Haemophilus somnus: A Comparison among Three Serological Tests and a Serological Survey in Beef and Dairy Cattle

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ABSTRACT

Serological tests for the detection of antibodies against *Haemophilus somnus* were carried out in herds of beef and dairy cattle using three different techniques: agglutination, complement fixation and counterimmunoelectrophoresis. The agglutination test appeared to detect more seroreactors than the complement fixation and counterimmunoelectrophoresis tests. Results of the three tests indicated that there were more positive reactors in beef cattle and dairy cattle from infected herds than in dairy cattle from clinically normal herds.

Key words: *Haemophilus somnus*, agglutination, complement fixation, counterimmunoelectrophoresis.

RÉSUMÉ

Cette étude consistait à rechercher des anticorps sériques contre *Haemophilus somnus*, dans des troupeaux de bovins de boucherie et de bovins laitiers, à l'aide des trois techniques suivantes: l'agglutination, la déviation du complément et l'électrosynérèse. La première technique s'avéra plus efficace que les deux autres. Les résultats de cette étude permirent d'identifier un plus grand nombre de réacteurs chez les bovins de boucherie et les bovins laitiers qui appartenaient à des troupeaux infectés, que chez les bovins laitiers qui appartenaient à des troupeaux apparemment sains.

Mots clefs: *Haemophilus somnus*, agglutination, déviation du complément, électrosynérèse.

INTRODUCTION

Haemophilus somnus is a Gram-negative, pleomorphic rod-shaped bacterium which produces a wide range of pathological conditions in cattle. The organism has been reported to be antigenically homogeneous (6). The disease is usually seen in feedlot cattle during the first months after the introduction of the young animals to the feedlots (8; D.A. Barnum, personal communication, 1977). Thromboembolic meningoencephalitis, pneumonia, pleuritis, laryngitis and arthritis are the manifestations in these animals. *Haemophilus somnus* infection is also common in cow-calf operations and it can cause abortions (3,14). A ten year retrospective study of losses in cattle herds in Western Canada showed that the infection is also present on a smaller scale in dairy operations (11).

Serological surveys for this disease in beef cattle operations have been performed using agglutination and complement fixation tests with varying results (7,10,13). Using the complement fixation test, almost 100% of cattle from herds in which one or more animals had died of *H. somnus* septicemia were found to be serologically positive in comparison to 23% for cattle from clinically normal herds (5). The counterimmunoelectrophoresis technique has been used to detect cross-reactions between *H. somnus* and other bacteria (6).

The purpose of this paper is to report the results of the evaluation of three serological tests for the detection of antibodies in cattle infected naturally with *H. somnus* and also to compare the serological status of beef cattle in feedlots, dairy cattle in clinically normal herds and dairy cattle in selected, infected herds.

MATERIALS AND METHODS

Haemophilus somnus strain 43826 was obtained from Dr. L.R. Stephens (13). This strain was isolated from the brain of a steer with naturally occurring thromboembolic meningoencephalitis. It was lyophilized and stored in small aliquots. Two day-old culture, grown on chocolate blood agar containing 1% of Isovitalex[1] and incubated at 37°C, was used for the preparation of the various antigens.

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Submitted May 10, 1982.

[1]Isovitalex, BBL, Becton Dickinson and Co., Mississauga, Ontario.

Blood samples were collected from three groups of cattle. The first group consisted of 1795 dairy cattle (D.C.) from 231 herds and the second group included 380 beef cattle (B.C.) from 14 feedlots. In these two groups of animals, there were no confirmed cases of *H. somnus* infection. The third group (I.C.) was comprised of 88 cattle from seven dairy herds in which one or more animals had died of *H. somnus* septicemia, confirmed by isolation, during the two months prior to collection of the blood samples. In the first two groups, herds were randomly selected by the veterinarians of the "Services Vétérinaires du Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec". Ten percent of cattle over one year old were randomly sampled in each herd of the three groups. Sera were harvested with sterile precautions and stored, without any preservative, at -20°C until used.

MICROAGGLUTINATION TEST USING HEATED ANTIGEN (MAT-H)

The test was carried out according to Shigidi and Hoerlein (12) with slight modification. A two day-old growth of the bacterium, grown on chocolate blood agar plates, was suspended in formol saline (0.3% formalin) pH 7 and washed twice by centrifugation at 3,000 RPM for 20 minutes in a clinical centrifuge. The bacterial sediment was suspended in formol saline and heated to 60°C and held for 60 minutes in a water bath with continuous shaking. The suspension was washed again in formol saline and resuspended in formol saline to an optical density of 0.5 at 540 nm. Serial, twofold dilutions of the test sera, beginning at 1 in 4, were made in 0.05 mL volume in U-buttom polystyrene microplates. An equal volume of antigen was added to each dilution and mixed well. The plates were incubated at 37°C for 18 hours, followed by a further incubation at 4°C for three hours. The titer was considered as the last serum dilution which gave at least 50% agglutination.

MICROAGGLUTINATION TEST USING BOILED ANTIGEN (MAT-B)

This test was done as described above except that the antigen employed was boiled for 90 minutes.

MICROAGGLUTINATION TEST USING FORMALINIZED ANTIGEN (MAT-F)

The bacterial pellet, suspended in formol saline, was kept at 4°C for 48 hours, not heat treated and used as antigen in the same concentration as above.

COMPLEMENT FIXATION TEST (CFT)

The antigen was prepared by ultrasonic disruption of the whole cells using an ultrasonic disintegrator[2] at high intensity for 20 minutes while being cooled by ice in a jacketed glass bowl. The sonicate was centrifuged at 4,000 × g for 20 minutes and diluted 1 in 32 for use as the antigen. A standard technique, as described by Casey (2), was used to perform the test in U-bottom polystyrene microtiter plates. The complement source was guinea pig serum and the diluent used throughout was 0.1 M Veronal buffer (pH 7.3). All the test sera were heat inactivated at 56°C for 30 minutes before testing. The titers were expressed as the reciprocal of the highest dilution where a complete inhibition of the hemolysis was seen.

COUNTERIMMUNO-ELECTROPHORESIS (CIEP)

The same antigen as used for CFT was also used for CIEP except that it was used undiluted. The test was carried out by the method described by Cho and Ingram (4). The samples were placed into the wells of the plate. The plate was orientated so that the antigen containing wells were those closest to the negative pole (cathode) and serum wells closest to the positive pole (anode). The test was performed at 200 volts for 18 minutes. The reactions were read immediately.

In all the three tests, rabbit hyperimmune serum and bovine fetal serum were always used as positive and negative controls respectively.

STATISTICAL ANALYSIS

The results were analysed with a Chi square test.

RESULTS

The comparison of the results obtained using the three different preparations of antigen in the microagglutination test are presented in Table I. The formalinized antigen was found to be the most sensitive antigen but had a tendency to autoagglutinate. With MAT-F, 71.3% of the sera showed a

TABLE I. Comparison of the Results of the Microagglutination Test using Three Different Preparations of Antigen (167 Cattle Sera)

	MAT-H[b]		MAT-B[c]		MAT-F[d]	
Titer[a]	N[e]	%	N	%	N	%
0[f]	64	38.3	64	38.3	48	28.7
4	33	19.7	25	15.0	18	10.8
8	11	6.6	32	19.1	12	7.2
16	18	10.8	25	15.0	18	10.8
32	19	11.4	16	9.6	16	9.6
64	18	10.8	5	3.0	28	16.7
128	4	2.4	0	0	20	12.0
256	0	0	0	0	7	4.2
Mean $\log_2$ titer	3.9029		3.4563		4.9412	

[a]The reciprocal of highest dilution of serum giving a positive reaction
[b]Heated antigen
[c]Boiled antigen
[d]Formalinized antigen
[e]N (Number of serum samples)
[f]0: Negative in 1:4

[2]MSE 100 watt ultrasonic disintegrator, Measuring Scientific Equipment Ltd., London, England.

TABLE II. Results of the Microagglutimation Test on 2239 Cattle Sera using the Heated Antigen (MAT-H)

	D.C.[a] (1795)[d]		B.C.[b] (380)		I.C.[c] (64)	
Titer[e]	N[f]	%	N	%	N	%
0[g]	801	44.6	145	38.2	22	34.4
4	203	11.3	83	21.8	7	10.9
8	144	8.0	27	7.1	6	9.4
16	238	13.2	31	8.2	11	17.2
32	215	12.0	26	6.8	11	17.2
64	115	6.4	30	7.9	2	3.1
128	53	3.0	24	6.3	3	4.7
256	23	1.3	6	1.6	0	0
512	3	0.2	5	1.3	2	3.1
1024	0	0	2	0.5	0	0
2048	0	0	1	0.3	0	0
Mean $\log_2$ titer	4.1620		4.1404		4.3333	
Negative	801		145		22	
Positive[h]	994		235		42	

Chi square: 7.433, degree of freedom: 2, ($P \leqslant 0.02$)

[a]D.C. (dairy cattle)
[b]B.C. (beef cattle)
[c]I.C. (dairy cattle from selected infected herds)
[d]Numbers in parenthesis indicate serum samples tested
[e]The reciprocal of highest dilution of serum giving a positive reaction
[f]N (Number of serum samples)
[g]O: negative in 1:4
[h]A titer of 4 or more was considered positive

positive reaction and the mean $\log_2$ titer was 4.9412. A titer of 4 or higher was considered positive. The MAT-H and MAT-B showed the same percentage of positive sera but the mean $\log_2$ titer of the MAT-H was higher than the mean $\log_2$ titer of the MAT-B. Thus the heated antigen was chosen as the test antigen.

MAT-H APPLIED TO THE THREE GROUPS OF CATTLE SERA

The test was used on 2239 sera of the three groups of cattle. Results are presented in Table II and show that 55.4% of D.C. 61.8% of B.C. and 65.6% of I.C. had titers of 1:4 or greater. The mean $\log_2$ titer of I.C. was higher than that of B.C. and D.C. Serological results and sources of sera were statistically dependent ($P \leqslant .02$).

CFT APPLIED TO THE THREE GROUPS OF CATTLE SERA

The test was performed of 96 sera randomly selected in the three groups (Table III). A titer of 4 or higher was considered positive. None of the 30 D.C. sera reacted positively. The percentages of positive sera were 69% for B.C. and 25% for I.C. The highest titer was observed in B.C. sera and this group had mean $\log_2$ titer of 3.3448. The I.C. sera reacted weakly: all the 24 sera tested had titers ranging from 0 to 8. The Chi square test was statistically significant.

CIEP APPLIED TO THE THREE GROUPS OF CATTLE SERA

None of the cattle sera produced more than one line of precipitation. The test was performed on 839 D.C. sera, 42 B.C. sera and 88 I.C. sera (Table IV). The percentages of positive sera were 1.8, 11.9 and 6.8 respectively. The Chi square test was not valid because more than 20% of theoretical numbers were less than 5.

RELATION BETWEEN SEROTESTS

The MAT-H and the CFT were compared on 70 cattle sera (Table V). Forty-eight sera had positive titers with MAT-H and 27 with CFT. A total of 58.3% of sera reacted positively with MAT-H, but were negative with CFT and 31.8% of sera which had CF-titers were negative with MAT-H.

The MAT-H and the CIEP were performed on 943 cattle sera. Only eight of the 585 MAT-H negative sera produced one line of precipitation in CIEP and only 16 of the 358 MAT-H positive sera were reactive in CIEP.

The CFT and the CIEP were carried out on 86 sera. The six CIEP positive sera were also positive with CFT: three animals presented a titer of 8 and the other three had a titer of 16.

DISCUSSION

Heat-treated antigen has been considered to be the antigen of choice by several workers to perform the agglutination test (6,7, 9,12,13). Our results are also sim-

TABLE III. Results of the Complement Fixation Test (CFT) on 96 Cattle Sera

	D.C.[a] (30)[d]		B.C.[b] (42)		I.C.[c] (24)	
Titer[e]	N[f]	%	N	%	N	%
0[g]	30	100	13	31	18	75
4	0	0	4	9.5	2	8.3
8	0	0	13	31	4	16.7
16	0	0	10	23.8	0	0
32	0	0	2	4.7	0	0
Mean $\log_2$ titer	0		3.3448		2.6667	
Negative	30		13		18	
Positive[h]	0		29		6	

Chi square: 37.83, degree of freedom: 2 ($P \leqslant 0.0000$)

[a]D.C. (dairy cattle)
[b]B.C. (beef cattle)
[c]I.C. (dairy cattle from selected infected herds)
[d]Numbers in parenthesis indicate serum samples tested
[e]The reciprocal of highest dilution of serum giving a positive reaction
[f]N (Number of serum samples)
[g]O: Negative in 1:4
[h]A titer of 4 or more was considered positive

TABLE IV. Results of Counterimmunoelectrophoresis (CIEP) on 969 Cattle Sera

	D.C.[a] (839)[d]		B.C.[b] (42)		I.C.[c] (88)	
Result	N[e]	%	N	%	N	%
Negative	824	98.2	37	88.1	82	93.2
Positive	15	1.8	5	11.9	6	6.8

Chi square test: not valid because more than 20% of theoretical number were less than 5
[a]D.C. (dairy cattle)
[b]B.C. (beef cattle)
[c]I.C. (dairy cattle from selected infected herds)
[d]Numbers in parenthesis indicate serum samples tested
[e]N (number of serum samples)

ilar. This antigen did not show spontaneous agglutination as was the case with formalinized not heat treated antigen. A total of 61.8% of the 380 beef cattle sera tested with MAT-H showed titers of 4 or more and 10% had titers of 128 or more. A previous report by Stephens *et al* (13) on 80 beef cattle sera showed that 91.2% animals had titers of 4 or greater and 6.3% with titers of 128 or greater. Another report on 1238 beef cattle sera described 24.2% with titers of 25 or more (7).

The percentage of positive sera and the mean $\log_2$ titer obtained with the MAT-H were greater in dairy herds in which one or more cattle had died of *H. somnus* septicemia than in the dairy herds with no such history. Moreover, 7.8% of the I.C. sera had titers of 128 or more in comparison to 4.5% of the D.C. sera. If high agglutinin titers do not persist for many weeks as previously reported (7), the high prevalence of 55.4% positive cattle sera in clinically normal dairy herds may suggest a frequent exposure of the dairy cattle to *H. somnus* or related antigens. It is not clear why the clinical manifestations of *H. somnus* infection are much more frequent in feedlots than in dairy operations but stress, management factors and immunosuppressive diseases could conceivably interfere, more in feedlots than in dairy operations, with the normal bovine defense mechanisms.

Of the 96 cattle sera tested with the CFT, 30% of the animals had titers of 8 or more. This was about the same as 23% and 28.3% positive reactors in two similar serological studies as previously reported (5,10). None of the 30 clinically normal dairy cattle sera tested reacted with the CFT; however, only six of these sera reacted with low titers (<8) in MAT-H. The CFT titers never exceeded 32 in beef cattle sera but the percentage of positive reactors was about the same as was obtained with MAT-H. Only 25% of cattle sera from selected infected dairy herds reacted positively and all the titers were <16. Relatively short-term persistence of high postinfection titers could explain the incidence of low titers (1).

The CIEP as used with sonicated extract was not sensitive enough for the detection of antibodies to *H. somnus* in naturally infected animals. Only 26 of the 969 sera tested gave one line of precipitation. Yet, with this test as with the other two, B.C. and I.C. sera reacted proportionately more often than D.C. sera.

When we compared the three tests, it appeared that MAT-H detected more seroreactors than the other two. It follows from the comparison between the MAT-H and the CFT that these two tests were complementary and to ensure the detection of the maximum number of animals having antibodies to *H. somnus* it is suggested that the two tests should be performed simultaneously. The value of using multiple serological tests in a research situation is well recognized (13).

TABLE V. Relations Between Microagglutination (MAT-H) Complement Fixation (CFT) and Counterimmunoelectrophoresis Tests (CIEP)

MAT-H[b]	CFT (70)[a]	
	Negative	Positive
Negative	15	7
Positive	28	20
MAT-H[c]	CIEP (943)	
	Negative	Positive
Negative	577	8
Positive	342	16
CFT[d]	CIEP (86)	
	Negative	Positive
Negative	57	0
Positive	23	6

[a]Numbers in parenthesis indicate serum samples tested with the two tests
[b]Chi square: 0.272, degree of freedom: 1 (P 0.6021)
[c]Chi square: 7.409, degree of freedom: 1 (P 0.0065)
[d]Chi square test: not valid because more than 20% of theoretical number were less than 5

ACKNOWLEDGMENTS

The authors thank Dr. L.R. Stephens for provision of the type strain, Dr. M. Bigras-Poulin for his cooperation with statistical analysis and all the veterinarians of the "Services Vétérinaires du Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec" who helped in the collection of blood samples. This project was funded by the "Conseil des Recherches et Services Agricoles du Québec".

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