Results of a Preliminary Trial with Sphaerophorus necrophorus Toxoids to Control Liver Abscesses in Feedlot Cattle

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ABSTRACT

A preliminary field experiment was undertaken to evaluate the efficacy of alum precipitated toxoids of Sphaerophorus necrophorus prepared from sonicated whole cells and cell fractions to reduce the incidence of bovine abscesses. A total of 108 calves were divided into five groups and treated as follows: I. uninoculated control, II. adjuvant inoculated control, III. 15.5 mg protein of sonicated (fragmented cells) toxoid, IV. 10.5 mg protein of cytoplasmic toxoid, and V. 15.5 mg protein of cytoplasmic toxoid. All animals were maintained under similar conditions to those prevailing in feedlots in Alberta. Livers were examined at slaughter. The most promising result was achieved with the injection of 15.5 mg protein of cytoplasmic toxoid. In this treatment group, no scars (healed lesions) were found in the liver and the incidence of liver abscesses was reduced to 10% from the average 35% liver abscesses and scars found in the uninoculated and adjuvant inoculated groups. The toxoid from sonicated whole cells did not reduce liver abscess incidence. These data suggest that the incidence of liver abscesses in cattle fattened in feedlots may be reduced by immunization.

RÉSUMÉ

On a réalisé, sur une ferme, une première expérience visant à évaluer l'efficacité de toxoïdes de Sphaerophorus necrophorus précipitées à l'alun et préparées avec des cellules entières ou des fractions cellulaires soumises aux ultrasons. On espérait ainsi contribuer à réduire la fréquence des abcès hépatiques chez les bovins de boucherie. On utilisa à cette fin 108 veaux que l'on répartit en cinq groupes, de la façon suivante: I - témoins non inoculés; II - témoins ne recevant que l'adjuvant; III - sujets recevant 15.5 mg de protéine d'un toxoïde à base de cellules fragmentées et soumises aux ultrasons; IV - sujets recevant 10.5 mg de protéine d'une toxoïde cytoplasmique; V - sujets recevant 15.5 mg de protéine d'une toxoïde cytoplasmique. On plaça tous ces veaux dans des conditions correspondant à celles qui prévalent dans les parcs d'engraissement de l'Alberta et on examina leur foie, lors de l'abattage. Le résultat le plus prometteur accompagna l'injection de 15.5 mg de protéine d'une toxoïde cytoplasmique. Les sujets de ce groupe ne présentèrent pas de lésions hépatiques cicatrisées et la fréquence des abcès hépatiques n'y atteignit que 10%, comparativement à une moyenne de 35% (abcès et cicatrices) observée chez les sujets des groupes témoins non inoculés ou n'ayant reçu que l'adjuvant. La toxoïde préparée à l'aide de cellules entières soumises aux ultrasons ne réduisit pas la fréquence des abcès hépatiques. Ces résultats laissent supposer que l'immunisation pourrait réduire la fréquence des abcès hépatiques chez les bouvillons des parcs d'engraissement.

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INTRODUCTION

Liver abscesses which develop in feedlot cattle represent an economically important disease caused in the greatest percentage of cases by *Sphaerophorus necrophorus*. In the western provinces of Canada where the beef cattle population is concentrated and the feedlot system of finishing cattle is a common practice the incidence of liver abscesses may average about 30-40% of slaughtered animals. It is believed that ulcerated lesions of the rumen and other organs of the digestive tract are produced by the feeding of coarse grain with little or no roughage and that *S. necrophorus* is the underlying bacterial cause (5). The organism is thought to enter the portal circulation when there is an inflammation and necrosis localized in these organs of the digestive tract. The post mortem findings consist of single to multiple, small or large abscesses in cattle being fattened in feedlots (5). Other species of bacteria such as *Streptococcus* spp and *Corynebacterium pyogenes* may be associated with *S. necrophorus* in some of the abscesses (2, 8).

There is no published report on the protective action of vaccines prepared from *S. necrophorus* against the infection of bovine livers by this organism. This study was carried out to evaluate the efficacy of immunizing cattle with toxoids of *S. necrophorus* sonicated whole cells and cell fractions to prevent or reduce the incidence of bovine liver abscesses.

MATERIALS AND METHODS

Strain

S. necrophorus strain designated as LA 19 was used in this study. The organism was isolated from a bovine liver abscess and identified by procedures used in a previous study (4).

Preparation of Sonicated and Cytoplasmic Toxoids

The cells were grown in bulk by the method described by Garcia and McKay (3). The sedimented cells were washed twice in saline (0.85% NaCl). The sediment was resuspended to a concentration of 20 mg/ml of dry weight in saline. Cells were ruptured ultrasonically for 18-20 min in a MSE 100 sonic vibrator. An almost complete disruption of the cells by this technique was observed by phase microscopy. The sonicated cells were centrifuged at 18,000 X g for 15 min and separated into the supernatant and sediment. The supernatant was considered to consist of the intracellular or cytoplasmic fraction and was designated as such. The sediment containing the crude cell walls was set aside for studies on *S. necrophorus* endotoxin.

Protein determinations were made of each fraction by the method of Lowry *et al* (7). The cytoplasmic and the sonicated cell fractions contained 5.0 mg/ml and 9.5 mg/ml protein respectively.

The toxoids used in vaccination were made from the sonicated, unfractionated cells (sonicated toxoid) and the cytoplasmic fraction (cytoplasmic toxoid). The latter fraction was relatively free of crude cell wall materials. Both preparations were treated with 0.06 M formaldehyde in the presence of 0.025 M lysine and incubated at 25°C for two to four weeks. Portions of these toxoided antigens were precipitated with 10% aluminum-potassium sulfate while maintained at pH 5.5 following the procedure of Kawamura (6). A previous study (1) involving various adjuvants indicated that alum precipitated *S. necrophorus* antigens elicited the highest serological response. The final concentration of the alum precipitated antigen suspension was adjusted to 1 mg/ml protein. Prior to field tests, the various preparations were injected subcutaneously in the neck of several calves in doses ranging from 1.0-20.0 ml. The larger doses produced severe reactions at the site of injection resulting in hot tender swellings. Doses of 5.0 and 10.0 ml produced smaller lumps which were found to dissipate in a few weeks and were not considered significant at slaughter by the meat inspector.

Immunization Procedures

In May of 1972, 108 beef animals approximately 500-600 lbs and composed of 76 steers and 32 heifers were purchased at auction from several owners at Lethbridge, Alberta. The calves were placed on pasture and randomly divided into five groups and treated as follows: I. uninoculated control

— 32 animals, II. adjuvant control — 17 animals, III. sonicated cell toxoid — 19 animals (total dose 15.5 mg protein), IV. cytoplasmic toxoid — 20 animals (total dose 10.5 mg protein) and V. cytoplasmic toxoid — 20 animals (total dose 15.5 mg protein). All animals were ear tagged with a distinctive color for each group and a separate number for each animal. The animals were bled and the sera used for the serological tests. The actual dosage for each group is included in Table I. On June 26, 1972, Groups III, IV and V were given an initial dose of toxoids subcutaneously in the neck region. Seventeen of the 49 control animals (Group II) received 10.0 ml of aluminum base adjuvant only (adjuvant control). On August 22, eight weeks later, a booster dose was given to the toxoid inoculated groups. The animals were placed in the feedlot and introduced to a high energy ration made up largely of grain without antibiotics. The grain ration consisted of 90% steam rolled barley, 5% beet pulp and 5% plain concentrate (32% protein without D.E.S.). The grain to roughage ratio was 9:1. On October 17, 16 weeks after the initial injection while in the feedlot, the toxoid inoculated groups were given a second booster dose of 0.1 mg/ml protein in 5.0 ml of saline. Sera were collected for serological tests prior to each injection. At various intervals the animals were observed for clinical signs of ill health. The animals were killed in December 1972 and January 1973 upon reaching 1000-1100 lbs live weight. All livers were inspected at slaughter and the numbers of scars and abscesses were noted. A few abscessed livers randomly chosen from the slaughtered animals were subjected to bacteriological examination. All were positive for the presence of *S. necrophorus*.

Serology

Immune response was assessed by a double immunodiffusion technique on 1% ionagar plates containing borate buffer (pH 8.0). Six peripheral wells, 5 mm in diameter were spaced 6 mm from the centre well. The titration wells were arranged so that each plate would contain titrated antigen or antibody (two-fold dilutions to 1/16) surrounding the centre well containing the homologous undiluted antibody or antigen. The undiluted antigen contained 4.2 mg/ml cytoplasmic protein. The plates were incubated at 25°C in a humid chamber for four days. Negative reactions were rechecked for seven days. Distinct precipitation lines were considered as a positive indication of immune response.

Statistical Analysis

Data were subjected to statistical analysis using the single classification analysis of variance (10).

RESULTS

The results of the experiments using the various toxoids are recorded in Table I. Thirty-five percent of the livers in the control group including the adjuvant control showed lesions, of which 27% were abscesses and 8% were healed lesions (scars). An interesting observation is the lower incidence of liver abscesses and scars in the adjuvant control group (29%) compared to 38% in the uninoculated controls. The livers of animals vaccinated with the sonicated toxoid had an incidence of 37% abscesses and scars. The cytoplasmic toxoid group receiving an initial dose of 5.0 mg protein followed by a second dose of 5.0 mg and a final dose of 0.5 mg showed a slight reduction (30%) in the incidence of abscesses and scars. However the average number of abscesses per infected liver was reduced to 1.4 compared to 6.5-7.0 in the sonicated toxoid and control groups. The most promising result was obtained with the injection of cytoplasmic toxoid containing 10.0 mg protein in the initial dose and followed with doses of 5.0 mg and 0.5 mg. Liver abscess incidence was markedly reduced to 10% in this group. This reduction was statistically significant ($P<.05$) compared to the uninoculated control. Moreover, no scars were found in the livers and the average number of abscesses per liver (2.0) was low. The significant decrease in the abscesses in the latter group and the decrease in the number of scars in both cytoplasmic treated groups constituted the main features of this experiment.

The results of the agar-gel immunodiffusion tests are listed in Table II. Most of the positive sera produced strong precipitin lines with the undiluted antigen and the half antigen dilution. None of the pre-

inoculation sera showed precipitin lines with the cytoplasmic antigen. When the sera were tested eight weeks following the initial dose of the toxoids, 35% of the sera of control animals not receiving toxoid showed precipitin lines against the antigen. The sera collected from the sonicated toxoid group showed 74% with lines, 70% in the cytoplasmic toxoid (smaller dose) and 95% with the larger dose. Following the second and third doses of the toxoids the number of animals showing precipitin lines dropped to 31% in the controls and to 35-42% in the toxoid groups. Seventy-five percent of animals with liver abscesses had sera which were negative for precipitins on the last bleeding date (October 16) prior to slaughter.

DISCUSSION

Results of this study suggest that cattle may be protected against liver abscesses with the use of *S. necrophorus* cell extracts. The cytoplasmic toxoid produced the most promising results. The amount of cytoplasmic toxoid injected appeared to be critical. For instance, the group receiving an initial dose of 10.0 mg protein and a total of 15.5 mg in the three doses had abscesses in only 10% of the livers without scars compared with 35% liver abscesses with scars in the control groups. Whereas in the group receiving a smaller dose of cytoplasmic protein (10.5 mg total) the abscess incidence was just slightly reduced to 30% and one scar was found. Smith and Jones (9) indicated that nonparasitic induced scars result from the healing of abscesses caused in most instances by *S. necrophorus*. The number of abscesses per infected liver in the cytoplasmic toxoid groups also was reduced markedly.

The inoculation of sonicated toxoid did not reduce the liver abscess incidence probably because insufficient cytoplasmic antigen was present to confer the same level of immunity or protection in animals as that produced by the cytoplasmic toxoid. Indeed, all animals with liver abscesses in this group had sera that were negative

TABLE I. Results of Experiments with *S. necrophorus* Toxoids

Group	Treatment	No. of Animals	Initial Dose (ml)	Total Dose[a] (mg protein)	Abscessed Livers No.	Abscessed Livers %	Fibrous[b] Scars No.	Fibrous[b] Scars %	Abscess and Scars No.	Abscess and Scars %	Abscesses per Infected Liver Ave. No.
I	Control (uninoculated)	32	0.0	0	10	32	2	6	12	38	7
II	Adjuvant Control[c]	17	10.0	0	3	18	2	12	5	29	6
	Total Controls	49			13	27	4	8	17	35	6.5
III	Sonicated Toxoid[d]	19	10.0	15.5	5	26	2	11	7	37	7.0
IV	Cytoplasmic Toxoid[e]	20	5.0	10.5	5	25	1	5	6	30	1.4
V	Cytoplasmic Toxoid	20	10.0	15.5	2	10	0	0	2	10	2.0

[a]Total Dose: Initial dose of toxoid (1 mg/ml protein) + 5.0 mg protein toxoid (8 weeks later) + 0.5 mg protein toxoid (16 weeks after initial dose). All doses given subcutaneously
[b]Healed abscess, not considered parasitic (9)
[c]10% aluminum-potassium sulfate, injected subcutaneously
[d]Whole organism disrupted ultrasonically and formalized, alum precipitated
[e]Crude bacterial cell extract minus the cell walls, alum precipitated

TABLE II. Results of the Agar-gel Immunodiffusion Tests

Group	Treatment	Preinoculation Sera Positive/Total	Preinoculation Sera %	Postinoculation Sera Positive/Total	Postinoculation Sera %
I.....	Uninoculated control	0/32	0	9/32[a]	28
II.....	Adjuvant control	0/17	0	8/17	47
	Total Controls	0/49	0	17/49	35.0
III.....	Sonicated Toxoid	0/19	0	14/19	74.0
IV.....	Cytoplasmic Toxoid	0/20	0	14/20	70.0
V.....	Cytoplasmic Toxoid	0/20	0	19/20	95.0

[a]Serum from uninoculated animals

for precipitins on the October 16 bleeding. Fewer abscesses developed in the control group inoculated with the alum adjuvant alone. There is at present no clear explanation for this since the sonicated toxoid, which also contained this adjuvant, did not provide any detectable protection.

It would be highly desirable to produce an efficient vaccine against *S. necrophorus* infections particularly bovine liver abscesses. Evaluation of the efficacy of such a vaccine is particularly difficult because it is not easy to reproduce consistently hepatic lesions by experimental inoculation of *S. necrophorus* (5). Moreover, the intraportal route may not be the sole mode of infection under natural conditions. Thus large groups of animals maintained under field conditions are required to obtain convincing evidence that vaccination is useful in the prevention of this disease. Since there is some indication that the amount of protein antigen given may be related to the degree of immunity produced experiments are now in progress using a larger number of cattle vaccinated with a more purified toxoid of cytoplasmic protein.

It must be emphasized that these results are preliminary and that much more data are required before a satisfactory toxoid may be devised to prevent this disease. The number of doses required, the frequency of administration, the amount of protein antigen in each dose, the type of antigen (mono- or polyvalent) most suitable and the age at which the calves should be vaccinated are only a few of the basic questions that must be answered by experimental trials. However, a start has been made along lines which, as far as we are aware, have not been attempted with success in the past.

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