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Fusobacterium necrophorum leukotoxoid vaccine for prevention of liver abscesses

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FUSOBACTERIUM NECROPHORUM LEUKOTOXOID VACCINE FOR PREVENTION OF LIVER ABSCESSSES ¹

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Summary

The efficacy of *Fusobacterium necrophorum* crude leukotoxoid vaccine to immunize and protect steers against experimentally induced liver abscesses was evaluated. The vaccine consisted of cell-free culture supernatant of a high leukotoxin-producing strain of *F. necrophorum*, inactivated with formalin and homogenized with an oil emulsion adjuvant. Vaccine was injected subcutaneously on days 0 and 21. Blood samples were collected weekly to monitor immune response. Three weeks after the second vaccination, steers were injected intraportally with *F. necrophorum* culture to induce liver abscesses. Three weeks later (day 63), steers were euthanatized and necropsied; livers were examined, and protection was assessed. Anti-leukotoxin antibody titers in the control steers generally did not differ from the baseline (week 0) titers. The titers in the vaccinated groups increased, more so after the second injection, and the increase was generally dose dependent. At necropsy, all steers in the control group had liver abscesses. In the vaccinated groups, two out of five steers in the 1.0 ml group and one each in the 2.0, 5.0, and 2.25 ml (concentrated) groups had liver abscesses. The difference suggests a protective effect of anti-

leukotoxin antibodies against experimentally induced liver abscesses.

(Key Words: Liver Abscesses, *Fusobacterium necrophorum*, Leukotoxoid Vaccine.)

Introduction

Liver abscesses are of economic concern to the feedlot industry because they cause liver condemnation, reduced feed efficiency, and reduced weight gain. *Fusobacterium necrophorum*, is the primary causative agent of liver abscesses. The incidence of liver abscesses averages 18 to 32% in feedlot cattle and is related to feeding high grain diets. Rapid ruminal fermentation of grain in the rumen results in ruminal acidosis and rumenitis, which are considered to be predisposing factors for liver abscesses. *Fusobacterium necrophorum*, a normal inhabitant of the rumen, colonizes the ruminal epithelial wall, reaches the liver via the portal circulation, and sets up infection. The ability of *F. necrophorum* to colonize ruminal epithelium and establish infection in the liver is attributed mainly to a potent leukotoxin that is toxic to leukocytes, macrophages, ruminal epithelial cells, and hepatocytes. Therefore, immunizing the animal against the toxin may

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prevent the onset of liver abscesses. Our objective was to determine the efficacy of *F. necrophorum* leukotoxoid vaccine to immunize steers and to provide protection against experimentally induced liver abscesses.

Experimental Procedures

Fusobacterium necrophorum, A25, a high leukotoxin producing strain, previously isolated from a liver abscess was used to prepare the vaccine, which consisted of cell-free supernatant (original or concentrated 5.2 fold) inactivated by adding formalin and homogenized with an oil emulsion adjuvant. The leukotoxicity of the original and concentrated culture supernatants, before and after formalin inactivation, was determined.

Twenty-five Holstein steers (mean body weight 860 lb), fed ad libitum a diet of alfalfa hay, were assigned randomly to five groups: control; three doses (1.0, 2.0, and 5.0 ml) of the culture supernatant; and a dose of the concentrated supernatant (2.25 ml) equivalent to the leukotoxin concentration in 4.5 ml of the original culture supernatant (Table 1). Each steer in the control group received 4.5 ml of phosphate buffer saline mixed with adjuvant. All injections were given subcutaneously on days 0 and 21.

Jugular blood samples were collected at weekly intervals after the first vaccination to monitor the immune response. Serum samples were assayed for anti-leukotoxin antibody titers. Three weeks after the second vaccination, steers were inoculated intraportally by an ultrasound-guided, percutaneous, catheterization procedure with *F. necrophorum* to induce liver abscesses. Steers were euthanatized 21 days after the intraportal challenge and examined for abscesses and other gross lesions in the liver and other organs.

Results and Discussion

The leukotoxin concentration in the original culture supernatant was 27,020 units/ml. In the culture supernatant concentrated 5.2-fold, the leukotoxin concentration was 54,040 units/ml. Apparently, the process of concentration reduced the leukotoxin activity. Therefore, 2.25 ml of the concentrated supernatant was used to equal the leukotoxin concentration in the 4.5 ml dose.

Antibody titers in the control steers injected with phosphate-buffered saline generally did not differ from the baseline throughout the 6-week sampling period. The titers in the vaccinated steers increased ($P < .01$) following the first vaccination and increased much more after the second injection (Figure 1). A significant treatment x week interaction ($P < .01$) occurred. Generally, the antibody titers appeared to be related to the dose of leukotoxoid, with the 1.0 ml dose eliciting the lowest antibody titers and the 5.0 ml dose eliciting the highest. The purpose of using the concentrated supernatant was to determine whether concentrating the culture supernatant to reduce the injection volume would alter its immunogenicity or protective effect. Apparently, concentrating the culture supernatant reduced its immunogenicity as evidenced by lower antibody titers.

At necropsy, all five steers in the control group had liver abscesses as compared to two out of five steers in the 1.0-ml-vaccinated group and one each in the 2.0, 5.0, and 2.25 ml (concentrated) groups (Table 2). Based on Fisher's exact test (2-tail), the incidence of liver abscesses was lower ($P < 0.01$) in the vaccinated groups (all four doses) than in the control. Steers that developed abscesses ($n=10$) had lower anti-leukotoxin titers ($P < 0.05$) during wk 1 to 6 than those steers

(n=15) that did not develop abscesses in the liver, regardless of the treatment (Figure 2).

Our results indicate that *F. necrophorum* culture supernatant was capable of eliciting anti-leukotoxin immunity that provided some

degree of protection against experimentally induced liver abscesses. However, further studies are required to determine the efficacy of the vaccine in feedlot cattle with naturally developing liver abscesses.

Table 1. Treatment Groups and Leukotoxin Concentration in the Culture Supernatant

Treatment	Culture Supernatant (ml)	Concentrated Supernatant ^a (ml)	Phosphate Buffered-Saline (ml)	Adjuvant (ml)	Leukotoxin Titer per Dose
Control	—	—	4.5	.5	—
Culture supernatant, 1.0 ml	0.9	—		.1	24,318
Culture supernatant, 2.0 ml	1.8	—		.2	48,636
Culture supernatant, 5.0 ml	4.5	—		.5	121,590
Concentrated supernatant, 2.25 ml		2.25	2.25	.5	121,590

^a Culture supernatant concentrated 5.2-fold

Table 2. Experimental Induction of Liver Abscesses in Control or Vaccinated Steers

Treatment	No. of Steers	Liver Abscesses	
		Necropsy ^a	Incidence (%)
Control	5	5/5	100
Culture supernatant, 1.0 ml	5	2/5	40
Culture supernatant, 2.0 ml	5	1/5	20
Culture supernatant, 5.0 ml	5	1/5	20
Concentrated supernatant, 2.25 ml ^b	5	1/5	20

^aFisher's exact test (2-tail), control vs. vaccinated P < .01.

^bCulture supernatant concentrated 5.2-fold.

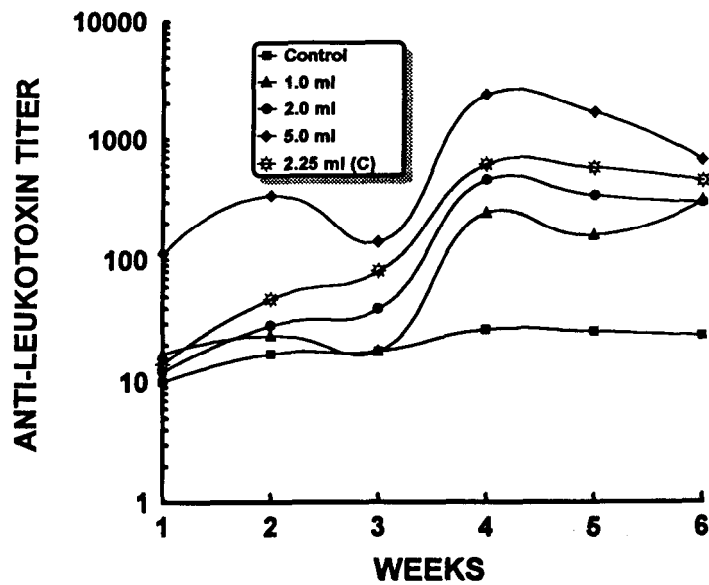


Figure 1. Serum Leukotoxin-Neutralizing Antibody Titers in Controls or Steers Vaccinated with 1.0, 2.0, or 5.0 ml Culture Supernatant and 2.25 ml of the Concentrated (C) Supernatant. SEM = 1.6, Treatment Effect $P < .01$, Week Effect $P < .01$, and Treatment x Week Interaction $P < .05$.

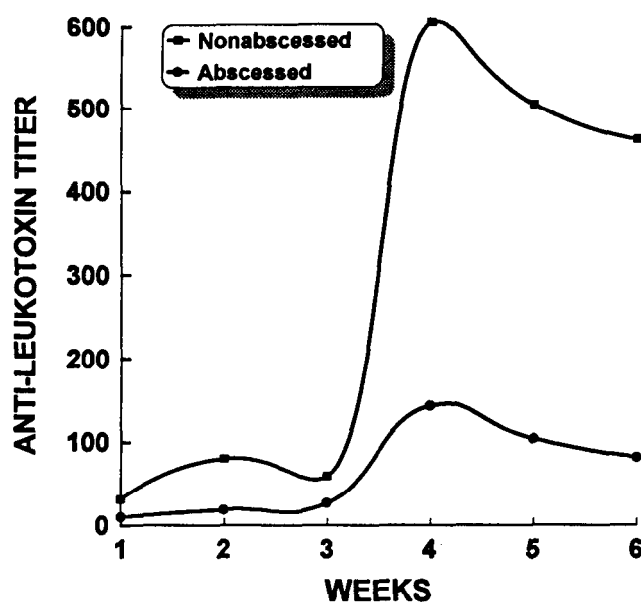


Figure 2. Serum Leukotoxin-Neutralizing Antibody Titers in Steers that Developed (N=10) or Did Not Develop (N=15) Liver Abscesses. SEM=1.4, Abscess Effect $P < .05$, Week Effect $P < .01$.