement with previous reports,[4,5] and consistent with the postulated role of *F. necrophorum* and *A. pyogenes* in the pathogenesis of liver abscesses. The reduction of liver abscess prevalence in these studies is in agreement with a previous study in which steers vaccinated twice with *F. necrophorum* culture supernatants or *F. necrophorum* purified leukotoxin were protected from experimental challenge.[5] Bacterins and toxoids which require two doses are usually given 14 to 28 days apart, as repeated doses given earlier or later often do not elicit a secondary or anamnestic response. This schedule does not fit well with current management practices for cattle in North American feedlots.

Three factors were considered in formulating the bacterin-toxoids as single dose vaccines: 1) the adjuvant; 2) antigen selection and preparation; and 3) antigenic mass. Growth of the bacterial cultures was optimized for maximum production of *F. necrophorum* leukotoxin and *A. pyogenes* pyolysin, and inactivation methods were developed that minimally reduced the antigenicity of the cultures and toxins. The bacterin-toxoids were then formulated with relatively large volumes of concentrated *F. necrophorum* leukotoxin and *A. pyogenes* pyolysin. A proprietary water-in-oil emulsion was selected as the adjuvant used in the bacterin-toxoids because of the strong, protective, and prolonged response it had elicited after a single dose when previously used with antigens of *Moraxella bovis, E. coli, Clostridium chauvoei,* or *C. novyi* (H. Jayappa, unpublished data). The dose response shown in Study 1 demonstrated that the antigenic mass, and hence, the selection, concentration, and processing of the antigens, were critical for the bacterin-toxoid success. Previous experience suggests the choice of adjuvant was also instrumental in the success of the single dose format.

The single dose, bivalent, bacterin-toxoid A1 reduced the prevalence and severity of liver abscesses in steers of Study 1, but steers vaccinated with a lower antigenic mass (bacterin-toxoid B1) were not protected, suggesting the presence of a dose response to the bacterin-toxoids. The liver abscess prevalence and liver scores of the high-antigen dose vaccinates did not differ from those of the tylosin-medicated steers, albeit the statistical power to detect a significant difference was limited. Steers in the second study that were vaccinated with the high-antigen (A2) or low-antigen (B2) dose bacterin-toxoids were protected from a severe natural challenge, and had lower liver abscess prevalence and severity than the placebo-vaccinated, non-medicated steers. The bacterin-toxoid A2 established the minimum release dose for a biological product currently in the process of licensure.

The studies were designed to test the efficacy of the bacterin-toxoids to reduce the prevalence and severity of liver abscesses in feedlot cattle. Vaccinated groups generally had more favorable measures of performance, but the differences were not statistically different, possibly because the study design had limited

power to detect differences at the pen level. The differences between the USDA yield grades of vaccinates and placebo-vaccinates in the second study, whether fed tylosin-medicated or non-medicated feed, were unexpected. These results raise the intriguing possibility that global metabolic processes of economic importance may be affected by subtle differences in inflammatory processes in the liver, but an estimate of the economic value of bacterin-toxoid A2 must await further studies.

There were two potential sources of bias in the study: pen effect and antibiotic medications for concurrent diseases. Treatment for concurrent disease was greatest in Study 2. Analysis of liver abscess prevalence after deletion of the data from treated steers demonstrated that the treatments had not biased the study. Both studies were designed to control pen effects by arranging pens in blocks, one pen per treatment group per block. Significant pen effects were not detected in the liver abscess prevalence in either study.

Conclusions

A single dose, bivalent *Arcanobacterium pyogenes–Fusobacterium necrophorum* bacterin-toxoid given to cattle entering a feedlot reduced the prevalence and severity of liver abscesses in a dose-dependent manner. Protection was demonstrated against a severe natural challenge. Vaccinates also had more favorable USDA yield grades than placebo-vaccinates. Measures of growth performance, although not significantly improved, also tended to be more favorable in vaccinates than in control groups. Accurate estimation of the potential economic benefit of the bacterin-toxoids must await further study.

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Footnotes

[a]Tylan® 100 Type A Medicated Pre-mix, Elanco Animal Health, Indianapolis, IN
[b]Rumensin®, Elanco Animal Health, Indianapolis, IN
[c]RapID ANA II®, Remel, Lenexa, KS
[d]BBL Crystal ID, Becton Dickinson, Sparks, MD

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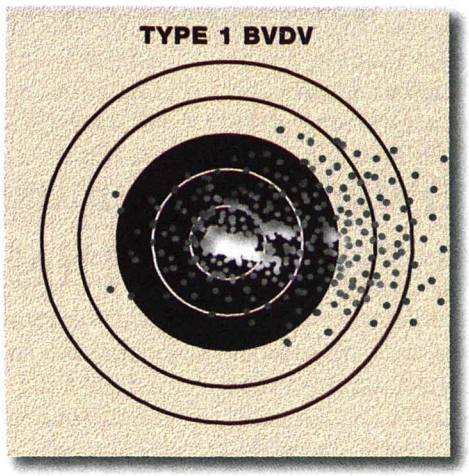

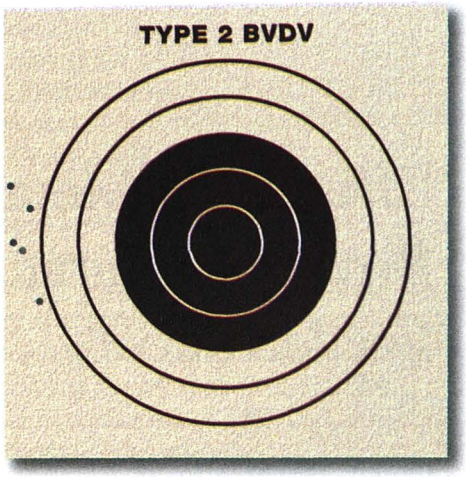

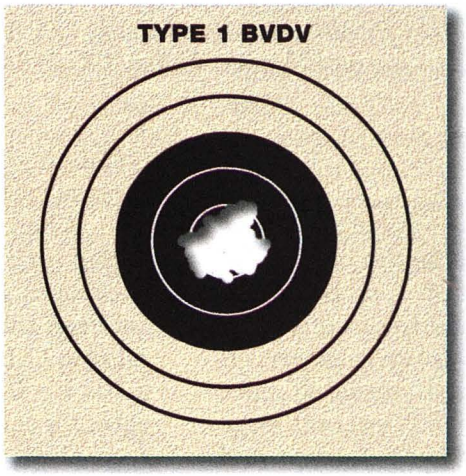

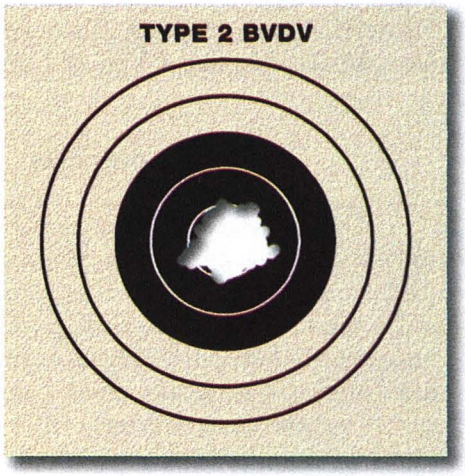

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