

AN IMMUNE RESPONSE TO RUMINAL STREPTOCOCCUS BOVIS,
AN ORGANISM IMPLICATED IN FEEDLOT BLOAT OF CATTLE

by *RLG*

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INTRODUCTION

Recently the feedlot bloat problem, in cattle, has become more important economically due to the extensive practice of fattening cattle on high-grain rations. The etiology of feedlot bloat is more uncertain than legume bloat, and the methods used to reduce legume bloat usually will not effectively relieve cattle suffering from feedlot bloat.

Some cattle are more susceptible to feedlot bloat. It has been shown (Hartman et al., 1962) that upon exchange of the rumen contents of a bloater and a non-bloater, the cattle returned to their respective status within three days. This suggested the possibility that an animal factor may be present which influences the severity of bloat in some animals.

Work was initiated to investigate the possibility of an immune response in cattle to Streptococcus bovis, a heavily encapsulated organism implicated in feedlot bloat. If there is an animal factor affecting S. bovis, how does it relate to the severity of bloat, and how is its effect manifested in the rumen?

REVIEW OF LITERATURE

Bloat

Hungate (1966) reviewed the literature extensively and gave a concise definition of bloat as the build-up of pressure in the rumen. Ruminant bloat is of two types, frothy and free-gas bloat. In either type the animal is unable to eructate the fermentation gases. Impetus has been given to feedlot bloat studies when a bloat-provoking ration consisting of 61% barley, 16% soybean oil meal, 22% alfalfa meal, and 1% NaCl was fed to cattle. Severe bloat resulted from the high carbohydrate ration, rather than from the consumption of fresh alfalfa, as in legume bloat. Jacobson and Lindahl (1955) suggested that feedlot bloat might sometimes be of a free-gas type, and Hungate (1966) states that frothy bloat has been observed in animals fed a high-grain ration.

Frothy bloat is scored from zero to five (Bartley and Yadava, 1961). A score of zero denotes no frothing and no distention of the rumen walls. A score of five represents frothy rumen ingesta, badly distended rumen walls on both sides, and other abnormalities such as defecation, urination, protruding anus, and respiratory distress.

Etiology of Feedlot Bloat

Hungate et al. (1955) advanced the possibility that slime production by rumen microorganisms could be a factor in frothy legume bloat. This subject will not be dealt with in this study. For a discussion of frothy legume bloat read the papers, Bloat in Cattle. I to XIV, by Bartley et al. Jacobson and Lindahl (1955) suggested that slime production in the rumen

traps fermentation gases and results in frothy digesta causing feedlot bloat. A high positive correlation was found by Jacobson et al. (1957) between the percentage of encapsulated microorganisms and the occurrence of bloat in cattle fed a bloat-provoking diet. Streptococcus bovis and Pectostreptococcus elsdonii were strongly implicated as agents responsible for feedlot bloat (Gutierrez et al., 1959). Hartman et al. (1962), however, found little correlation between numbers of streptococci and bloat in the rumen of animals on feedlot bloat-provoking rations. Bryant et al. (1961) also found little, if any, correlation between the occurrence or numbers of any predominant groups of bacteria and feedlot bloat. The etiology of feedlot bloat remains indefinite.

MATERIALS AND METHODS

Animals

Twenty-eight head of cattle receiving various treatments for the prevention of bloat* were furnished by the Department of Dairy and Poultry Science. They were of different breeds and ages. The cattle were changed from hay to a bloat-provoking ration (composition in appendix). The ratio of the bloat ration to hay was steadily increased until the hay was eliminated from the diet. Bloat severity was determined $1\frac{1}{2}$ hours after feeding times of 8:00 A.M. and 5:00 P.M. Bloat scores from zero to five (Bartley and Yadava, 1961) were used.

The cattle were divided into five treatment groups: one group of five cattle was given daily treatments of mineral oil; another group of six was given poloxalene; a third group of five received magnesium oxide; a fourth group of five older cattle, and a fifth group of six young cattle were used as controls.

Collection of Blood

The cattle were bled at predetermined intervals. The first bleeding was denoted as "0" time or before the bloat ration was started. Samples of jugular vein blood were collected in large test tubes by Dr. Erle E.

*They were used in a simultaneous experiment by Drs. Erle E. Bartley and RoNel Meyer of the Dairy Science Department. These compounds were being tested for ability to break up foam in feedlot bloat (See Table 1, Page 8).

Bartley, his staff, and the author. The blood samples were held at room temperature for two to three hours and allowed to clot. Complete clot retraction occurred by placing the tubes in a refrigerator for several hours. After this treatment, clear serum was obtained by centrifugation with a Servall angular head centrifuge at 3500 rpm. for 25 minutes and the serum was decanted into pre-labeled sterile tubes. The serums were stored at -4 C.

Collection of Saliva

Saliva was collected from two bloating cattle and two non-bloaters. A rubber tube was inserted into the animal's mouth to stimulate salivation. The resulting excess of oral saliva was allowed to drip into a funnel held above a glass tube. A sufficient amount of saliva for immunological tests was collected by this method.

Organisms Used for Antigen Preparations

Two strains of Streptococcus bovis, 7H4 and 7H4SV, were obtained from Dr. Marvin P. Bryant of the University of Illinois, Urbana. Three strains, 18C2, 18M2, and 2B, were obtained from Dr. Peter N. Hobson and Mr. S. O. Mann of the Rowett Research Institute, Aberdeen, Scotland. All strains checked out according to Bergey's Manual of Determinative Bacteriology (Breed et al., 1957). The cultures were maintained on trypticase-soy agar slants plus 0.5% soluble starch. Cultures for antigens were prepared as follows: Each strain was grown in 500 ml aliquots of dextrose or sucrose broth. Organisms were also harvested from Blake bottles of trypticase-soy agar or sucrose agar. For maximum capsule production, all cultures were incubated at 37 C for three to four days.

A strain of Streptococcus, presumable bovis species, was isolated from the rumen of a fistulated cow being fed the same bloat-provoking ration. The strain showed some atypical characteristics.

Antigen Preparations

The cells were harvested from the broth cultures by centrifugation at 10,000 rpm. in a Servall angular head centrifuge for ten minutes. The supernatant was decanted, and the cells were resuspended in 0.5% phenolized saline. The cell concentration was adjusted to Nephelometer tube 1. The antigens were neutralized with 1N NaOH. The agar-grown cultures were washed from the surface with ten ml of phenolized saline, diluted to the same concentration and neutralized as described above.

In order to develop a rapid serum plate antigen the cells from Blake bottles containing sucrose agar were harvested by suspending them in saline to a concentration 200X Nephelometer tube 1. Aniline dyes were added to 25 ml of suspension at the following ratio: To each liter of the product add two ml of 1% aqueous crystal violet and four ml of 1% brilliant green.

Antigen-Antibody Tests

A standard bacterial tube agglutination test (see appendix for procedure) was used. In each test the serum was diluted 1:25, 1:50, 1:100, 1:200, 1:400. Also the standard rapid serum plate agglutination test (see appendix for procedure) was used with the antigen obtained as described above. The complement-fixation test was employed to determine the antibody titer of saliva. The procedure was altered by changing the first incubation time from 30 minutes to approximately 12 hours, and the first

incubation was carried out in the refrigerator instead of in a 37 C water bath.* The saliva was tested unaltered. The antigen used was a saline suspension of S. bovis strain 7H4 grown on sucrose agar.

Note: See appendix page 27 for procedures in which negative results were obtained.

RESULTS

Bloat

The animals showed signs of bloat approximately six weeks after the bloat-provoking ration was started. The young cattle did not show any bloat symptoms throughout the entire experiment.

The chemicals administered by Drs. Erle E. Bartley and RoNel Meyer did not appear to have any effect on bloat. This can be seen in table 1 which lists the bloat scores of the cattle receiving various treatments. Hence, these compounds should not alter research findings, as the controls and test animal bloat scores in no way reflected treatments described.

Isolation of a Rumen Streptococcus

The organism isolated from the rumen digested starch on starch agar plates. This was shown by a clear zone around the colonies after flooding the plate with a Gram's iodine solution. The cocci appeared slightly larger than the Streptococcus bovis and chain formation was indefinite.

* Complement-fixation tests using bacterial antigens require longer incubation periods (Carpenter, 1965).

Table 1. Bloat scores¹ of experimental cattle receiving various foam-reducing treatments.

Experimental animals	Treatment	Time of bleeding (wks.)			
		0	3	8	21
036D	None	0	0	5	3
0285C	None	0	0	5	2
0169E	None	0	0	3	3+
011D	None	0	0	5	2+
0161E	None	0	0	6	5
025C	Mineral oil	0	0	5	4
098C	Mineral oil	0	0	1	2+
0174E	Mineral oil	0	0	2	0
0173E	Mineral oil	0	0	3	3
046C	Mineral oil	0	0	4	4
0110F	Poloxalene	0	0	2	0
0109F	Poloxalene	0	0	1	1
0116F	Poloxalene	0	0	0	2
0190E	Poloxalene	0	0	0	0
047D	Poloxalene	0	0	5	3
69D	Poloxalene	0	0	6	4
0194E	MgO	0	0	2	0
0112F	MgO	0	0	2	1
0118F	MgO	0	0	3	4
0113F	MgO	0	0	1	3+
048D	MgO	0	0	6	4
68D	MgO	0	0	7	3+
94B	None	0	0	0	- ²
97C	None	0	0	0	-
B115	None	0	0	0	-
B130	None	0	0	0	-
169B	None	0	0	0	-
295C	None	0	0	0	-

¹The bloat score is the sum of the morning and evening scores on the day of bleeding.

²The last six cattle in the table were under eight months of age and were not bled the last time. All other cattle were adults.

The isolated organism and Streptococcus bovis strains 7H4, 18M2, 18C2, and 2B, showed heavy capsule production in dextrose broth, sucrose broth, and on sucrose agar. The method used to stain the capsule was that of White (1947). S. bovis strain 7H4SV occasionally showed capsule formation. None of the organisms appeared to form extensive capsular material on standard trypticase-soy agar.

Bacterial Tube Agglutination Results

The results of the tube agglutination tests are given in tables 2 through 5. In tables 2 and 3 all the animals are listed and the titer of the serum for each of the antigens shown across the top of the tables. From these tables it is evident that the titer was higher when S. bovis strain 7H4 was grown in dextrose broth and on sucrose agar. In subsequent tests ten representative animals were compared using the best antigens described previously. These test results are shown in tables 4 and 5.

Rapid Serum Plate Agglutination Results

The results of the rapid plate agglutination tests are shown in tables 6, 7 and 8. The antigens used were suspensions of S. bovis strains 7H4 and 18M2 and the isolated Streptococcus species which were grown on sucrose agar. Ten representative animals were compared as above. The results in these tests were obtained in a much shorter time and the results were much stronger than the agglutinations in the tube tests.

Complement-Fixation Results

Preliminary tests of the saliva samples from the bloaters and non-bloaters showed perceptible but very small amounts of antibody against the antigen. The antigen in this test was a saline suspension of S. bovis strain 7H4 grown on sucrose agar. The titer of the antibody was approximately 1:48. Further confirmatory tests were not performed at this time.

Note: See appendix page 27 for results that were not used. The test procedures did not prove applicable to this work but are included to illustrate blind or false starts.

Table 2. Titer of antibody against various antigens¹ at "0" time.

Experimental animals	Bloat scores	Antigens					
		1	2	3	4	5	6
0285C	0	1:200	0	1:50	0	0	1:100
0161E	0	1:400	1:25	1:100	0	0	1:200
0109F	0	1:100	1:25	1:50	0	0	1:50
0116F	0	1:200	0	1:25	0	0	1:100
0190E	0	1:200	1:25	1:25	0	0	1:50
69D	0	1:400	1:50	1:100	0	0	1:200
0113F	0	1:100	0	1:50	0	0	1:50
68D	0	1:400	1:100	1:100	0	0	1:200
94B	0	1:100	0	1:25	0	0	1:25
295C	0	1:400	1:50	1:100	0	0	1:100
036D	0	1:400	1:25	1:50	0	0	1:200
0169E	0	1:200	1:50	1:100	0	0	1:100
011D	0	1:400	1:25	1:100	0	0	1:200
025C	0	1:200	1:25	1:50	0	0	1:400
098C	0	1:200	1:25	1:25	0	0	1:50
0174E	0	1:200	1:25	1:25	0	0	1:25
0173E	0	1:200	0	1:50	0	0	1:100
046C	0	1:100	0	1:100	0	0	1:200
0110F	0	1:100	0	1:25	0	0	1:200
047D	0	1:200	1:25	1:50	0	0	1:400
0194E	0	1:100	0	1:25	0	0	1:50
0112F	0	1:200	1:25	1:50	0	0	1:50
0118F	0	1:200	1:25	1:100	0	0	1:200
048D	0	1:400	1:50	1:100	0	0	1:400
97C	0	1:200	1:25	1:25	0	0	1:400
B115	0	1:200	1:25	1:50	0	0	1:200
B130	0	1:200	1:25	1:100	0	0	1:200
169B	0	1:100	1:25	1:25	0	0	1:100

¹The antigens used in this experiment are as follows:

1. S. bovis strain 7H4 grown in dextrose broth.
2. S. bovis strain 7H4SV grown in dextrose broth.
3. S. bovis strain 7H4 grown on standard trypticase-soy agar.
4. Escherichia coli B grown in dextrose broth.
5. S. bovis strain 7H4SV grown on trypticase-soy agar.
6. S. bovis strain 7H4 grown on sucrose agar.

Table 3. Titer of antibody against various antigens¹ after three weeks on bloat ration.

Experimental animals	Bloat scores	Antigens					
		1	2	3	4	5	6
0285C	0	1:200	0	1:25	0	0	1:100
0161E	0	1:400	1:25	1:100	0	0	1:100
0109F	0	1:100	1:25	1:25	0	0	1:50
0116F	0	1:200	0	0	0	0	1:50
0190E	0	1:200	1:25	1:25	0	0	1:50
69D	0	1:400	1:25	1:50	0	0	1:200
0113F	0	1:100	0	1:50	0	0	1:50
68D	0	1:400	1:100	1:100	0	0	1:400
94B	0	1:100	0	1:25	0	0	1:50
295C	0	1:400	1:25	1:100	0	0	1:100
036D	0	1:400	1:25	1:50	0	0	1:200
0169E	0	1:200	1:25	1:100	0	0	1:100
011D	0	1:400	1:25	1:50	0	0	1:100
025C	0	1:200	1:25	1:25	0	0	1:200
098C	0	1:200	1:25	0	0	0	1:100
0174E	0	1:200	1:25	1:25	0	0	1:50
0173E	0	1:200	1:25	1:50	0	0	1:100
046C	0	1:100	0	1:50	0	0	1:200
0110F	0	1:100	0	1:25	0	0	1:200
047D	0	1:200	1:25	1:50	0	0	1:400
0194E	0	1:100	0	1:50	0	0	1:100
0112F	0	1:200	1:25	1:50	0	0	1:50
0118F	0	1:200	1:25	1:100	0	0	1:200
048D	0	1:400	1:50	1:100	0	0	1:400
97C	0	1:200	1:50	1:25	0	0	1:400
B115	0	1:200	1:25	1:25	0	0	1:200
B130	0	1:200	0	1:50	0	0	1:200
169E	0	1:100	1:25	1:25	0	0	1:100

¹The antigens used in this experiment are as follows:

1. S. bovis strain 7H4 grown in dextrose broth.
2. S. bovis strain 7H4SV grown in dextrose broth.
3. S. bovis strain 7H4 grown on standard trypticase-soy agar.
4. Escherichia coli B grown in dextrose broth.
5. S. bovis strain 7H4SV grown on trypticase-soy agar.
6. S. bovis strain 7H4 grown on sucrose agar.

Table 4. Titer of antibody against various antigens¹ after eight weeks on bloat ration.

Experimental animals	Bloat ₂ scores	Antigens	
		1	2
0285C	5	1:200	1:200
0161E	6	1:200	1:100
0109F	1	1:200	1:100
0116F	0	1:400	1:100
0190E	0	1:100	1:50
69D	6	1:400	1:200
0113F	1	1:200	1:100
68D	7	1:400	1:400
94B	0	1:100	1:50
295C	0	1:400	1:200

¹The antigens used in this experiment are as follows:

1. S. bovis strain 7H4 grown in dextrose broth.
2. S. bovis strain 7H4 grown on sucrose agar.

²The bloat score is the sum of the morning and evening scores on the day of bleeding.

Table 5. Titer of antibody against various antigens¹ after 21 weeks on bloat ration.

Experimental animals	Bloat ₂ scores	Antigens	
		1	2
0285C	2	1:100	1:100
0161E	5	1:200	1:100
0109F	1	1:200	1:50
0116F	2	1:200	1:50
0190E	0	1:100	1:50
69D	4	1:200	1:100
0113F	3+	1:100	1:50
68D	3+	1:100 ₃	1:100
94B	0	—	—
295C	0	—	—

¹The antigens used in this experiment are as follows:

1. S. bovis strain 7H4 grown in dextrose broth.
2. S. bovis strain 7H4 grown on sucrose agar.

²The bloat score is the sum of the morning and evening scores on the day of bleeding.

³No serums were available because the young cattle were not bled the last time.

Table 6. Titer of antibody against S. bovis strain 18M2 as determined by the rapid plate agglutination test.

Experimental animals	Time of bleeding (wks.)			
	0	3	8	21
0285C	1:400	1:200	1:200	1:200
0161E	1:200	1:400	1:400	1:200
0109F	1:100	1:200	1:400	1:200
0116F	1:100	1:200	1:200	1:200
0190E	1:200	1:200	1:200	1:100
69D	1:400	1:400	1:200	1:200
0113F	1:200	1:200	1:200	1:100
68D	1:200	1:400	1:200	1:200 ₁
94B	1:100	1:200	1:200	—
295C	1:400	1:400	1:400	—

¹No serums were available because the young cattle were not bled the last time.

Table 7. Titer of antibody against S. bovis strain 7H4 as determined by the rapid serum plate agglutination test..

Experimental animals	Time of bleeding (wks.)			
	0	3	8	21
0285C	1:400	1:200	1:200	1:200
0161E	1:400	1:200	1:200	1:200
0109F	1:100	1:200	1:400	1:200
0116F	1:100	1:200	1:200	1:100
0190E	1:200	1:400	1:200	1:100
69D	1:400	1:400	1:400	1:200
0113F	1:200	1:200	1:200	1:100
68D	1:200	1:200	1:400	1:200 ¹
94B	1:100	1:200	1:100	—
295C	1:400	1:400	1:400	—

¹No serums were available because the young cattle were not bled the last time.

Table 8. Titer of antibody against isolated Streptococcus species as determined by the rapid serum plate agglutination test.

Experimental animals	Time of bleeding (wks.)			
	0	3	8	21
0285C	1:400	1:200	1:200	1:200
0161E	1:200	1:200	1:200	1:100
0109F	1:100	1:200	1:200	1:100
0116F	1:100	1:200	1:200	1:200
0190E	1:200	1:400	1:200	1:200
69D	1:200	1:200	1:400	1:200
0113F	1:200	1:100	1:100	1:100
68D	1:200	1:200	1:200	1:100 ₁
94B	1:100	1:100	1:200	—
295C	1:400	1:400	1:400	—

¹No serums were available because the young cattle were not bled the last time.

DISCUSSION AND SPECULATIONS

The results indicate that an immune factor is present in the serum of most cattle, whether bloating or not, against Streptococcus bovis. There is a stronger response with certain strains of the organisms as indicated by the difference in the titers using the antigens made with strains 7H4 and 7H4SV. The type of media on which the bacteria were grown for antigen preparation was another factor affecting the strength of the immune response. The highest titers were obtained from strain 7H4 grown in the presence of dextrose followed by the same strain grown on sucrose.

Using the capsule stain, it was shown that the greatest capsular material was present around strain 7H4 when it was cultured on dextrose. This suggests the possibility that the capsule is the site of the antigen against which the antibody was produced. Further work could be done by isolating the capsular material to determine its antigenic specificity.

No correlations existed between the titer of the antibody in the serums and the severity of bloat. All animals showed some antibody response throughout the experiment. Some of the titers increased during the onset of bloat while others decreased. Possibly a correlation could be made if the serum titers were observed at closer intervals during a period in which an animal developed bloat.

The development of the rapid serum plate antigen could provide a means for a fast test to determine the titer of antibody in any animal against a particular strain of S. bovis. The tube agglutination test was time consuming and the precipitation and gel diffusion tests gave negative results (see appendix page 27 for negative procedures).

The only way the antibody could affect the number of S. bovis would be its presence in the rumen. There are two possibilities by which antibodies could get into the rumen. One is across the rumen wall and the other is salivary antibodies being transported to the rumen. No work was done on the first possibility. Preliminary investigations using the complement-fixation test showed a small amount of antibody in the saliva tested. There is a need for further confirmation. This could be the route by which antibodies enter the rumen. If this were true, a high antibody titer could possibly inhibit the specific organisms or capsule formation in the rumen, and the animal could be spared severe bloat. In these experiments the animals that bloated severely showed a high antibody titer at the time blood was taken, but closer observations might show a rise and fall in the titer just as the severity of bloat rises and falls over a period of time. If the animal's immune response was overwhelmed by a large increase in the streptococci, causing immune antibody paralysis, the antibody titer might drop too low to have an effect and severe bloat would result. This must not be the case though because the titers stayed about the same throughout the experimental period.

Secretory or salivary immunoglobulins have been found in cattle and sheep (Tomasi, 1968). The immunoglobulins are transudated from serum to saliva in these animals. The most prevalent type seems to be immunoglobulin G (IgG) which fixes complement. It has been shown that agammaglobulinemic newborn calves show a decrease in the selective transport of Ig into their saliva. Further work with saliva needs to be done to determine the type and concentration of secretory antibody present in bloaters and non-bloaters.

SUMMARY

Studies were carried out on the serums and saliva of cattle being fed a feedlot bloat-provoking ration. Some cattle were suffering from feedlot bloat. The following results were obtained:

1. An immune response to Streptococcus bovis, an organism implicated in feedlot bloat, is present in cattle.
2. Strains of S. bovis, showing extensive capsule formation on dextrose or sucrose, exhibit a greater immune response than strains showing little capsule formation.
3. The titer of antibody does not appear to correlate with the severity of bloat.
4. The S. bovis rapid serum plate antigen developed provides a fast method of determining antibody titer in cattle.
5. Preliminary investigations point to a perceptible although very small amount of antibody to S. bovis in ruminant saliva.

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LITERATURE CITED

- BARTLEY, E. E. 1965. Bloat in cattle. VI. Prevention of legume bloat with a nonionic surfactant. *J. Dairy Sci.* 48:102-104.
- BARTLEY, E. E., AND R. BASSETTE. 1961. Bloat in cattle. III. Composition of foam in legume bloat. *J. Dairy Sci.* 44:1365-1366.
- BARTLEY, E. E., T. J. CLAYDON, L. R. FINA, C. HAY, I. S. YADAVE. 1961. Bloat in cattle. II. Its development on alfalfa pasture after inoculation of empty rumina with autoclaved or fresh rumen fluid. *J. Dairy Sci.* 44: 553-555.
- BARTLEY, E. E., H. LIPPKE, H. B. PFOST, R. J. NIJWEIDE, N. L. JACOBSON, R. M. MEYER. 1965. Bloat in cattle. X. Efficacy of poloxalene in controlling alfalfa bloat in dairy steers and in lactating cows in commercial herds. *J. Dairy Sci.* 48:1657-1662.
- BARTLEY, E. E., AND I. S. YADAVA. 1961. Bloat in cattle. IV. The role of bovine saliva, plant mucilages, and animal mucins. *J. Animal Sci.* 20: 648-653.
- BREED, R. E., E. D. G. MURRAY, AND N. R. SMITH. 1957. *Bergey's Manual of Determinative Bacteriology*. 7th Ed. Williams and Wilkins, Baltimore.
- BRYANT, M. P., I. M. ROBINSON, AND I. L. LINDAHL. 1961. A note on the flora and fauna in the rumen of steers fed a feedlot bloat-provoking ration and the effect of penicillin. *Appl. Micro.* 9:511-515.
- CARPENTER, PHILIP L. 1965. *Immunology and serology*. W. B. Saunders Company, Philadelphia and London.
- FINA, L. R., C. A. HAY, E. E. BARTLEY, B. MISHRA. 1961. Bloat in cattle. V. The role of rumen mucinolytic bacteria. *J. Animal Sci.* 20:654-658.
- GUTIERREZ, J., R. E. DAVIS, I. L. LINDAHL, E. J. WARWICK. 1959. Bacterial changes in the rumen during the onset of feedlot bloat of cattle and characteristics of Peptostreptococcus elsdenii n. sp. *Appl. Micro.* 7:16-22.
- HARTMAN, P. A., R. H. JOHNSON, L. R. BROWN, N. L. JACOBSON, R. S. ALLEN, P. R. SHELLENBERGER, H. H. VAN HORN, JR. 1962. Relationship of rumen facultative anaerobes to feedlot and pasture bloat. *Iowa State J. Sci.* 36:217-231.
- HELMER, L. G., E. E. BARTLEY, R. M. MEYER. 1965. Bloat in cattle. IX. Effect of poloxalene, used to prevent legume bloat, on milk production, feed intake, health, reproduction, and rumen fermentation. *J. Dairy Sci.* 48:575-579.

- HUNGATE, R. E. 1966. The rumen and its microbes. Academic Press, New York and London.
- JACOBSON, D. R., AND I. L. LINDAHL. 1955. Studies on biochemical, physical, and bacteriological factors involved in feedlot bloat. Univ. Md. Misc. Pub. 238:9-15.
- JACOBSON, D. R., I. L. LINDAHL, J. J. MCNEILL, J. C. SHAW, R. N. DOETSCH, R. E. DAVIS. 1957. Feedlot bloat studies. II. Physical factors involved in the etiology of frothy bloat. J. Animal Sci. 16:515-524.
- MEYER, R. M., E. E. BARTLEY, C. W. DEYOE. 1965. Bloat in cattle. VII. Relation to amino acid composition of alfalfa as affected by maturity. J. Dairy Sci. 48:213-216.
- MEYER, R. M., E. E. BARTLEY, J. L. MORRILL, W. E. STEWART. 1964. Salivation in cattle. I. Feed and animal factors affecting salivation and its relation to bloat. J. Dairy Sci. 47:1339-1345.
- MEYER, R. M., L. G. HELMER, E. E. BARTLEY. 1965. Bloat in cattle. VIII. Extent of elimination in milk and body tissues of C¹⁴-labeled poloxalene used to prevent legume bloat. J. Dairy Sci. 48:503-505.
- MISHRA, B. D., E. E. BARTLEY, L. R. FINA, M. P. BRYANT. 1968. Bloat in cattle. XIV. Mucinolytic activity of several anaerobic rumen bacteria. J. Animal Sci. 27:1651-1656.
- MISHRA, B. D., L. R. FINA, E. E. BARTLEY, T. J. CLAYDON. 1967. Bloat in cattle. XI. The role of rumen aerobic (facultative) mucinolytic bacteria. J. Animal Sci. 26:606-612.
- STILES, D. A., E. E. BARTLEY, A. B. ERHART, R. M. MEYER, F. W. BOREN. 1967. Bloat in cattle. XIII. Efficacy of molasses-salt blocks containing poloxalene in control of alfalfa bloat. J. Dairy Sci. 50:1437-1443.
- THOMAS, T. B., J. BIENENSTOCK. 1968. Secretory immunoglobulins. Adv. in Immunology. 9:1-96.
- VAN HORN, JR., H. H., E. E. BARTLEY. 1961. Bloat in cattle. I. Effect of bovine saliva and plant mucin on frothing rumen contents in alfalfa bloat. J. Animal Sci. 20:85-87.
- WHITE, P. BRUCE. 1947. A method for combined positive and negative staining of bacteria. J. Path. Bact. 59:334.

APPENDIX

Bacterial Tube Agglutination Test

1. For each sample of serum to be tested, label 5 agglutination tubes consecutively 1 to 5.
2. With B.A.I. pipette add the following amounts of the serum to be tested to each tube as follows:

Tube	1	0.08 ml
	2	0.04
	3	0.02
	4	0.01
	5	0.005

3. Pipette 2 ml of antigen into each serum tube and add 2 ml of antigen to an empty tube as an antigen control. Mix the serum and antigen well.
4. Incubate the tubes at 37 C for 42-48 hours.
5. Read and record the results as follows:

- + indicates complete agglutination
- indicates no agglutination
- I indicates incomplete agglutination

The highest dilution showing complete agglutination is the titer of the serum.

Rapid Blood Serum Plate Agglutination Test

1. Accurately measure upon a ruled glass plate the following quantities of sera for the desired dilutions:

Dilution desired	1:25	1:50	1:100	1:200	1:400
serum, ml	0.08	0.04	0.02	0.01	0.005

2. Place 0.03 ml of thoroughly mixed plate antigen with each quantity of serum. Place 0.03 ml of antigen on the plate with no serum to check the antigen for non-specific clumping.
3. Stir slowly and thoroughly with a clean toothpick or glass rod beginning with the mixture of highest dilution.

4. Rock or rotate the plate slowly 3 or 4 times, then place it in a 100% humidity chamber at room temperature for 7 minutes.
5. Again slowly rock or rotate the plate 3 or 4 times, then read the results as negative (-), incomplete (I), or positive (+), depending on the degree of clump formation for each serum-antigen ratio.

Bloat-provoking ration used by Drs. E. E. Bartley and Ronel M. Meyer to produce feedlot bloat in cattle.

60%	Corn and sorghum grain
22%	Dehydrated alfalfa meal
16%	Soybean meal
1%	Dicalcium phosphate
1%	Sodium chloride

Procedures Giving Negative Results

Each serum sample was tested for an immune response to S. bovis by means of the precipitation and gel diffusion tests. All the serums gave negative responses to each of the antigens tested. The antigens were the same as those used in the tube agglutination tests. The particulate or insoluble nature of these antigens may be a factor in the negative responses obtained by the two tests. The saliva samples were also tested by means of the precipitation and gel diffusion tests and they showed no immune response to the S. bovis antigens.

AN IMMUNE RESPONSE TO RUMINAL STREPTOCOCCUS BOVIS,
AN ORGANISM IMPLICATED IN FEEDLOT BLOAT OF CATTLE

by

RICHARD LEE GETTINGS

B. S., Kansas State University, 1968

AN ABSTRACT OF A MASTER'S THESIS

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Twenty-eight head of cattle were gradually changed from a hay diet to a high-grain bloat-provoking ration. Moderate to severe bloat developed in some cattle approximately six weeks after feeding of the bloat ration was started, while others showed no symptoms of bloat throughout the experimental period of 21 weeks.

Blood serums were collected from the cattle at predetermined intervals to test for an immune response against antigens prepared from various known ruminal strains of Streptococcus bovis, an organism implicated in feedlot bloat, and a Streptococcus species isolated from an experimental animal. By means of a standard bacterial tube agglutination test, an immune factor was found to be present against the strains of S. bovis which exhibited extensive capsule formation on dextrose and sucrose. A rapid serum plate antigen was developed which provided a fast method of determining the antibody titer in serum against S. bovis.

Preliminary investigations, utilizing the complement-fixation test, showed a very small but perceptible amount of antibody against S. bovis in the saliva of several experimental cattle. Complete confirmation of this result was not done at this time, but this may be the means by which the antibody to S. bovis manifests itself in the rumen. This may offer a new approach to the study of the etiology of feedlot bloat in cattle.