

***Haemophilus somnus* Infections II. A Canadian Field Trial of a Commercial Bacterin: Clinical and Serological Results**

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SUMMARY

To evaluate the efficacy of a *Haemophilus somnus* bacterin a total of 1114 beef calves or yearling bulls were used in the province of Saskatchewan. The six herds were included in a vaccination trial in which the vaccine was administered subcutaneously in one or two doses. The prevalence of *H. somnus* in nasal swabs of these animals at the time of the initial vaccination was 0.35%. Postvaccination information on morbidity and mortality for a four month period was requested from the five ranches where there were nonvaccinated control calves. Postvaccination outbreaks of infectious thromboembolic meningoencephalitis occurred in two of these herds and, although the numbers were limited, there was a trend to reduced morbidity and mortality in the vaccinated animals compared to controls. Seroconversion rates, as determined by the complement fixation test, in the six herds were 28.3% for control calves, 57% for animals vaccinated once and 80.3% for animals vaccinated twice. On the basis of these results the bacterin was considered to be sufficiently efficacious to warrant its further evaluation under field conditions.

RÉSUMÉ

Les infections à *Haemophilus somnus* II. Les résultats cliniques et sérolo-

giques de l'essai d'une bactérine commerciale, au Canada

Cette étude consistait à évaluer l'efficacité d'une bactérine contre *Haemophilus somnus*, en Saskatchewan. On utilisa à cette fin 1114 animaux à boeuf: veaux et taureaux d'un an. Ces sujets appartenaient à six troupeaux où on effectua une expérience de vaccination qui comportait une ou deux injections sous-cutanées de la bactérine. Au moment de la première vaccination, les écouvillonnages nasaux de ces bovins recelaient *H. somnus*, dans la proportion de 0.35%. Au cours des quatre mois ultérieurs à la vaccination, on fit recueillir les informations relatives à la morbidité et à la mortalité, sur les cinq fermes où il y avait des veaux témoins. Des éclosions de méningo-encéphalite thrombo-embolique se produisirent effectivement dans deux de ces troupeaux; bien que leur nombre s'avéra limité, on nota une tendance à une morbidité et à une mortalité plus faibles chez les sujets vaccinés, par rapport aux témoins. La recherche d'anticorps sériques, chez les sujets des six troupeaux, à l'aide de l'épreuve de la déviation du complément, donna des résultats positifs chez 28.3% des témoins, 57% des sujets vaccinés une seule fois et 80.3% de ceux qui avaient reçu deux injections de bactérine. De tels résultats permirent de considérer cette bactérine suffi-

samment efficace pour justifier d'autres études similaires.

INTRODUCTION

Infectious thromboembolic meningoencephalitis (ITEME) was first diagnosed in the U.S.A. in 1956 (6). It was first reported in Canada during 1969 (10, 18) and its frequency in western Canada increased 25 fold during the decade 1969-1978 (17). It is recognized that meningoencephalitis is only one of the clinical signs caused by *Haemophilus somnus* infection. However, it is the syndrome that is least likely to be confused with other clinical entities if a necropsy is conducted (2, 14, 17).

Though *Haemophilus* septicemia is a more accurate description of the disease and its varied manifestations, ITEMÉ is the syndrome that causes most concern. Previously, the morbidity of ITEMÉ in eight outbreaks in 1971 had been determined to be 0.7% with a range of 0.3 to 4%. (16). A survey of outbreaks in Alberta in 1977-1978 found the reported morbidity of ITEMÉ to be 7.5% (Green, P.D. personal communication, 1979). This same investigation found the case fatality rate to be 68%, considerably lower than that commonly reported (14). Treatment with antibiotics of individual cases of ITEMÉ early in the clinical course of the disease often results in satisfactory outcomes (1). Chronic cases usually remain recumbent (9, 10) and without good nursing care can become complicated by frostbite or starvation.

Until the fall of 1978, there had been only two methods of handling ITEMÉ outbreaks that occurred in calves six to 11 months old (10). The feedlot operator could increase the frequency of observation hoping thereby to identify and treat affected calves earlier. The other method was to mass medi-

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cate a pen of calves, either by treating them all individually or by addition of antibiotics to the feed or water. The third option, a *H. somnus* bacterin¹ became available to Canadian feedlot operators in September 1978 although information on the efficacy of the bacterin was limited, (7, 13, 19). The purpose of this paper is to report the results of a field trial of this bacterin in the production of humoral antibody and the prevention of ITEME in some herds defined as endemically infected.

MATERIALS AND METHOD

Selection of Six Herds — The selection of herds was primarily based on laboratory records available from the veterinary diagnostic laboratories at Regina and Saskatoon, Saskatchewan. From these records, four herds considered "endemic" for *H. somnus* infection were selected (Table I). There were confirmed diagnoses of ITEME in herds A and B the previous two years and in herds C and D during the previous winter. Herd E, the University of Saskatchewan's beef herd, was established in 1975 and had not had any recognized disease problems associated with *H. somnus*. Herd F was the Central Saskatchewan Record of Performance (ROP) Bull Test Station. The bulls presented to the station came from 84 breeders throughout the pro-

vince of Saskatchewan. During the winter of 1977-78 four bulls died of ITEME while on test, therefore, the station was also considered "endemic" through the source herds.

Assignment, Immunization and Sampling Procedures of Treatment Groups — When the farm or ranch was visited for the first time, all calves with the exception of the bulls in herd F, were systematically assigned to one of three treatment groups as they were processed through the chute (see Table I). In all herds except D and F this visit was made one to three weeks before weaning. A large plastic ear tag provided identification. Calves assigned to groups 2 and 3 were vaccinated subcutaneously with the bacterin and group 1 calves were not vaccinated. In addition, all calves were bled for serological evidence of prior exposure to *H. somnus*. The nasal flora of the calves were sampled using a guarded nasal swab² and in cases where the ranch was greater than two hours driving time from the laboratory, swabs were placed in transport media³.

Calves assigned to treatment group 3 were revaccinated at an interval of not earlier than ten days and not later than 27 days after the initial vaccination. After the second vaccination all calves were bled again to determine

their immune response to the bacterin.

Measuring the Occurrence of Disease

— Following vaccination the owners were requested to manage the cattle as they had in the past, recording morbidity and mortality for all disease conditions. Suspected cases of *H. somnus* infection were to be referred to the local veterinarian. On all cattle that died, a necropsy was conducted by the veterinarian or a diagnostic laboratory. Field necropsy examinations were to be completed with appropriate submissions to the nearest diagnostic laboratory.

Contingency Plans — The calves available to us in this investigation were all privately owned and in the fall of 1978 their value was increasing markedly. Therefore, our plan in the event of more than one confirmed case of ITEME called for mass medication with antibiotics in the feed along with simultaneous vaccination of unvaccinated controls.

Cultural Examinations — On arrival at the laboratory the nasal swabs were immediately inoculated onto plates of 5% bovine blood agar⁴ with 1% yeast extract⁵ (BBAY) which were incubated in a CO₂ incubator for an initial 18 to 24 hours and a subsequent 24 hours. Suspected *H. somnus* colonies were identified by standard procedures (3).

After being cultured on BBAY plates the nasal swabs were put into screw-capped vials containing 5 mL of MEM medium⁶ (with antibiotics) and frozen at -70°C. These samples were subsequently thawed and assayed for cytopathogenic viruses by one passage in fetal calf kidney (FCK) cells (15).

Serological Studies — The sera were kept frozen at -10°C until they were tested. After preliminary investigations of three tests for measuring *H. somnus* antibodies, namely the agglutination test (8), the gel-diffusion test

TABLE I
EXPERIMENTAL DESIGN OF A FIELD VACCINATION TRIAL WITH A *H. SOMNUS* BACTERIN
IN SIX HERDS OF BEEF CATTLE FROM SEPTEMBER 1978 — MARCH 1979

Herd Type	Total Calves	Number of Controls ^a /Vaccinates ^b		Days from 1st Dose to 2nd Dose/2nd Bleeding	
APB ^c	79	27	52	22	36
BPB	77	26	51	27	41
CComm ^d /PB	358	120	238	23	50
DComm	90	30	60	10	51
EComm	186	62	124	27	55
FPB	324(Bulls)	0	324	NA	31
Totals	1114	265	849		

^aControls (no vaccination) = Treatment Group 1.

^bVaccinates divided into two equal groups, #2 (vaccinated once) and #3 (vaccinated twice) with exception of Herd F where all bulls received one vaccination.

^cPB = Purebred.

^dComm = Commercial type.

¹Somnugen TM, Bio-ceutic Laboratories, Inc. St. Joseph, Missouri 64506. Available in Canada, M.T.C. Pharmaceuticals, 1890 Brampton Street, Hamilton, Ontario.

²Teigland Swabs (Modified), Haver-Lockhart Laboratories, Shawnee, Kansas 66201.

³Bacto-Transport Medium Amies, Code 0996, Difco Laboratories, Detroit, Michigan 48201.

⁴Bacto-Blood Agar Base (B45), Difco Laboratories, Detroit, Michigan 48201.

⁵Bacto-Supplement B (B276), Difco Laboratories, Detroit, Michigan 48201.

⁶Gibco Canada, P.O. Box 484, Calgary, Alberta T2G 4B7.

TABLE II
NECROPSY FINDINGS AND COMPLEMENT-FIXATION TITERS
IN CALVES DYING POSTVACCINATION IN HERDS C AND D

Herd	Calf No.	Group ^a	Lab. Diagnosis	CF Titer
C(Alta)	166	1 ^a	ITEME	--- ^b
	128	2	ITEME	1/16
	41	2	Pneumonia	neg
	107	2	Pneumonia and Tracheitis	1/128
C(Sask)	238	1	ITEME	neg
D	363	1	ITEME	---
	11	2	Ventricular Empyema ^c	---
	42	3	Meningo- encephalitis and Thrombosis	---

^aTreatment groups:

1. Control.
2. One vaccination.
3. Two vaccinations.

^b--- Postvaccination serum not available.

^c*Corynebacterium pyogenes* isolated from lesion.

(19) and the complement-fixation (CF) test (2, 5, 11, 13), we selected the latter.

Haemophilus somnus strain 8025⁷, maintained in egg yolk suspension was used for immunization of two rabbits for reference antisera (5) and for preparation of CF antigens (2). The microtiter CF technique was as described (11) except that serum dilutions tested ranged from 1:8 to 1:1024.

RESULTS

Clinically suspected and laboratory confirmed cases of ITEM E occurred in only two (herds C and D) of the five herds where non-vaccinated controls were included in the treatment groups. The other four herds (A, B, E and F) reported no sickness or deaths from ITEM E.

Herd D — These calves had been vaccinated two weeks after weaning. The owner found the first affected calf dead 12 days after the second vaccination. An additional calf died within five days and another in a week. The postmortem findings and postvaccination CF titers on these calves are recorded in Table II. This ranch only reported one additional calf treated and in this case it was from the non-vaccinated control group.

Herd C — Calves on this ranch were first vaccinated three weeks prior to weaning and treatment group 3 was

revaccinated two days postweaning. In the 27 day period between this vaccination and the postvaccination bleeding a total of 50 of the 358 calves were treated for undifferentiated bovine respiratory disease. These were almost equally divided among the three treatment groups.

Ninety of the best steer calves were shipped approximately 400 miles by truck to a custom feedlot in Alberta 32 days after weaning (five days after bleeding). The first death due to ITEM E in these calves occurred one day after arrival at the Alberta feedlot. The predominant pathological findings in four calves that died within a three week period in this feedlot are also included in Table II under the

heading C (Alta.). When the first calf died in the Alberta feedlot, all control calves in this group were immediately vaccinated.

Two hundred and sixty-eight calves remained home on the ranch in Saskatchewan. Four days after the steers were transported to Alberta, a calf (number 238, Table II) found moribund, was euthanized and the clinical diagnosis of ITEM E was confirmed by necropsy and cultural procedures. An additional five calves became recumbent. Three of these five calves recovered uneventfully while the other two recovered with serious sequelae (permanent blindness and ataxia) and were therefore marketed. All six affected calves were from the unvaccinated control group. Three other calves, that were not identified by treatment group because of loss of ear-tags, also died but were not necropsied and were excluded from the totals. A summary of the ITEM E-associated morbidity and mortality experienced in herds C and D is shown in Table III.

Microbiology and Virology — *Haemophilus somnus* was recovered from less than 0.35% of nasal swabs (Table IV)

Three isolations of parainfluenza-3 virus were made in FCK cell cultures from nasal swabs from three calves of herd B at the initial sampling. No other isolations of cytopathic or hemadsorbing viruses were made.

Serology — The *H. somnus* CF seroconversion rates for the three treat-

TABLE III
MORBIDITY AND MORTALITY FROM ITEM E IN POSTVACCINATION
OUTBREAKS IN TWO HERDS

Herd	Morbidity In		Mortality In	
	Controls	Vaccinates	Controls	Vaccinates
C	Feedlot	1/23 ^a	1/23	1/67 ^b
	Ranch	1/97	0/171	0/171
D		2/30	1/30	1/60 ^c
Totals	9/150	2/298 ^d	3/150	2/298 ^e

^aNo. affected

No. in group

^bCalf vaccinated once.

^cCalf vaccinated twice.

^dValue highly significant ($P \leq 0.01$) in comparison to controls.

^eValue not significant ($P \leq 0.1$) in comparison to controls.

⁷Obtained from the Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa 50010.

TABLE IV
RECOVERY OF *H. SOMNUS* FROM DEEP NASAL SWABS OF CATTLE
AT TIME OF VACCINATION AND POSTVACCINATION BLEEDING

Herd	At First Vaccination	At Postvaccination Bleeding
A ^a	2/203 ^b	0/ 76
B, C	0/710	0/701
D, E		
F	2/319	--- ^c

^aCattle of all age groups on this ranch were sampled on the initial visit and both isolations were from cows.

^bNo. of isolations

No. of cattle sampled

^c---Not sampled.

ment groups in the six herds are shown in Table V. The seroconversion rate for the control group was 28.3%, compared to 57% for the once-vaccinated group and 80.3% for the twice-vaccinated group.

The CF titers for seroconverting once-vaccinated animals compared to twice-vaccinated animals is presented in Table VI.

Herd E was the only one in which it was possible to collect sera at five months postvaccination. The changes in CF titers to *H. somnus* over this period are presented in Table VII.

DISCUSSION

The prevaccination recovery rate of 0.35% for *H. somnus* from deep nasal swabs is less than the 8.8% reported for prevaccination nasal swabs from calves on a similar vaccination trial (7) or the 3.2% for tracheal swabs from feedlot cattle (3). In another study the initial time of colonization of the respiratory tract of beef calves with *H. somnus* was satisfactorily determined by the culturing of nasal swabs (4). Our data from herds C and D tended to support the view that the capability to transmit infection is likely of short duration (14) and is stress associated (4, 14).

Herds where ITEME had been diagnosed in previous years were selected to evaluate the vaccine. The rationale assumed that the probability of ITEME recurring on a ranch next year is greater than for any ranch chosen at random (17). The reappearance of ITEME on two of the four ranches with positive diagnoses the previous year supports the validity of this assumption. The nearly simultaneous occurrence in herd C of

ITEME among those steer calves shipped to Alberta and those remaining on the home ranch suggests that factors other than transportation predispose to the development of the disease, as has been suggested by other authors (3, 5, 12, 18). The occurrence of ITEME in calves of herds C and D

indicates that the use of cultural procedures to identify nasal carriers a few weeks prior to an outbreak is generally unsatisfactory as a predictive procedure. This result is somewhat at variance with work reported by others (3, 7) but may merely reflect the low prevalence of *H. somnus* carriers in ranch calves prior to weaning or prior to admission to feedlots as opposed to the increased prevalence of nasal carriers shown in such calves after admission to feedlots (7).

Conclusions as to the efficacy of the *H. somnus* bacterin to induce resistance to ITEME under field conditions are limited because of the small number of calves affected and incomplete information available on two herds, C and D, in which postvaccination outbreaks occurred. Calves 11 and 42 in herd D were submitted frozen 17 days after they died (Table II) and in both cases the necropsy findings

TABLE V
COMPLEMENT-FIXATION TITERS OF VACCINATED AND CONTROL CATTLE
BEFORE AND AFTER VACCINATION WITH *H. SOMNUS* BACTERIN

Herd No.	Paired Sera	Positive CF Test by Time Group:			
		Pre	Post #1 ^a	Post #2 ^a	Post #3 ^a
A	71	0	3/ 27 ^b	1/ 21	18/ 23
B	67	4	6/ 21	8/ 20	20/ 22
C ^c	341	0	18/108	42/115	107/118
D ^c	84	4	21/ 28	23/ 26	23/ 26
E	171	11	20/ 56	28/ 55	23/ 49
F	307	91	---	156/216	---
Total	1041	110	68/240	258/453	191/238
Percentage		7.8%	28.3%	57.0% ^d	80.3% ^d

^a1 — controls, 2 — vaccinated once, 3 — vaccinated twice.

^bNo. positive

No. in group

^cConfirmed ITEME postvaccination and postvaccination sera were collected after the outbreak in herd D.

^dThese values highly significant ($P \leq 0.01$) in comparison to controls.

TABLE VI
COMPLEMENT-FIXATION TITERS OF SEROCONVERTING CATTLE
VACCINATED ONCE OR TWICE WITH *H. SOMNUS* BACTERIN

Number Seroconverted	No. Cattle Developing CF Titres of:				
	16	32	64	128	≥256
Vaccinated once 258/453 ^a	97(38%)	86(33)	45(17)	21(8)	9(4)
Vaccinated twice 191/238	45(24%)	75(39)	42(22)	23(12)	6(3)

^aNo. seroconverted

No. vaccinated

TABLE VII
COMPLEMENT-FIXATION TITERS OF CALVES IN HERD E
AT ONE AND FIVE MONTHS AFTER VACCINATION WITH *H. SOMNUS* BACTERIN

No. Calves Reacting at One Month	Changes in CF Titres at Five Months		
	None	Increase	Decrease
Positive 43	25	3	15
Negative 41	14	27	0

could be considered equivocal because *H. somnus* was not isolated. On another three calves from herd C, necropsies were not done and identification as to treatment group was impossible. On the positive side, the observed morbidity rate (Table III) in these two herds in controls versus vaccinates was 6.1% versus 0.7% ($P \leq 0.01$) while the mortality rate was 2% for controls versus 0.3% (1/298) for vaccinates ($P \leq 0.05$, with calves 11 and 42 excluded as ITEMÉ cases) or 1% (3/298, not significant, with calves 11 and 42 included as ITEMÉ cases). Also, outbreaks did not occur in the other four vaccinated herds (A, B, E and F) in spite of the fact that non-vaccinated controls were included in herds A, B and E and that *H. somnus* was isolated on prevaccination samplings from two herds (A and F). In addition to the demonstration of *H. somnus* in prevaccination nasal swabs from these two herds, it appears certain, on the basis of the 28.6% and 35.7% seroconversion rates in the controls in herds B and E (Table V), that *H. somnus* was also present in these groups but did not produce clinical disease.

The seroconversion rates in the six herds in our study were 68/240 (28.3%) for the controls, 258/453 (57.0%) for animals vaccinated once ($P \leq 0.01$), 191/238 (80.3%) for animals vaccinated twice ($P \leq 0.01$) and 449/691 (65.0%) for both vaccinated groups ($P \leq 0.01$). The seroconversion rate for controls ranged from a low of 11.1% in herd A to a high of 75.0% in herd D. In the latter herd postvaccination bleeding was not done until after the clinical disease subsided, and thus the seroconversion rates in controls and vaccinates are artificially high. The exclusion of this herd results in actual seroconversion rates of

47/212 (22.2%) for controls, 235/427 (55%) for cattle vaccinated once, 168/212 (79.3%) for cattle vaccinated twice and 403/639 (63.1%) for both vaccinated groups.

The seroconversion rate was significantly higher for the calves vaccinated twice compared to those vaccinated once as was also shown in the only other report of a field trial of this bacterin (7). In our study animals vaccinated twice tended to have higher titres (74.4% $\geq 1:32$) than those vaccinated once (62.4% $\geq 1:32$), thereby raising the possibility of greater protection in the face of natural exposure, as has been shown to be the case for experimental exposure (19). Our CF test results could not be compared to the immunodiffusion test results from the previous field trial of the bacterin (7) because the latter test is qualitative rather than quantitative.

Other data that supported the clinical and serological results reported here were challenge of immunity studies done on 20 calves (seven controls, 13 vaccinates) purchased from herds C and E (Janzen, E.D. and Saunders, J.R., unpublished data 1979). The response to intravenous or intracerebral exposure with *H. somnus* generally correlated with CF antibody titres and the two calves that died were both nonvaccinated controls.

The seroconversion rates by immunodiffusion tests in the previously cited field trial (7) of this bacterin in one group of ranch calves entering a field lot were: 27% for nonvaccinated controls, 24% for calves subcutaneously vaccinated once and 93% for calves subcutaneously vaccinated twice. In comparison, our seroconversion rates by the CF test, using an antigen similar to that of the immunodiffusion test, were 28%, 57% and 80% for the respective groups.

Infectious thromboembolic meningoencephalitis did not develop postvaccination in controls or vaccinates in the other trial, although ITEMÉ was diagnosed in other cattle in the feedlot during the trial period (7). One advantage of our study was the development of ITEMÉ in two herds postvaccination and the opportunity to compare relatively crude morbidity and mortality rates in control and vaccinated calves. The trend to reduced morbidity and mortality in the vaccinates, the acceptable seroconversion rate of 80% in animals vaccinated twice, the higher antibody titers in animals vaccinated twice and the results from our limited challenge-of-immunity study give credence to the view that this bacterin, when administered in two doses under prescribed conditions of management, is sufficiently efficacious to justify its further evaluation in the field.

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LETTER TO THE EDITOR

Practical Vacuum System for Rapid Blood Collection

DEAR SIR:

Since the replacement of vacuum bottles by plastic bags for collection and storage of blood for transfusions, harvesting blood from a donor has become a slow tedious process. To correct this I use my surgical suction pump to create a vacuum around the plastic collection bag.

This is accomplished by using a glass jar that can be sealed with a metal lid. The jar must have the capacity to accommodate a full bag of blood. Its mouth must be wide enough to allow a

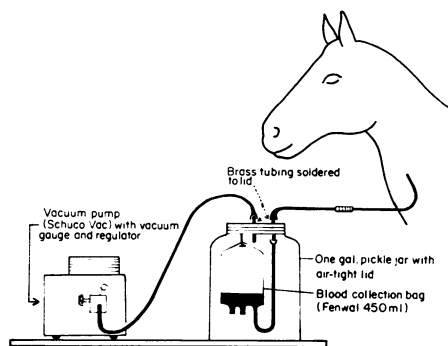


FIGURE 1. Vacuum system for rapid collection of blood from a donor.

full bag of blood to be taken out. The only required modification is to have

two small caliber brass tubes soldered into the lid. One is to be attached to the pump tube and the other to the blood tube (Figure 1). The collection tube of the bag has to be cut a few centimeters from the bag, one end is attached to the brass tube from inside the lid and the other end on the outside of the lid.

To operate, the needle is placed in the donor's vein before the pump is turned on. The use of this technique has cut blood collection time by two-thirds.

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