DIAGNOSTIC PROCEDURES IN EXPERIMENTAL HEMOPHILUS SOMNUS INFECTION IN CATTLE

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Introduction

Infectious thromboembolic meningoencephalitis (ITEME) was first described in 1956 (5). *Hemophilus somnus* has been cultured and characterized as the causative agent (1, 6, 10). Other syndromes associated with this organism include respiratory disease (2, 4), polyarthritis and tendinitis (7), septicemia (7, 8), weak-calf syndrome (12) and abortion (3, 11).

ITEME can be confused clinically with other bovine neurological disorders (13). The disease was reproduced in this study to develop better methods of diagnosis. Cerebrospinal fluid (CSF), joint fluid and blood were cultured to determine their value. Serum creatinine phosphokinase (CPK) have been reported to be useful in diagnosis of central nervous disorders (9). In this study the CPK was determined in CSF and serum.

Materials and Methods

Hemophilus somnus (A-74-65) was isolated from the brain of a calf and maintained at −70°C in egg yolk. Infection of Calf 1 was established with this strain and all subsequent infections were induced with organisms isolated from Calf 1. Bacteria were injected into the yolk of ten-day-old embryonated eggs, harvested at 48 hours and 1 ml aliquots placed into vials for storage at −70°C until use. Organisms were cultured on 5% sheep-blood agar in 10% CO_2 at 37°C. Organisms for challenge were removed from blood agar after 24 hours growth, suspended in saline, adjusted to an optical density of 0.25 at 600 nm in a 12.4 mm tube. This was equivalent to 9×10^7 colony forming units/ml.

Calves secured from various farms (five grade Holstein, one grade Hereford, one grade Angus) were more than eight months of age and weighed 250–275 kg, with the exception of one Holstein which was less than six months of age and weighed 200 kg. The disease was reproduced in the summer in semi-isolation, in a room maintained at approximately 25°C.

Calves 1 through 4 received challenge intravenously and calves 5 through 7 were challenged by aerosol (Table I). Aerosol exposure was performed using a box (1.2 m × 0.6 m × 0.6 m) placed over the head and fitted closely round the neck. Organisms (100 ml × 1.8×10^7/ml) were aerosolized using a hand spray gun[1] inserted into an opening in the box. Calves 5 and 6 were exposed for six minutes (one min spray, one min pause, two min spray, two min pause). Calf 7 was exposed for three minutes (one min spray, one min pause, one min spray) of 50 ml suspension.

Sample collections are listed in Tables II–IV. Blood was withdrawn from the jugular vein, CSF obtained by aspiration through the lumbosacral space and joint fluid taken from the carpal joint, all with aseptic techniques.

Blood collected in EDTA was used for total and differential leukocytes counts. CPK in cerebrospinal fluid and serum was determined by measuring the rate of a coupled enzyme reaction[2].

Blood, cerebrospinal fluid and joint fluid were cultured for *H. somnus*. Five ml of blood was injected into a blood culture vial containing 50 ml of trypticase soy broth[3], with 5% CO_2 in air in atmosphere of vial. The vials were incubated for 48 hours before subculture into 5% sheep blood agar plates. Samples of CSF and joint fluid were inoculated immediately to 5% sheep blood agar plates. All the plates and culture vials were incubated in 5–10% CO_2 at 37°C. The plates were examined at 24, 48 and 72 hours for growth of *H. somnus*.

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[1]Plastic atomizer bought from a local hardware store.

[2]Abbott Bichromatic Analyzer, South Pasadena, California.

[3]BBL, Becton, Dickinson & Co. Canada Ltd., Mississauga, Ontario.

TABLE I

CLINICAL DISEASE AND ROUTE OF ADMINISTRATION OF EXPERIMENTAL *Hemophilus somnus* INFECTION IN SEVEN CALVES

Calf. No.[a]	Challenge Method	Dosage	Disease Classification	Clinical Findings
1 and 4	IV IV	18×10^7 9×10^7	peracute speticemia	Signs developed zero to eight hours postexposure. Depression, somnolence, watery nasal and ocular discharges, fever, panting, twitching of muscles, head shaking, sternal recumbency, strabismus, opisthotonus, reduced rumen function, bloat, constipation, lateral recumbency, twitching of head and legs, coma.
2 and 3	IV IV	9×10^7 9×10^7	subacute septicemia	Signs developed <8 hours postexposure. Depression, panting, fever, weak ruminal contractions, bloat, constipation.
5 and 6	Aerosol Aerosol	18×10^7/ml (100 ml in 6 minutes)	subacute bacteremia	Signs developed 24 hours postexposure. Depression, panting, increased respiratory sound, watery nasal discharge, bloating, constipation.
7	Aerosol	18×10^7/ml (50 ml in 3 minutes)	none	No signs noticed during the period of observation.

[a]Calves 1, 2, 3, 5, 6, 7 were >8 mo age and weighed 250–275 kg. Calf 4 was <6 mo age and weighed 200 kg.

TABLE II

HEMATOLOGY AND BACTERIOLOGY OF PERACUTE SEPTICEMIC DISEASE DUE TO *H. somnus* (CALF I)

	Time of Sampling in Hours						Normal Values
	−24	0	8	24	48	72	
WBC per μl	8500	ND	700	1100	3300	9000	4000–12000
Neutro "	2550	ND	56	99	1419	7100	600– 4000
Bands "	0	ND	28	44	66	90	0– 120
Eosino "	850	ND	0	0	0	0	0– 2400
Lympho "	4420	ND	616	957	1749	1530	2500– 7500
Mono "	680	ND	0	0	66	270	25– 840
BACTERIOLOGY							
Blood	ND	ND	+ +[a]	+ +[b]	−	−	
CSF	ND	ND	ND	−	+	ND	
Joint Fluid	ND	ND	ND	−	−	ND	

Animals exposed at 0 hours to 18×10^7 *H. Somnus* iv
0 = none − = negative ND − not done
[a]18 hours.
[b]36 hours.

RESULTS

More severe disease was observed in the calf given 18×10^7 bacteria (Calf 1) as compared to the calves given 9×10^7 (Calves 2 and 3). A younger and smaller calf (Calf 4) given 9×10^7 developed peracute septicemia similar to Calf 1 (Table I). This animal died 24 hours after challenge and had thromboembolic lesion in the brain, liver, spleen, lungs, kidney, intestine and throughout voluntary muscles. Onset of signs developed in less than eight hours after IV challenge in calves 1–4.

Aerosol exposure produced signs of depression, ataxia, head shaking, bloat and constipation in animals challenged six min. Three min. challenge produced no clinical evidence of disease.

All animals with subacute disease (Calves 2, 3, 5 and 6) yielded *H. somnus* from blood cultures 24 hours after exposure. CSF yielded *H. somnus* in 30 hours (Calf 2), and 48 hours (Calf 1 and 4). All other culture attempts of CSF did not yield *H. somnus*. *H. somnus* was isolated only once from joint fluid (at 48 hrs, Calf 2) despite repeated culture attempts.

Calves challenged intravenously (1, 2, 3, 4) had a profound neutropenia in eight hours.

TABLE III

HEMATOLOGY AND BACTERIOLOGY OF SUBACUTE SEPTICEMIC DISEASE DUE TO *H. somnus* (CALF II)

	Time of Sampling in Hours						Normal Values
	−24	0	8	24	48	72	
WBC per μl	9400	ND	1600	8000	10300	9800	4000–12000
Neutro "	2632	ND	128	3440	5047	3626	600– 4000
Bands "	0	ND	16	0	0	0	0– 120
Eosino "	564	ND	0	160	721	392	0– 2300
Lympho "	5734	ND	1456	4240	4120	4900	2500– 7500
Mono "	470	ND	0	160	412	882	25– 840
BACTERIOLOGY							
Blood	ND	ND	− +[a]	− +[b]	+	−	
CSF	ND	ND	ND	−	+	−	
Joint Fluid	ND	ND	ND	−	+	−	

Animals exposed at 0 hours to 9×10^7 *H. somnus* iv
0 = none − = negative ND = not done
[a]20 hours
[b]36 hours.

TABLE IV

HEMATOLOGY AND BACTERIOLOGY OF SUBACUTE BACTEREMIC DISEASE DUE TO *H. somnus* (CALF VI)

	Time of Sampling in Hours						Normal Values
	−24	0	8	24	48	72	
WBC per μl	10900	ND	10700	10800	11800	12000	4000–12000
Neutro "	2071	ND	4280	2592	3658	3840	600– 4000
Bands "	0	ND	0	0	118	0	0– 120
Eosino "	436	ND	321	324	236	512	0– 2400
Lympho "	7630	ND	5778	7128	6844	8192	2500– 7500
Mono "	763	ND	321	756	944	256	25– 840
BACTERIOLOGY							
Blood	ND	ND	−	+ +[a]	−	−	
CSF	ND	ND	ND	−	+	−	
Joint Fluid	ND	ND	ND	−	−	−	

Animals exposed at 0 hours with 1.8×10^7 *H. somnus* by aerosol
0 = none − = negative ND = not done
[a]36 hours.

Calves challenged by aerosol (5, 6, 7) had no changes in leukon eight to 72 hours after exposure although *H. somnus* was isolated from the blood of 5 and 6 (Tables II, III, IV).

Serum CPK levels were normal (<50 IU/dl) in all calves sampled 24 hours before and 24 hours after challenge. Cerebrospinal fluid CPK levels were elevated in three of five calves 24 hours after challenge (Table V). Calf 6 had 4396 and 4832 IU/dl at 48 and 72 hours after challenge. The respective serum levels were 22.2 and 25.4 IU/dl.

Necropsy of calves 1 and 4 which died at 72 and 24 hours after challenge respectively revealed lesions varying from mild to severe thrombosis, hemorrhage and necrosis in respiratory, muscular and nervous systems. *H. somnus* was cultured from muscles, lymph nodes, lungs, joints and brain. Surviving calves (2, 3, 5, 6, 7) were not examined at necropsy.

DISCUSSION

Successful establishment of infection by aerosol route confirms postulations (2, 6) that the portal of entry is by way of respiratory tract. The bacteremia without apparent clinical signs (Calf 7) is interesting since it may represent a possible type infection that might occur in field outbreaks.

Impaired rumen function, bloat and constipation represent an observation not described in natural cases. Thromboembolic lesions seen in the gastro-intestinal tract of the animals necropsied may be an explanation of this phenomenon.

TABLE V
SERUM AND CEREBROSPINAL FLUID CREATININE PHOSPHOKINASE LEVELS[a]

Time of Collection − preexposure + postexposure		Calf No. II	III	V	VI	VII	Normal Values
Serum	− 24 hrs	5.7	3.7	ND	ND	ND	<50
	+ 24 hrs	3.5	5.5	11.0	13.0	7.6	
CSF	− 24 hrs	43	33.4	ND	ND	ND	unknown
	+ 24 hrs	391	17.7	460	324	31.7	

[a]Expressed as IU per dl.
ND—samples not done.

Results of this trial indicate that culture of blood within 24 hours of evidence of clinical disease would be the most effective laboratory diagnostic tool. Culture of CSF and joints is unreliable.

The occurrence of elevated CPK levels in CSF represents a new finding (9), however the usefulness of this procedure awaits confirmation on natural cases of *H. somnus* disease.

SUMMARY

Hemophilus somnus disease of varying degrees of severity was reproduced in calves by intravenous (IV) and aerosol challenge. The severity of disease was dependent upon route of administration (more severe by IV), dose of organisms (more severe with higher dosage), age and size of calf (more severe in a younger, smaller calf). In two calves that died, *H. somnus* could be isolated from most tissues, indicating generalized septicemia. Thromboembolic lesions were observed in brain, lungs, and muscles. Signs of disease included: depression, somnolence, watery nasal and ocular discharge, fever, panting, twitching of muscles, reduced rumen function, bloat and constipation.

Evaluation of diagnostic methods indicated that blood culture at 24 hours yielded *H. somnus* in six of seven calves exposed. The one calf which was negative did not show any signs of disease. Cerebrospinal fluid and joint fluid, when positive, yielded *H. somnus* at 30 and 48 hours respectively. Creatinine phosphokinase (CPK) levels in CSF were increased (approximately ten times) in three of five calves tested 24 hours after exposure. Serum CPK levels were not elevated in any calves.

RÉSUMÉ

Cette expérience visait à provoquer une maladie plus ou moins grave, chez des veaux, au moyen d'injections intra-veineuses et de vaporisations de *Hemophilus somnus*. La gravité de la maladie varia selon la voie d'infection, la quantité de microbes, l'âge et le poids des veaux. On réussit à isoler *H. somnus* de la plupart des organes de deux des sujets qui succombèrent à cette infection, indice d'une septicémie. On nota la présence de thromboembolies dans le cerveau, les poumons et les muscles. Les signes cliniques de la maladie se traduisirent par de la dépression, de la somnolence, un écoulement oculaire et nasal séreux, de l'hyperthermie, de l'essoufflement, des contractions musculaires, de l'atonie du rumen, du météorisme et de la constipation.

Une évaluation des méthodes de diagnostic révéla qu'une hémoculture, effectuée 24 heures après l'infection, permit d'isoler *H. somnus*, chez six des sept veaux expérimentaux. Celui dont l'hémoculture donna un résultat négatif ne manifesta pas de signes cliniques. Lorsque la culture des liquides cérébro-spinal et articulaire donna un résultat positif, ce fut respectivement à 30 et à 48 heures après l'infection. La teneur du liquide céphalo-rachidien en créatinine phosphokinase se révéla environ dix fois supérieure à la normale, chez trois des cinq veaux qu'on éprouva à cette fin, 24 heures après l'infection. Aucun des veaux n'accusa une élévation de la créatinine phosphokinase sérique.

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BOOK REVIEW

Nutrient Requirements of Beef Cattle, 5th Revised Edition. Published by National Academy of Sciences/National Research Council, Washington. 1976. 56 pages. Price $3.75.

This is the 5th revision of these requirements, the most recent having been in 1970. Among the changes in this revision, veterinarians will be interested in the fact that only those signs of nutritional deficiency "generally discernible by visual observation" are included, not those obtained by "clinical observation". Presumably this is because "the descriptions of the more common signs of nutritional deficiency can aid (the farmer or rancher) in the recognition of faulty nutrition." This is unfortunate for the practicing veterinarian, for whom there are few other sources of modern, reliable nutritional information in the concise form so characteristic of N.R.C. publications. The principal sections are: Feed consumption and rates of gain, Nutrient requirements and signs of deficiency, Nonnutrient additives and implants, Water, Composition of feeds and Formulating diets. In addition, several valuable tables of data and a bibliography are included.

The information on growth promoters is based on U.S. Food and Drug Administration regulations. Canadian readers should obtain appropriate information from the Bureau of Veterinary Medicine, Health Protection Branch, Ottawa. On the other hand, the feed composition tables are appropriate to Canada because they are based on U.S.-Canadian data.

This booklet is a necessary part of the library of any practitioner involved in the beef cattle industry; its value is the nutritional standards and the tables, not the descriptions of deficiency states which are now practically useless to the veterinarian. Some examples: the "convulsive seizures [of severe vitamin A deficiency are] probably caused by increased cerebrospinal fluid pressure"; "blindness ... results from stenosis of optic nerves ..."; polioencephalomalacia responds to "thiamine (sic) at a level of 2.2 mg per kg body weight" (a low dose in our experience; 6 mg/kg should be considered). The text section on signs of selenium deficiency refers to a photograph which is labelled "selenium toxicity." A pleasing change is the expunging of that hoary term "ration", a favorite of animal scientists, and its apparent replacement by "diet", a shorter, more sensible, and universally-understood term. There remains a legitimate and useful role of this publication for the veterinarian, however, and I recommend it. *F. M. Loew.*