A Comparison of Various Haemophilus somnus Strains

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ABSTRACT

Sixty-eight Haemophilus somnus strains isolated from the bovine in Canada and the U.S.A. were compared. In media enriched with 5% ovine serum, 5% bovine serum and 10% yeast extract, H. somnus fermented glucose, levulose, maltose, mannitol, mannose, sorbitol, trehalose and xylose, but failed to ferment arabinose, dulcitol, galactose, inositol, lactose, raffinose, rhamnose, salicin and sucrose. The organisms acidified litmus milk, produced cytochrome oxidase, indole and hydrogen sulfide (H_2S) and reduced nitrates to nitrites. The motility, methyl-red, acetylmethyl-carbinol urease catalase, citrate, malonate, lysine, ornithine and arginine tests were negative. Haemophilus somnus was resistant to lincomycin, neomycin and triple sulfa, but susceptible to ampicillin, chloramphenicol, streptomycin, penicillin and tetracycline. No antigenic differences were noted between strains when tested against rabbit antisera of eight strains using agglutination, complement-fixation, immunodiffusion and counterimmunoelectrophoresis tests. Low titre cross-reactions were found in the agglutination tests with some of the anti-H. somnus rabbit sera with Actinobacillus lignieresi and Moraxella bovis. No distinct antigenic similarities to nine other species of pathogenic bacteria of animal origin were found. No difference was observed between H. somnus isolates from Ontario and those from western Canada and the U.S.A.

RÉSUMÉ

Cette expérience visait à comparer 68 souches de Haemophilus somnus, isolées chez des bovins du Canada et des États-Unis. Dans des milieux de culture enrichis de 5% de sérum ovin, de 5% de sérum bovin ou de 10% d'extrait de levure, le microbe fermenta le glucose, le lévlulose, le maltose, le mannitol, le mannose, le soribtol, le tréhalose et le xylose; il ne fermenta toutefois pas l'arabinose, le dulcitol, le galactose, l'inositol, le lactose, le raffinose, le rhamnose, la salicine ou le sucrose. Il acidifia le lait tournesolé, produisit de la cytochrome oxydase, de l'indole, ainsi que du sulfure d'hydrogène (H_2S), et il réduisit les nitrates en nitrites. Les épreuves de la motilité, du rouge de méthyle, de l'acétyl-méthyl-carbinol, de l'uréase, de la catalase, du citrate, du malonate, de la lysine, de l'ornithine et de l'arginine s'avérèrent négatives. H. somnus se révéla résistant à la lincomycine, à la néomycine et au triple sulfa, mais sensible à l'ampicilline, au chloramphénicol, à la streptomycine, à la pénicilline et à la tétracycline. On ne décela pas de différences antigéniques entre les souches, lorsqu'avec des antisérums préparés chez le lapin, on les soumit aux épreuves suivantes: agglutination, déviation du complément, immunodiffusion et contre-immuno-électrophorèse. En effectuant des épreuves d'agglutination avec Actinobacillus lignieresi et Moraxella bovis et des antiserums, préparés chez le lapin

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Based in part on thesis submitted by senior author to University of Guelph in partial fulfillment for M.Sc. degree.

Submitted May 13, 1976.

contre H. somnus, on réalisa que certains de ces antisérums donnaient une faible réaction croisée. On ne décela pas de similarités antigéniques distinctes, avec neuf autres espèces de bactéries pathogènes d'origine animale. On ne décela pas non plus de différences entre les souches de H. somnus isolées en Ontario, dans l'Ouest du Canada ou aux États-Unis.

INTRODUCTION

Haemophilus somnus produces a wide range of septicemic conditions in the bovine which include infectious thromboembolic meningoencephalitis (ITEME), pneumonia, arthritis, genital infections and abortion. Panciera *et al* (20) noted three clinical manifestations of *H. somnus* infections, peracute (neurological), acute (respiratory) and chronic (arthritic), with frequent overlapping of the syndromes. Waldhalm (26) suggested a role of *H. somnus* in the weak calf syndrome. Reports on abortions associated with this organism have been published (7, 25). At necropsy, brains of the neurological cases show multiple reddish foci of necrosis and inflammation with no apparent pattern of distribution (13). Similar lesions are found in cardiovascular, respiratory, urinary, muscular and cutaneous systems and joints (19, 20). *Haemophilus somnus* infections have been increasingly found among feedlot cattle in Canada and the U.S.A. MacDonald *et al* (17) reported that the disease was first seen in Alberta in 1969, while van Dreumel *et al* (24) first reported the disease and isolated the organism in Ontario. Dukes (12) described the ocular lesions of some ITEME cases in Ontario. In a survey in 1975, Little (personal communication), determined that ITEME was a disease of major concern to the Ontario beef producer and that some means of prevention and control were needed.

Kennedy *et al* (15) first isolated and identified the organism and found similarities with other *Haemophilus*, particularly *H. agni*. They described the bacterium as *Haemophilus*-like. Bailie (1) suggested the name *H. somnus*, which has been continued in the literature. However, this name was not validly published and in 1973, Bailie *et al* (2) concluded that their results did not justify the inclusion of the organism in the genus *Haemophilus* as presently defined (27).

Serologically, *H. somnus* has some similarities with *H. agni* (11, 22) and with *H. oakley* (19). Shigidi (22) found that seven isolates of *H. somnus* were antigenically homologous and that a definite cross-agglutination occurred with *H. agni* and *Bordetella bronchiseptica* but not with *H. influenzae, H. aegyptius, H. parainfluenzae, Antinobacillus actinodes, Brucella abortus* and *Moraxella bovis*. In contrast, Dierks *et al* (11) found cross reactions with *H. somnus, H. aphorphilus, H. aegyptius, H. influenzae* and *parinfluenzae*. They also noted one-way cross reactions between *H. somnus* and *Actinobacillus actinoides* and *M. bovis*. Miller *et al* (18) found cross reactions with *H. somnus* whole cell and crude polysaccharide antigens and *H. agni* and *A. lignieresi* cell suspensions. The objective of this work was to collect a series of *H. somnus* isolates for bacteriological and serological study, to determine their antibiotic susceptibility patterns, and to characterize the antigens possessed by the isolates using different serological techniques.

MATERIALS AND METHODS

Source Of Isolates

Sixty-eight isolates of *H. somnus* were obtained as indicated in Table I. Nine of the isolates from the U.S.A. were used by workers in previous reports (1, 11, 15, 20).

The strains were grown initially on brain heart infusion agar plates containing 5% calf blood and 0.5% yeast extract (BHICBYE plates) under 10% CO_2 at 37°C for 48 hours. Examination for capsules was made following Hiss' method (16, 18) after growing *H. somnus* on BHICBYE plates containing 4% lactose. The isolates were inoculated into the yolk sac of five to seven day old chick embryos incubated for 18 hrs, then the infected yolks were harvested and stored at -70°C. Viable bacterial counts were made on solid media.

The effect of different media on the growth of eight isolates of *H. somnus* was studied on blood agar (BA) base, choco-

TABLE I. Source of 68 *Haemophilus somnus* Strains

Region	No. Strains	Source — Tissue				
		Brain	Lung	Vagina	Fetus	Nose
Ontario	30	20	4	4	1	1
Alberta	28	22	6			
California	1	1				
Iowa	2	2				
Kansas	1	1				
Michigan	1	1				
Oklahoma	5	5				

late agar (CHA), trypticase soy agar (TSA), heart infusion agar (HIA) and brain heart infusion agar (BHIA). All media contained either 5% calf blood alone or plus 0.5% yeast extract. Inoculated plates of each media were incubated in aerobic, anaerobic and 5 to 10% CO_2 atmospheric conditions. After 48 hours of incubation at 37°C, subjective criteria such as the size of colonies and the total amount of growth were used in determining the effect of the media and graded 1 + (minimum growth) to 4 + (maximum growth). Twenty-four isolates of *H. somnus* were used to compare BHI, cystine heart agar (CYH) and eugonagar[1] (EUA) under 5% CO_2.

The effect of some supplementary factors on the growth of *H. somnus* was determined by seeding five isolates on BHA and HIA agar plates containing final concentrations of: a) 10% bovine blood, b) 5% bovine blood, c) 10% bovine serum plus 0.5% yeast extract (YE), d) 10% bovine serum, 0.5% YE and 1 mg/ml hemin, e) 10% bovine serum, f) 10% serum plus 1 mg/ml hemin, g) 1% YE, h) 0.5% YE, i) 0.5% YE plus 1 mg/ml hemin and j) 1 mg/ml hemin. All plates were incubated 48 hours at 37°C under 5% CO_2. The growth present on the plates was compared as above.

For the determination of fermentation reactions by *H. somnus*, 17 carbohydrates were used in basal media containing 1% of the carbohydrate, 5% bovine serum, 5% ovine serum and 0.5% YE. The tubes were incubated three days at 37°C under 10% CO_2, then held at room environment until the noninoculated controls returned to the original pH. The reactions were read as positive (+), doubtful (±) and negative (−).

The media for biochemical reactions contained 5% bovine serum, 5% ovine serum and 0.5% YE in addition to the basal ingredients. Standard diagnostic bacteriology procedures were employed to perform the tests (3, 5, 10, 16). In all cases, sterile negative controls as well as known positive controls were treated and incubated under identical conditions.

The susceptibility of *H. somnus* to 13 chemotherapeutic agents was assessed using single, low concentration discs on inoculated BHICBYE plates which was incubated at 37°C in 10% CO_2.

Eight isolates, one from Iowa (strain 09), two from Alberta (strains 48, 49) and five from Ontario representing one nasal isolate (strain 38), one cervical (strain 41) and three from ITEME (strains 17, 37, 39) were selected to immunize rabbits for production of anti-*H. somnus* sera. Bacteria, washed from BHICBYE plates and standardized to Brown's nephelometer No. 10, were injected i.v. in increasing doses once a week for six weeks.

The agglutination tests were performed using a technique modified from that described by Shigidi (22). The antigens for agglutination tests were prepared by washing a 48 hour old growth of each isolate with an equal solution of pH 8 physiological saline solution (PSS) and distilled water. The washed cultures were held in a water bath at 60°C for 90 minutes. The suspensions were diluted with an equal volume of 1% phenolized PSS pH 8 to an optical density of 0.39 at 550 millimicrons (40% transmittance) in a colorimeter.[2] The test was performed by making twofold serial dilutions in 0.5 ml of PSS pH 8, starting with a 1:10 dilution and adding 0.5 ml of antigen. The tubes were examined for agglutination after 12 hrs at 37°C and a further 12 hrs at room temperature.

Antigens for agglutination were prepared similarly with *Actinobacillus equuli, Actinobacillus lignieresi, Bordetella bronchi-*

[1]Eugonagar, BBL Laboratories. All other media and yeast extract, Difco Labs.

[2]Spectronic 20, Bausch & Lomb Inc., Rochester, N.Y.

TABLE II. Fermentation Reactions of 68 Strains of *H. somnus* Grown in Various Carbohydrates[a]

Carbohydrate	Positive %	Negative %	Doubtful %	Doubtful and Negative %
Arabinose[a]	1.5	66.2	32.4	98.5
Dulcitol	0	100	0	0
Galactose	0	82.4	17.6	100
Glucose	100	0	0	0
Inositol	0	94.1	5.9	100
Lactose	0	95.6	4.4	100
Levulose	98.5	0	1.5	1.5
Maltose	95.6	0	4.4	4.4
Mannitol	97.5	0	2.94	2.94
Mannose	100	0	0	0
Raffinose	0	100	0	100
Rhamnose	0	98.5	1.5	100
Salicin	0	98.5	1.5	100
Sorbitol	94.1	0	5.9	5.9
Sucrose	0	95.6	4.4	100
Trehalose	100	0	0	0
Xylose	100	0	0	0

[a]1% carbohydrate in phenol red broth base containing 5% bovine serum, 5% ovine serum and 0.5% YE

septica, Brucella abortus, Moraxella bovis, Neisseria catarrhalis, Pasteurella haemolytica, Pasteurella multocida and *Yersinia enterocolitica.* They were tested in the same manner against the anti-*H. somnus* rabbit sera.

The complement-fixation (CF) was performed utilizing the standard microtiter system as described by Casey (6) and Dierks (11). The CF antigen was prepared by washing the *H. somnus* isolate with sterile phosphate buffered saline (PBS) solution (0.005 molar, pH 7.4). The suspension was sonicated for 20 minutes at high intensity and centrifuged at R.C.F. 4,000 x g for 20 minutes. The supernatant was titrated for CF and stored in small aliquots in a −20°C freezer. The tests were performed in microtiter plates in dilutions of 1:2 to 1:256 of the serum.

The antigens for the immunodiffusion tests were prepared by washing 48 hour-growth of *H. somnus* from BHICBYE plates with sterile physiological saline solution, then centrifuging at 8,000 x g for 45 minutes. The cells were washed three times and the final suspension was made in 1% phenolized saline solution. Antigens were prepared from the other bacteria as above, plus *H. gallinarum* and *H. parasuis.*

The double immunodiffusion (Ouchterlony) technique was performed using commercial plates.[3] After filling the wells with the reactants, the plates were incubated at 37°C in a moist chamber, and the readings were made every 24 hours for three days for precipitation bands.

The counterimmunoelectrophoresis (CIEP) technique was carried out by the method of Scheidegger (21) as described by Cho (8). Antigens prepared as for the immunodiffusion tests were used against the anti-*H. somnus* rabbit sera. The samples were placed into the wells of the chamber and the electrophoresis was performed at 200 volts for 40 minutes, then the reactions were read.

RESULTS

Cultural and Biochemical Characteristics

The colonies of *H. somnus* on BHICBYE plates were convex, circular with entire edge, moist, smooth and glistening, reaching one to two mm in size in three days; they were butyrous in consistency, showing a slight yellow colour. About 50% showed a small zone of partial haemolysis when the colonies were removed, with the remainder nonhaemolytic except four isolates which had clear haemolysis around the colonies.

All strains were Gram-negative, small and pleomorphic, ranging from coccoid forms of 0.7 to 0.9 microns in diameter, to

[3]Immunodiffusion plates, pattern C Hyland, Div. Travenol Labs. U.S.A.

the most common coccobacillary forms of 0.8 to 1.1 microns in diameter and a predominant length of 1.2 microns. Occasional larger filamentous forms were present. A capsule could not be observed.

The inoculation of *H. somnus* into the yolk sac of the chick embryos consistently killed the embryos within 24-48 hours. The harvested yolks contained 2 to 6 x 10^6 viable organisms per ml, and the organisms could be recovered from the yolks after storage up to 18 months at –70°C.

The results of different media and atmospheric conditions showed that (a) 0.5% YE and 10% bovine blood were the best supplements to the basic medium, (b) the use of hemin 1 mg/ml did not enhance growth, (c) the media BHIA yielded growth similar to EUA followed by CYH, HIA, TSA, BA and CHA and (d) maximum growth was obtained under 10% CO_2 with all strains growing sparsely under aerobic and anaerobic conditions.

The results on fermentation of carbohydrates are summarized in Table II. If the doubtful and negative reactions are combined, there are two defined groups with eight carbohydrates being fermented by over 95% of the strains.

All isolates of *H. somnus* were consistent in the pattern of biochemical results which are summarized in Table III. The strains were positive for H_2S production when lead acetate-impregnated filter paper strips were placed on top of culture tubes while there was not enough production of H_2S to darken either TSI or SIM tubes. All isolates were positive for indol production when Kovacs reagent was added to tryptone broth while only 50% were positive when the reagent was added to SIM medium. Two of the isolates were negative for the indol test by these and all other methods tried.

The chemotherapeutic susceptibility results showed that the isolates were susceptible to ampicillin, bacitracin, cephalordin, chloramphenicol, erythromycin, novobiocin, penicillin, polymixin B, dihydrostreptomycin (one exception) and tetracycline. *H. somnus* was resistant to lincomycin (two exceptions), neomycin (12 exceptions, 17%) and triple-sulfa.

TABLE III. Summary of Reactions by 68 Isolates of *H. somnus* on Various Biochemical Tests

Tests with Positive Reaction	Tests with Negative Reaction
Cytochrome Oxodase Production	Arginine dihydrolization
Fermentative in 0/F Test	citrate utilization
Hydrogen Sulfide (H_2S) Production	Enzyme catalase detection
Indole production[a]	Lysine decarboxylation
Litmus milk (acidity)	Malonate utilization
Nitrate reduction	MacConkey growth
	Methyl red test
	Motility
	Ornithine decarboxylation
	Urea hydrolysis
	Voges-Proskauer test

[a]Two of 68 isolates were negative

SEROLOGY

The final titres obtained with homologous and heterologous systems of eight isolates are presented in Table IV. The results of testing the antigens of 68 isolates against eight antisera are given in Table V.

The overall results show that when a hyperimmune serum had a given titre against an homologous *H. somnus*, it would give approximate results against antigen made from other *H. somnus* isolates. Three sera gave reactions at 1/40 against *Actinobacillus lignieresi*, one serum showed similar results against *M. bovis*, and another against *Yersinia enterocolitica*.

TABLE IV. Titre of Agglutinating Antibodies in Hyperimmune Sera Against Eight Strains of *H. somnus*

	Antigens							
Antiserum	09	17	37	38	39	41	48	49
09	320[a]	640	160	320	160	160	160	160
17	320	640	1280	640	320	320	320	640
37	1280	1280	1280	1280	640	640	640	1280
38	640	320	320	1280	320	320	320	320
39	320	320	160	160	320	160	320	320
41	640	640	160	320	640	640	640	160
48	640	640	640	640	640	640	1280	640
49	320	320	320	320	640	230	320	320

[a]Expressed as reciprocal of dilution

TABLE V. Number and Titre of Reactions in Agglutination Tests Using *H. somnus* Isolates and Sera from Immunized Rabbits

Antigens	Antiserum	Dilution of Serum Samples						
		1/20	1/40	1/80	1/160	1/320	1/640	1/1280
68 Isolates *H. somnus*	09	—	—	12[a]	30	21[b]	5	—
"	17	—	—	—	10	23	30[b]	5
"	37	—	—	—	1	7	32	28[b]
"	38	—	—	—	2	40	23	3[b]
"	39	—	—	—	31	33[b]	4	—
"	41	—	—	1	24	32	10[b]	1
"	48	—	—	—	1	8	37	22[b]
"	49	—	—	—	8	45[b]	15	—

[a]Number of the 68 isolates having 1/80 as the highest reacting dilution
[b]Dilution at which the homologous antigen-antibody reaction occurred

In the immunodiffusion tests, one line of precipitation was found in most positive sera tested with homologous and heterologous antigens. None of the sera gave a positive reaction with bacteria other than *H. somnus*.

The hyperimmune sera reacted to all homologous and heterologous in the counter-immunoelectrophoresis tests by the production of at least a one-line precipitation band. A few two- and three-line reactions were observed. None of the sera reacted with the antigens of bacteria other than *H. somnus*.

DISCUSSION

The strains used in this study were considered to be representative of different sources of isolation and of geographic areas in Canada and the U.S.A. where ITEME has been diagnosed.

The characteristics of *H. somnus* colonies were similar to those described earlier in the literature (1, 22, 24). The clear haemolysis seen in four isolates in this work is a new observation. These haemolytic isolates did not share any other characteristics. The microscopic features of *H. somnus* were also in agreement with earlier reports. However, while Miller *et al* (18) observed a capsule on *H. somnus* strain 8025, no capsule could be clearly observed on any of the strains reported here.

Brain heart infusion agar was found to be the most suitable medium for growth of *H. somnus*, while Shigidi and Hoerlein (23) indicated CYH as optimum and Bailie (1) used TSA. However, both failed to include BHI in their studies. Different media have been used by different workers for growth of *H. somnus* for pathological or serological studies (9, 15, 19, 20, 24). The finding that 10% CO_2 was a most favourable atmospheric condition was in agreement with the findings of Bailie (1) and Shigidi (22). Bailie failed to secure growth of fresh isolates under atmospheric conditions. He considered that aerobic adaptation was associated with altered colony size and possibly represented a mutation; thus, these aberrant colonies were considered nontypical. However, the strains used in this study have been successfully cultivated in the laboratory under aerobic conditions with apparent unchanged colonial characteristics. This adaptation to aerobiosis was found by Kennedy *et al* (15) and van Dreumel *et al* (24).

In order to obtain reliable data on carbohydrate fermentation, 10% serum and 0.5% YE was required in the medium for adequate growth. The negative results observed by Panciera *et al* (14), Olander *et al* (19) and Shigidi and Hoerlein (23) may have resulted from the use of only 2% serum or bovine blood digest in their carbohydrate media, as 1% was shown to be inadequate (1).

When the fermentation results of this work are compared with other workers (Table VI) there is agreement on the fermentation of glucose, maltose, mannose, sorbitol, trehalose and xylose. The variability between the findings of the various authors and those of this study on the biochemical reactions could be due to the techniques employed rather than differences in the organisms (Table VII). For example,

in this study, H_2S production was positive when the paper strip method of determination was used, but negative in the tube medium, and cytochrome oxidase was much less sensitive by the Pathotec method than by the addition of the suitable reagent to isolated colonies on solid media.

Two isolates of *H. somnus* failed to produce indole by all methods. One of these isolates was received from Bailie (1), who reported the strain as positive. The other strain that failed to react was a vaginal isolate from Alberta. It has been observed in the diagnostic laboratory of the Veterinary Services Branch, Guelph, Ontario that indole negative strains of *H. somnus*-like organisms have been isolated from cattle. Their significance is yet to be assessed.

The results of antibiotic susceptibility tests correspond in general with previous

TABLE VI. Reported Carbohydrate Fermentation Reactions of *H. somnus*

Carbohydrate	Kennedy *et al* (15)	Bailie (1)	van Dreumel *et al* (24)	MacDonald *et al* (17)	Present Study
Arabinose	+	−	+	v	−
Dulcitol	+ ¼ v	−	−	−	−
Galactose		+		v	−
Glucose	+	+	+	+	+
Inositol	−	−	−	−	−
Lactose	−	−	−	v	−
Levulose				v	+
Maltose	+	+	+	+	+
Mannitol	−	+	+	v	+
Mannose	+	+		v	+
Raffinose	−	−	−	−	−
Rhamnose	+	−	−		−
Salicin		−		−	−
Sorbitol	+	+	+		+
Sucrose	+ ¼ v	−	−	v	−
Trehalose		+		+	+
Xylose	+	+	+	v	+

\+ Positive reaction
− Negative reaction
v Variable results
¼ One of four isolates negative

TABLE VII. Reported Biochemical Reactions of *H. somnus*

Test	Kennedy *et al* (15)	Panciera *et al* (20)	Shigidi (22)	Bailie (1)	Olander *et al* (19)	van Dreumel *et al* (24)	MacDonald *et al* (17)	Present Study
Arginine								—
Catalase			−	−	+	−	v	−
Citrate		−	−	−	−			−
Oxidase			+	+	+	+	v	+
Gelatine	−		−	−	−			−
Hydrogen Sulfide (H_2S medium)	−		−	−	−	−		−
Hydrogen sulfide (H_2S paper)				+		+		+
Indole	+	−	+	+	−	+	v	+
Litmus milk (acidity)	+		+	−				+
Lysine								−
Malonate								−
Methyl red			−	−				−
Nitrate	+	−	+	+	+			+
Ornithine								−
Urea			−	−	+			−
Voges-Proskauer			−	−				−

\+ Positive reaction
− Negative reaction
v Variable results

TABLE VIII. Reported Susceptibility Pattern of *H. somnus* to Chemotherapeutic Agents

Antibiotic	Kennedy *et al* (15)	Shigidi (22)	Bailie (1)	van Dreumel *et al* (24)	MacDonald *et al* (17)	Present Study
Ampicillin					+	+
Bacitracin	−		+	−		+
Cephaloridine						+
Chloramphenicol	+	+	+	+	+	+
Dihydrostreptomycin		−				+
Erythromycin	+	+	+	+	+	+
Lincomycin					+	−
Neomycin	−	−	+	−		−
Novobiocin		+	+			+
Penicillin	+	+	+[a]	+	+	+
Polymixin B	+		+	+		+
Tetracycline		+	+	+	+	+
Triple sulfa		−				−

\+ Susceptible
− Resistant
[a]Four of Bailie's isolates were resistant to penicillin

reports although other workers had tested fewer strains (Table VIII). Bailie (1) did not report the number of isolates susceptible to neomycin, nor the amount of antibiotic used in the test, to compare with the 84% resistant to neomycin in the present study. However, the isolate from Bailie was susceptible by our method. MacDonald *et al* (17) found two isolates susceptible to lincomycin while only two of the 68 isolates we tested were susceptible.

SEROLOGY

Immunization against *H. somnus* must be considered in light of the increasing incidence of the disease and the difficulty of diagnosis and treatment before loss has occurred. To be of benefit, an immunizing agent is most effective when all strains share common antigens. Thus, the antigenic inter-relationships of the isolates available in this study were determined by using antibody prepared against eight strains. When the sera were tested against the 68 heterologous antigens, there was a distribution of reactions about the homologous titres. No antigen appeared distinct from others, thereby indicating that all strains were very similar in their agglutinating antigens. These findings agree with the reports on agglutination tests by Shigidi (22) and Hoerlein *et al* (14).

The nine species of other bacteria used for the preparation of agglutinating antigens are common animal bacteria and *A. lignieresi, B. abortus, M. bovis, P. haemolytica* and *P. multocida* are bovine pathogens. Three of the sera reacted with *A. lignieresi,* and another serum with *M. bovis,* but in low titres. The report of Shigidi (22) on cross reaction with *B. bronchiseptica* was not confirmed. Dierks and Hanna (11) using the CF test, reported one-way cross reactions with *A. actinoides* and *M. bovis.* When the titres of the rabbit sera against the 68 strains of *H. somnus* were compared with titres against *A. actinoides* and *M. bovis,* only low level titres were observed. It was concluded that these bacteria do not share antigens common to *H. somnus.*

The mean antibody levels of both the CF and agglutination tests followed similar trends after antigenic stimulus. Brown *et al* (4) qualifies the CF test as valuable for selection of susceptible experimental calves, for evaluation of immune response for diagnostic purposes and for epidemiological studies. Dierks and Hanna (11) used it with success in a serological survey in bovine sera in the field.

The precipitin reaction as conducted by the immunodiffusion method was applied to antigens in an effort to seek reactions of either identity, variance or partial identity. It was found that there was always a line of identity between the eight antigens and their corresponding rabbit antisera. In some cases, a high (agglutinating) titre antisera produced a second line which was usually too short and faint to indicate identity. These results gave evidence that these eight strains shared common precipitating antigens.

The CIEP was used to examine the antigens of all the strains of *H. somnus* in this

study. The results confirmed the findings of immunodiffusion tests as there were identity lines of reaction between the eight antigens and the eight antisera of the representative strains. When the antigens of the other 60 strains were tested against the eight antisera, at least one precipitin line occurred. The line occurred with antigens of eleven other bacteria of animal origin.

ACKNOWLEDGMENTS

The authors appreciate the help of the diagnostic bacteriology laboratory of the Veterinary Services Branch, OMAF, in supplying many of the Ontario isolates of *H. somnus* and other laboratories that furnished cultures. Dr. Garcia-Delgado received a scholarship from Consejo Nacional de Ciencia y Tecnologia, Mexico, to pursue graduate studies at the Ontario Veterinary College.

This work was supported in part by the Ontario Ministry of Agriculture and Food and a grant from the Ontario Cattleman's Association.

REFERENCES

1. **BAILIE, W. C.** Characterization of **Haemophilus somnus** (new species), a microorganism isolated from infectious thromboembolic meningoencephalomyelitis of cattle. Ph.D. Dissertation, Kansas State University, Manhattan, Kansas. 1969.
2. **BAILIE, W. E., E. H. COLES and K. D. WEIDE.** Deoxyribonucleic acid characterization of a microorganism isolated from infectious thromboembolic meningoencephalitis in feed lot cattle. Int. J. syst. Bact. **23**: 231-237. 1973.
3. **BRANSON, D.** Methods in Clinical Microbiology. Springfield, Illinois: Charles G. Thomas. 1972.
4. **BROWN, L. N., C. REGGIARDO, W. M. KHAN, P. G. ENESS and H. L. SELF.** Applications of the complement-fixation test to the study of **Haemophilus somnus** infections of cattle. Proc. a. Meet. U.S. Anim. Hlth Ass. **76**: 502-508. 1972.
5. **CARTER, G. R.** Diagnostic Procedures in Veterinary Microbiology, 2nd Edition. Springfield, Illinois: Charles G. Thomas. 1973.
6. **CASEY, L.** Standardized diagnostic complement-fixation method and adaptation to micro test. I. Laboratory branch complement-fixation method. II. Adaptation of LBCF method to micro technique. Public Health Monograph No. 74 (PHS Publication No. 1228). Washington, D.C.: U.S. Government Printing Office. 1965.
7. **CHLADEK, D. W.** Bovine abortion associated with **Haemophilus somnus**. Am. J. vet. Res. **36**: 1041. 1975.
8. **CHO, H. J.** Aleutian disease of mink: Host responses and isolations of the viruses. Thesis, University of Guelph, Guelph, Ontario, Canada. 1973.
9. **CORSTVET, R. E., R. J. PANCIERA, H. B. RINKER, B. L. STARKS and C. HOWARD.** Survey of tracheas of feed lot cattle for **Haemophilus somnus** and other selected bacteria. J. Am. vet. med. Ass. **163**: 870-873. 1973.
10. **COWAN, S. T. and K. J. STEEL.** Manual for the Identification of Medical Bacteria, 2nd Edition. Cambridge, England: Cambridge University Press. 1974.
11. **DIERKS, R. E. and S. A. HANNA.** Epizootiology and pathogenesis of **Haemophilus somnus** infection. J. Am. vet. med. Ass. **163**: 866-869. 1975.
12. **DUKES, T. W.** The ocular lesions in thromboembolic meningoencephalitis (ITEME) of cattle. Can. vet. J. **12**: 180-182. 1971.
13. **GRINER, L. A., R. JENSEN and W. W. BROWN.** Infectious embolic meningoencephalitis in cattle. J. Am. vet. med. Ass. **129**: 417-421. 1956.
14. **HOERLEIN, A. B., K. GOTO and S. YOUNG. Haemophilus somnus** agglutinins in cattle. J. Am. vet. med. Ass. **163**: 1375-1377. 1973.
15. **KENNEDY, P. C., E. L. BIBERSTEIN, J. A. HOWARTH, L. M. FRAZIER and D. L. DUNGWORTH.** Infection meningoencephalitis in cattle, caused by a **Haemophilus**-like organism. Am. J. vet. Res. **21**: 403-409. 1960.
16. **LENETTE, E. H., E. H. SPAULDING and J. P. TRUANT.** Manual of Clinical Microbiology. 2nd Edition. Washington, D.C.: American Society for Microbiology. 1974.
17. **MacDONALD, D. W., R. G. CHRISTIAN and G. A. CHALMERS.** Infectious thromboembolic meningoencephalitis: literature review and occurrence in Alberta, 1961-1971. Can. vet. J. **14**: 57-61. 1973.
18. **MILLER, R. J., H. W. RENSHAW and J. A. EVANS. Haemophilus somnus** complex. Antigenicity and specificity of fractions of **Haemophilus somnus**. Am. J. vet. Res. **36**: 1123-1127. 1975.
19. **OLANDER, H. J., A. M. GALLINA, B. DIETRA and M. MORROW.** Observations on thromboembolic meningoencephalitis in cattle in Indiana feed lots. Proc. a. Meet. U.S. Anim. Hlth Ass. **74**: 589-600. 1970.
20. **PANCIERA, R. G., R .D. DAHLGREN and H. B. RINKER.** Observations on septicemia of cattle caused by a **Haemophilus**-like organism. Pathologia vet. **5**: 212-226. 1968.
21. **SCHEIDEGGER, J. J.** Une micro-méthode de l'immunoelectrophorese. Int. Archs Allergy appl. Immun. **7**: 103-110. 1955.
22. **SHIGIDI, M. T. A. Haemophilus** of bovine encephalitis. Thesis, Colorado State University, Microbiology, Fort Collins, Colorado. 1969.
23. **SHIGIDI, M. T. A. and A. B. HOERLEIN.** Characterization of the **Haemophilus**-like organism of infectious throembolic meningoencephalitis of cattle. Am. J. vet. Res. **31**: 1017-1022. 1970.
24. **VAN DREUMEL, A. A., R. A. CURTIS and H. L. RUHNKE.** Infectious thromboembolic meningoencephalitis in Ontario feedlot cattle. Can. vet. J. **11**: 125-130. 1970.
25. **VAN DREUMEL, A. A. and M. KIERSTEAD.** Abortion associated with **Hemophilus somnus** infection in a bovine fetus. Can. vet. J. **16**: 367-370. 1975.
26. **WALDHALM, D. G., R. F. HALL, W. A. MEINERSHAGEN and F. W. FRANK. Haemophilus somnus** infection in the cow as a possible contributing factor to weak calf syndrome: isolation and animal inoculation studies. Am. J. vet Res. **35**: 1401-1403. 1974.
27. **ZINNEMANN, K.** Subcommittee on the taxonomy of **Haemophilus**. Report 1962-1966. Int. J. syst. Bact. **17**: 165-166. 1967.