EXPERIMENTAL INFECTION ON RABBITS WITH *HAEMOPHILUS SOMNUS* (NEW SPECIES)

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WILLIAM RODGER RAPP

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Approved by:

L. D. Anthony
Major Professor

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INTRODUCTION

An encephalitic disease of cattle, first reported as a feedlot entity in Colorado (Griner et al., 1956), was subsequently recorded as occurring in feedlots in California (Kennedy et al., 1960), Kansas (Weide et al., 1964 and Bailie et al., 1966), and Illinois (Case et al., 1965 and Gossling, 1966). In Oklahoma and Texas the disease was reported in cattle grazing pastures as well as in feedlots (Panciera et al., 1968).

The encephalitic clinical syndrome was of short duration and sudden death. The principal macroscopic lesions were single or multiple foci of hemorrhage and necrosis of various size, located in the brain without any specific anatomical pattern. Microscopic studies revealed meningoencephalitis characterized by suppurative inflammation and presence of emboli and/or thrombi (Bailie et al., 1966).

Microbiologic studies of brain tissues revealed a small gram-negative bacillus in a significant number of cases. The organism was described as Haemophilus-like (Kennedy et al., 1960 and Case et al., 1965), Actinobacillus-like (Gossling, 1966), and Actinobacillus actinoides-like (Bailie et al., 1966). Extensive studies indicated characteristics which warranted classification of the organism within the genus Haemophilus and Bailie (1969) proposed a new species designation of Haemophilus somnus.

This research was designed to test the reliability of the rabbit as a laboratory animal for pathogenic studies of this organism. The research objectives were: (1) to determine and characterize the gross and microscopic lesions of infection in rabbits; (2) to determine the method of inoculation and the infective dose necessary to reliably reproduce signs and lesions of encephalitis; (3) to determine alterations of specific hemogram and clinical parameters; (4) to test for endotoxic manifestations of the microorganism; and (5) to investigate the effects of serial passage of the microorganism in rabbits.

REVIEW OF THE LITERATURE

Griner et al., (1956) described an encephalitic syndrome in cattle as "infectious embolic meningo-encephalitis" which occurred in feedlot cattle, 12 outbreaks between 1949 and 1956, and involved 36 animals. This syndrome was characterized principally by acute progressive neurologic signs and death. Necropsy revealed that the only consistent macroscopic lesions were located in the brain and meninges. The lesions were described as "multiple reddish-brown foci of necrosis and inflammation". There was no apparent consistent pattern to location of the lesions and the overlying meninges were inconsistently involved. Histopathologic studies of the brain tissue revealed multiple lesions of inflammation, hemorrhage, and necrosis, the inflammatory reaction being primarily suppurative. Vasculitis was prominent with the formation of thrombi composed of leukocytes and fibrin.

Bacteriologic examination of brain tissues revealed Corynebacterium sp. and Streptococcus sp. as possible pathogens from several specimens, but because of lack of consistency of isolation, Griner et al., (1956) concluded that the syndrome was not related to either microorganism.

In California, Kennedy et al., (1960) reported a disease in feedlot cattle with encephalitic lesions of vasculitis, thrombosis, and necrosis similar to those reported by the Colorado workers (Griner et al., 1956). Their report offered evidence of a specific disease entity suggested by morbidity

rate, similar pathologic manifestations, and isolation of a pleomorphic, gram-negative bacterium from a significant number of cases. The authors considered the bacterial isolate to be a Hemophilus-like organism.

Weide et al., (1964) summarized the occurrence and diagnosis of bovine encephalitides in Kansas. Their report included polioencephalomalacia, listeriosis, streptococcal meningitis, and infectious embolic meningoencephalitis. The latter disease was the most frequently encountered encephalitic disease of feedlot cattle during a two-year period immediately preceeding the report. The gross and microscopic lesions were consistent with those previously reported. Microbiologic studies did not reveal the Hemophilus-like organism of Kennedy et al., (1960) nor other organisms of considered pathologic significance.

The report by Case et al., (1965) indicated the occurrence of a disease in Illinois with characteristics similar to those previously described, and a single isolation of the Hemophilus-like organism of Kennedy et al., (1960).

Gossling (1966) described characteristics of bacteria isolated from cattle in Illinois with lesions of "embolic meningoencephalitis" and suggested that ". . . this organism may probably be best described as an Actinobacillus species . . .".

Bailie et al., (1966) reported the occurrence of "infectious thromboembolic meningoencephalitis" in feedlot

cattle as ". . . a disease of primary importance . . ." in Kansas. The clinical features, gross and histopathologic lesions were similar to those previously described (Griner et al., 1956; Kennedy et al, 1960; Weide et al., 1964; and Case et al., 1965). In addition, they isolated a gram-negative bacillus from a significant number of cases. This organism was similar to both the Hemophilus-like organism of Kennedy et al., (1960) and Case et al., (1965) and to Actinobacillus actinoides. Similarities to the latter organism were considered significant and a tentative identification as Actinobacillus actinoides-like organism was made.

In 1968, Panciera et al. described a septicemia occurring in cattle in Oklahoma and in the Texas Panhandle caused by a Hamophilus-like organism, which was manifested by acute, subacute, and chronic syndromes involving the central nervous system, the respiratory system, and joints. The central nervous system manifestations parallelled those reported previously as embolic or thromboembolic meningoencephalitis. Their report provided evidence of the disease entity occurring in animals pastured on winter wheat as well as in feedlots. In addition, their report suggested significant lesions in tissues other than those in the central nervous system and the extraneural lesions appeared related to vascular injury. The Oklahoma workers considered that these lesions were not attributable to embolism, but to a vasculitis which may lead

to thrombosis. Their report indicated that polyarthritis, polyserositis, and psuedomembranous or ulcerative laryngitis were commonly associated with central nervous system lesions. The authors successfully reproduced the disease experimentally in 2 cattle utilizing a Hamophilus-like organism isolated from the brain of a naturally-occurring field case.

Bailie (1969) summarized the occurrence of thromboembolic meningoencephalomyelitis (TEMEM) among the suspected bovine encephalitis cases which he investigated. He reports TEMEM occurring in 60.5% (193) of 259 brains with encephalitic lesions. The cases were from Kansas (157), Nebraska (20), Illinois (6), Texas (4), Oklahoma (4), and Missouri (2). Extensive morphologic, physiologic, and biochemic examinations of a microorganism isolated from brain tissue of affected animals were conducted by the author and he concluded that the microorganism ". . . possesses much in common with the genus Haemophilus and therefore the name Haemophilus somnus (new species) is proposed."

MATERIALS AND METHODS

Rabbits

A total of 116 ten to twelve week-old New Zealand white rabbits of similar breeding were used in the experiments. Random distribution of rabbits according to the 4 primary research objectives was:

Experiment I: Optimal Infective Dosage and Hemogram Alteration Study (32).

Experiment II: Optimal Inoculation Method Study (51).

Experiment III: Endotoxin Study (21).

Experiment IV: Serial Passage Effect on Virulence Study (12).

The rabbits were housed individually in cages, under as nearly identical conditions as possible, in isolation facilities. All animals received a commercial rabbit food and water ad libitum. An equal number of males and females were selected by random sampling for each test group and each group included an appropriate number of control animals.

The normal health status of each rabbit was determined for a minimum of 2 weeks immediately preceeding each test. It was evaluated by daily examination of general appearance, appetite, and rectal temperature. A minimum of 2 fecal samples were collected from each rabbit during this period and examined by the zinc sulfate flotation method (Ewing, 1967) for evidence of parasitic infection. For those tests to evaluate specific hemogram alterations, blood samples were collected as described under hematologic examination. Only

rabbits which appeared healthy and whose status was considered normal were utilized in this investigation.

Hematologic Examination

One phase of the experimental design was to evaluate specific hemogram alterations following inoculation of H. somnus (n. sp.) described under inoculum preparation. During the 2 week period preceeding testing a minimum of 4 blood samples were collected by intracardiac puncture. The skin puncture site was prepared by shaving the hair and cleansing with 70% alcohol. A one inch 20 gauge needle with a 2 cc. glass syringe was used. Dipotassium ethylenediamine tetra-acetate* was utilized as the anticoagulant. The examination of each blood sample included: hemoglobin, hematocrit, total erythrocyte count, total leukocyte count, differential leukocyte count, and bacteriologic culture. Following inoculation of the test rabbits, and according to the experimental design, blood samples were collected and examined as described.

Hemoglobin was determined by the photometric cyanmethemoglobin method (Wintrobe, 1961) and the packed cell volume by the microhematocrit method (Schalm, 1965). The total erythrocyte and total leukocyte counts were made with the

*Mallinckrodt Chemical Works, St. Louis, Missouri.

Coulter Counter.* The differential leukocyte counts were made from smears utilizing standard techniques (Coles, 1967) and Wrights stain.**

Bacteriologic Examination

Bacteriologic examination of the blood samples was done by inoculating 0.5 ml. into 10.0 ml. of brain-heart infusion (BHI) broth.*** An aliquot from this was then inoculated into thyoglycolate broth for anaerobic incubation. The BHI broth sample was divided into 2 eaual parts, one was incubated aerobically and the other was incubated in an atmosphere of 10% CO_2.**** The broths were incubated for 48 hours at 37 C. One drop aliquots from each tube was then inoculated into blood agar***** plates and incubated under one of the 3 atmospheres described above. Plates were checked for growth at intervals of 48, 72, and 168 hours. Negative plates were discarded after 168 hours.

Inoculum Preparation

The inoculum was prepared from bovine brain

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*Coulter Electronics, Hialeah, Florida.

**Matheson Coleman and Bell, East Rutherford, New Jersey.

***Difco Laboratories, Detroit, Michigan.

****NAPCO, National Appliance Co., Portland, Oregon.

*****Sterile defibrinated sheep blood.

tissues* from which H. somnus (n. sp.) had been previously isolated. The brain tissue was cultured on typtose agar containing 20% sterile defibrinated sheep blood.

The cultures utilized had the following reactions: gram-negative; growth under microaerophilic conditions (approximately 5-10% CO_2), but without growth under aerophilic conditions; catalase-negative; production of acid fermentation in OF media with serum; oxidase-positive; and Mac Conkeys agar growth negative.

A bacterial suspension was prepared by washing the growth from the surface of the agar plates with sterile saline. The inoculum was standardized in a spectrophotometer** to conform with MacFarland nephlometer tube no. 3 equivalent to 3×10^6 organisms per ml. and confirmed by poured plate counts. Each inoculum was utilized within one hour after preparation.

Inoculation Procedure

The study was designed in 4 experiments and the inoculation procedure was determined for each experiment. A total of 116 rabbits were utilized with various numbers of rabbits in each experiment according to the investigation objectives. Without regard to the objectives of a specific experiment, each rabbit was examined at the termination of the experiment

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*Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, Kansas.

**Coleman, Jr. Coleman Instruments Corp. Maywood, Illinois.

by the methods described for necropsy, histopathologic, and bacteriologic examinations.

Experiment I

Optimal Infective Dosage and Hemogram Alteration Study

The aim was to establish the optimal infective dosage and any alteration of the specific hemogram parameters.

Thirty two rabbits were divided into 4 equal groups and each consisted of 4 males and 4 females, 3 test and 1 control of each sex. Each group received a different dosage calculated as milliliters of standardized inoculum per kilogram of body weight. Each pair of a group were further identified as "High Dose" (HD), "Mid Dose" (MD), "Low Dose" (LD), and "Control" according to dosage. The rabbits were inoculated as follows:

Rabbit Number	Identification	Dosage (ml./kg. of body wt.)
Group I		
001 005	Control	0.2
002 006	LD	0.1
003 007	MD	0.2
004 008	HD	0.3

Group II		
009 013	Control	0.45
010 014	LD	0.40
011 015	MD	0.45
012 016	HD	0.50
Group III		
017 021	Control	0.60
018 022	LD	0.40
019 023	MD	0.60
020 024	HD	0.80
Group IV		
025 029	Control	0.60
026 030	LD	0.40
027 031	MD	0.60
028 032	HD	0.80

Each rabbit was injected via the lateral marginal ear vein with a one-half inch 26 gauge needle and 1.0 ml. tuberculin syringe. Prior to inoculation, the hair was clipped from the injection site and the area cleansed with

70% alcohol. The control rabbits were injected in the same manner with an equal volume of the inoculum suspending medium.

Experiment II

Optimal Inoculation Method Study

Prior to the initiation of this experiment, a preliminary trial was conducted to evaluate the viability and pathogenicity of the available bacterium. The perliminary study utilized 3 rabbits which were inoculated intracerebrally. Two rabbits received a standarized inoculum and one received the suspending medium of the inoculum and served as a control.

The preliminary study group of rabbits were inoculated as follows:

Rabbit	Identification	Dosage (ml. of stand. inoculum)
A	Control	0.15
B	LD	0.1
C	HD	0.2

Forty-eight rabbits were divided into 4 groups of 12. Each group consisted of 3 dose levels, with 3 rabbits per dose level and 3 animals served as controls. The dosage was calculated as milliliters of standardized inoculum per kilogram of body weight. The control rabbits in each group were inoculated with the inoculum suspending medium. The amount of inoculum and method of inoculation corresponded to

that for each group.

Group I

The rabbits were inoculated intravenously via the lateral marginal ear vein as follows:

Rabbit Number	Identification	Dosage (ml./kg. of body wt.)
101	LD	0.3
102	LD	0.3
103	MD	0.5
104	MD	0.5
105	HD	0.7
106	HD	0.7
107	LD	0.3
108	MD	0.5
109	HD	0.7
110	Control	0.3
111	Control	0.5
112	Control	0.7

The volume of inoculum was injected at a rate which required 30 seconds to complete.

Group II

The rabbits were inoculated via the conjunctiva by swabbing each eye 3 times with a dry sterile cotton swab 2 minutes prior to inoculation. One-half of the calculated dose of inoculum was instilled into the conjunctival sac of each eye, with the head and eyelids held in a manner which provided retention of the maximal contact of the inoculum with the conjunctiva for 2 minutes.

The 12 rabbits in this group were inoculated as follows:

Rabbit Number	Identification	Dosage (ml./kg. of body wt.)
201	LD	0.08
202	LD	0.08
203	MD	0.12
204	MD	0.12
205	HD	0.16
206	HD	0.16
207	LD	0.08
208	MD	0.12
209	HD	0.16
210	Control	0.08
211	Control	0.12
212	Control	0.16

Group III

Nasal inoculation was performed by inserting a sterile catheter 2 cm. into each nasal passage and injecting one-half of the calculated dose into each nostril.

The 12 rabbits were inoculated as follows:

Rabbit Number	Identification	Dosage (ml./kg. of body wt.)
301	LD	0.07
302	LD	0.07
303	MD	0.14
304	MD	0.14
305	HD	0.21
306	HD	0.21
307	LD	0.07
308	MD	0.14
309	HD	0.21
310	Control	0.07
311	Control	0.14
312	Control	0.21

Group IV

This group was inoculated orally by intubation. A sterile catheter was inserted per os into the stomach and the calculated dose was injected, followed by 2 ml. of sterile physiological saline.

The rabbits were inoculated as follows:

Rabbit Number	Identification	Dosage (ml./kg. of body wt.)
401	LD	1.0
402	LD	1.0
403	MD	2.0
404	MD	2.0
405	HD	3.0
406	HD	3.0
407	LD	1.0
408	MD	2.0
409	HD	3.0
410	Control	1.0
411	Control	2.0
412	Control	3.0

Experiment III

Endotoxin Study

Twenty-one rabbits were utilized to test for the presence of endotoxin which was suggested by preliminary studies. Endotoxin was evaluated by the local and generalized Shwartzman reactions (Shwartzman, 1937 and 1953).

Local Shwartzman Reaction:

Twelve rabbits were divided into 4 groups of 3. Each

rabbit was initially injected intradermally and then 24 hours later intravenously. The inoculum used was different for each group and is described below. The intradermal site was located approximately 2 cm. lateral to the dorsal midline of the third lumbar vertebrae on the left side. This site was selected as it offered less opportunity for self-mutilation which could mask skin changes. The injection site was prepared by clipping and shaving a 5 cm. circular area, and cleansing with 70% alcohol. The injection was accomplished with a one-half inch 26 gauge needle and a 1.0 ml. tuberculin syringe. The intravenous injection was given via the lateral marginal ear vein after clipping and cleansing with 70% alcohol.

Group 1 was injected intradermally with 0.25 ml. of a filtrate obtained by Seitz-filtration* of a standardized inoculum followed 24 hours later by 0.1 ml. intravenously. The intradermal site was examined carefully for any visible change for a 48 hour period following the intravenous or provoking injection.

Groups 2, 3, and 4 were injected similarly except that group 2 received standardized inoculum, group 3 was given bacterial suspension which had been heated for 5 minutes in a water bath at 100 C, and group 4 was injected with a filtrate obtained by the filtering process described for group 1 utilizing the heated suspension used for group 3.

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*Republic Seitz Filter Corp., Milldale, Conn.

Generalized Shwartzman Reaction:

Nine rabbits were divided into 3 groups of 3. Each rabbit was injected intravenously and 24 hours later received a second intravenous injection.

Group 1 was injected with standardized inoculum. Group 2 was injected with the heated suspension previously described. Group 3 was injected with a filtrate of heated suspension which was filtered as previously described.

The rabbits were inoculated as follows:

Rabbit Number	Inoculum No. 1	Inoculum No. 2
Group 1		
701	S.I.,* 1.0 ml.	Saline
702	S.I., 1.0 ml.	S.I., 1.0 ml.
703	S.I., 1.0 ml.	S.I., 2.0 ml.
Group 2		
704	S.I., heated at 100 C for 5 min.	Saline
705	S.I., heated at 100 C for 5 min.	As Inoc. No. 1, 1.0 ml.
706	S.I., heated at 100 C for 5 min.	As Inoc. No. 1, 2.0 ml.
Group 2		
707	S.I., filtered	Saline
708	S.I., filtered	As Inoc. No. 1, 1.0 ml.
709	S.I., filtered	As Inoc. No. 1, 2.0 ml.

*S.I. = Standardized Inoculum.

Beginning 24 hours after the second inoculation, a rabbit from each group was killed and necropsied. A rabbit from each group was similarly examined at 48 hours and 72 hours following the second or provoking injection.

Experiment IV

Effect of Serial Passage on Virulence Study

Twelve rabbits were utilized to investigate the virulence of H. somnus (n. sp.) for rabbits following serial passage. A culture of H. somnus (n. sp.) was obtained by isolation from the brain tissue of a rabbit (103) used in Experiment II. The rabbit exhibited manifestations of central nervous system disturbances and the brain tissue had microscopic lesions of nonsuppurative meningoencephalitis. A standardized inoculum was prepared from this culture by the method previously described.

Twelve test rabbits were divided into 2 groups of 6, 4 test and 2 control. The first group were inoculated with standardized inoculum intravenously and the 2 control rabbits received suspending medium.

The animals were inoculated intravenously as follows:

Rabbit Number	Identification	Dosage (ml./kg. of body wt.)
801 804	Control	0.4
802 805	LD	0.3
803	HD	0.5

The second group were injected with a culture obtained from the brain tissue of one rabbit (802) from group 1. Six

rabbits were inoculated intravenously as follows:

Rabbit Number	Identification	Dosage (ml./kg. of body wt.)
807 810	Control	0.4
808 811	LD	0.3
809 812	HD	0.5

Post-Inoculation Procedures

General physical examinations were made of all rabbits as frequently as dictated or at intervals not exceeding 12 hours. The basic examination included general appearance and rectal body temperature. Changes in feed and/or water consumption rates were noted. In the first experiment blood samples were collected at specific times according to the method described.

Disposition of Rabbits

Test rabbits were necropsied immediately after death or if *in extremis*. Test rabbits which survived were killed by carbon dioxide narcosis and exsanguination, and then necropsied. The control rabbits were killed and necropsied at the time as the surviving test rabbits.

Necropsy examination was conducted on each rabbit and the following tissue sections were collected and fixed in 10% buffered neutral formalin: all macroscopic pathologic lesions, brain, spinal cord, eyes, nasal mucous membranes and turbinates, liver, kidney, stomach, duodenum, lung, heart, and lymph nodes (parotid, retropharnygeals, mandibular, gastric, and mesenteric). Representative sections of all macroscopic pathologic lesions, central nervous system tissues, liver, kidney, and lung were frozen and held at -70 C* for subsequent bacteriologic culture.

Histopathologic Examination

Emphasis of the histopathologic examination was placed on tissues from the central nervous system. Sections from the cerebral cortex, caudate nucleus, hippocampus, thalmus, hypothalmus, corpora quadrigemina, pons, medulla oblongata, cerebellum, and spinal cord were processed by the paraffin block method, sectioned at 5-6 microns, and routinely stained with hematoxylin and eosin. Sections with lesions were stained with the Brown and Brenn modification of the Gram stain for bacteria (Thompson and Hunt, 1966).

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*Revco Inc., Industrial Products Division, Deerfield, Michigan.

RESULTS

Experiment I

Optimal Infective Dosage and Hemogram Alteration Study

Group I

The average pre-inoculation values for the specific hemogram parameters were determined (Appendix, Table I). The values, for each of 7 samples of blood collected during this period, were considered within normal limits for all rabbits. Rectal body temperatures were recorded for each rabbit twice daily at 12 hour intervals (Appendix, Table II). Each rabbit was observed daily to determine the normal clinical attitude of movement, alertness, color of mucous membranes, and respiratory rates. No abnormalities were noted. Examination of fecal samples by the zinc sulfate flotation method revealed oocysts of hepatic coccidia (Eimeria sp.) in 4 rabbits (004, 006, 007, and 008) and intestinal coccidia (Eimeria sp.) oocysts in 2 (004 and 006). Microbiologic examination of blood samples collected from this group revealed Staphylococcus aureus in the sample taken 4 days prior to inoculation of animal 005. This was considered a contaminant because the rabbit had no clinical signs of disease.

The post-inoculation (P.I.) values of the specific hemogram parameters were determined (Appendix, Table I). At 8 hours P.I., an increase in the number of large lymphocytes was observed in all rabbits receiving H. somnus (n. sp.).

Other hemogram parameters did not reveal any significant change. The hemogram parameters of the control rabbits were not significantly different from the pre-inoculation status. Hemogram values at 20 hours P.I. were nearly identical with the values obtained at 8 hours P.I. At 32 hours P.I. the total leukocyte count and percentage of pseudoeosinophils and large lymphocytes were altered, the former decreased and the latter increased; the other values remained constant. The altered pseudoeosinophil and large lymphocyte relationship was also evident at 48 hours P.I., when the highest total leukocyte values were established. These values remained relatively constant until 96 hours P.I. when there was evidence of return to normal cellular relationship levels and a decrease in total leukocyte numbers (Appendix, Table I).

Rectal temperatures of rabbits in Group I increased following inoculation. The increase was observed initially at 2 hours P.I. and was maintained for 46 hours before returning to the pre-inoculation level. Test rabbits developed a temperature increase within 2 hours P.I. which was maximal at 24 hours P.I. and returned to normal at 50 hours P.I. Differences in temperature change resulting from the various dosage levels were not significant (Appendix, Table II).

The clinical appearance of control rabbits was regarded as slightly depressed. The appearance of test rabbits varied considerably, and without any apparent correlation with the dosage. There was a sudden increase in the respiratory rate

following inoculation which developed within 5 minutes and lasted for periods ranging from 30 minutes to 2 hours. Some rabbits became recumbant for periods of 5 to 18 hours. A decrease in the consumption of feed and water was noted in all test rabbits. A watery and fetid diarrhea developed in rabbits 002, 003, 006, and 008 and was evident from 4 hours P.I. to the termination of the experiment (Appendix, Table III).

The microbiologic examination of blood samples revealed H. somnus (n. sp.) at 24 hours and until the termination of the experiment at 96 hours in 2 rabbits, at 24 hours through 72 hours in 1 rabbit, at 48 hours through 96 hours in 1 rabbit, at 72 hours through 96 hours in 1 rabbit, and at 72 hours only in 1 rabbit (Table 1).

TABLE 1

MICROBIOLOGIC EXAMINATION OF BLOOD SAMPLED FOLLOWING INOCULATION OF RABBITS WITH HAEMOPHILUS SOMNUS (n. sp.)

Experiment I

Rabbit Number	Hours Post-Inoculation			
	24	48	72	96
Group 1				
001	-	-	-	-
002	+	+	+	+
003	-	+	+	+
004	+	+	+	+
005	-	-	-	-
006	+	+	+	-
007	-	-	+	-
008	-	-	+	+

- = Negative.
+ = Hemophilus somnus (n. sp.) isolated.

All rabbits in Group 1 survived the 96 hour period and were killed. Necropsy revealed large subepicardial hemorrhage in animals 002, 005, and 006 as the only visible gross lesions. These lesions were considered to result from the intracardial puncture technique utilized to collect blood samples.

Histopathologic examination of the brain tissues revealed lesions in all test rabbits. The lesions consisted of a meningoencephalitis characterized by a mononuclear inflammatory cellular infiltration with perivascular cuffing, glial nodule formation, satellitosis, neuronophagia, and necrosis. There was an apparent hyperplasia of the reticular cells of the vascular adventitia (Appendix, Plate I). The appearance of some lesions closely resembled those occurring in field cases of infectious thromboembolic meningoencephalitis in cattle. The lesions were primarily confined to the cerebral hemispheres. There was considerable variation in degree of histologic alteration between rabbits and no microorganisms were observed. No lesions were noted in the brain tissues of the control animals (Table 2).

Microscopically, the other tissues revealed a nonsuppurative perioportal hepatitis in all rabbits, a nonsuppurative interstitial nephritis in 6 (002, 003, 004, 005, 007, and 008), and an interstitial pneumonia in 5 (001, 002, 003, 005, and 008). Minor inconsistent microscopic lesions were observed in various tissues of individual rabbits.

TABLE 2

MICROSCOPIC LESIONS IN THE BRAIN OF RABBITS FOLLOWING INOCULATION WITH HAEMOPHILUS SOMNUS (n. sp.)

Experiment I

Lesions	Rabbit Number and Identification							
	001 Cont.*	002 LD	003 MD	004 HD	005 Cont.	006 LD	007 MD	008 HD
Encephalitis	-	+	+	+	-	+++	++	+
Meningitis	-	+	+	+	-	+++	++	+
Reactive Cells	-	L	L	L	-	L	L	L
Perivascular Cuffing	-	+	+	+	-	+++	++	+
Glial Nodules	-	+	-	+	-	++	+	+
Satellitosis	+	+	+	+	-	+	+	+
Neuronophagia	+	+	+	+	-	+	+	+
Necrosis	-	+	-	+	-	+	-	-

* = Control.
+ to +++ = Relative severity of lesion.
- = Not observed.
L = Lymphoid.

Group II

The average pre-inoculation values for the specific hemogram parameters (Appendix, Table I) and normal rectal temperatures are tabulated (Appendix, Table II). Coccidial oocysts of the intestinal form (Eimeria sp.) were found in 3 rabbits (012, 015, and 016) and the hepatic form (Eimeria sp.) in 2 rabbits (012 and 013).

The P.I. values of the specific hemogram parameters were determined (Appendix, Table I). The values were similar to those observed for Group I. An initial increase in total

leukocytes due to an increase of pseudoeosinophils occurred after 8 hours P.I. and prior to 32 hours P.I. After this time, the ratio of pseudoeosinophils to large lymphocytes was reversed and persisted until after 96 hours P.I. when a reduction in total leukocytes became evident. Two rabbits (009 and 011) died of cardiac tamponade, resulting from intracardiac puncture after 32 hours P.I. A third rabbit (016) was euthanatized at 32 hours P.I. because of a broken leg.

The post-inoculation rectal temperatures in Group II were initially similar to those found in Group I, but persisted for a longer period (Appendix, Table II). Clinical manifestations (Appendix, Table III) in this group were also similar to those observed in Group I, with the exception of rabbit 014. This rabbit exhibited marked dyspnea, anorexia, and ataxia which progressed to recumbancy at 32 hours P.I. and death by 48 hours P.I. Feed and water consumption rates decreased for this group similar to Group I. Microbiologic examination of blood samples from this group during the P.I. period were negative. The surviving rabbits in Group II were maintained for 16 hours beyond the established period of 96 hours without any significant changes.

Necropsy examination of rabbits in this group revealed gross lesions only in number 014. These lesions were: enlarged, friable, and congested liver; excess pericardial fluid; and enlarged and congested spleen. Histopathologic examination

of tissues from all rabbits in this group revealed an interstitial pneumonia and additional lesions in 014. These lesions in rabbit 014 were: interstitial and alveolar edema of the lungs; congested liver with dilatation of the sinusoids and degeneration of the hepatocytes which was primarily centrolobular; and congested spleen with apparent depletion of lymphoid elements. Bacteriologic examination of the tissues was negative.

Group III

Pre-inoculation hemogram parameters (Appendix, Table I), rectal temperatures (Appendix, Table II), clinical manifestations (Appendix, Table III), and other data identical to that of Group I and Group II were within normal limits. During the P.I. period for this group, the hemogram, body temperatures, and clinical manifestations closely parallelled those observed for Groups I and II with the exception of significantly reduced feed and water consumption rates for Group III.

Death occurred in less than 24 hours P.I. in 1 rabbit at each dosage level (018, 023, and 024). Each rabbit exhibited dyspnea, lethargy, progressive weakness and recumbancy with spasmotic muscular contractions until death. Recumbancy lasted for 17 hours in rabbit 018, 12 hours in 023, and less than 6 hours in 024. Microbiologic examination of blood samples for this group were negative.

Necropsy examination of Group III revealed lesions in 3 animals (018, 023, and 024) and splenomegaly, hepatomegaly and congestion, and pulmonary edema were noted in each. In addition, there was increased pericardial fluid and a soft flabby heart in 018. Histopathologic examination of selected specimens from this group failed to reveal any significant lesions except in the brain of rabbit 019 which had a marked meningoencephalitis similar to that observed in rabbits of Group I (Table 3). Bacteriologic studies of the tissues from this group were negative.

TABLE 3

MICROSCOPIC LESIONS OF THE BRAIN OF RABBITS FOLLOWING INOCULATION WITH HAEMOPHILUS SOMNUS (n. sp.)

Experiment I

Lesions	Rabbit Number and Identification							
	017 Cont.	018 LD	019 MD	020 HD	021 Cont.	022 LD	023 MD	024 HD
Encephalitis	-	-	+++	-	-	-	-	-
Meningitis	-	-	+++	-	-	-	-	-
Reactive Cells	-	-	L	-	-	-	-	-
Perivascular Cuffing	-	-	++	-	-	-	-	-
Glial Nodules	-	-	++	-	-	-	-	-
Satellitosis	-	-	+	-	-	-	-	-
Neuronophagia	-	-	+	-	-	-	-	-
Necrosis	-	-	++	-	-	-	-	-

+ to +++ = Relative severity of lesion.
- = Not observed.
L = Lymphoid.

Group IV

Pre-inoculation data were similar to Groups I, II, and III and were within normal limits (Appendix, Tables 1, 2, and 3). The P.I. data for Group IV (Appendix, Tables 1, 2, and 3) were similar to that recorded for the previous groups. Death occurred in 36 hours P.I. in 2 rabbits. At the mid-dose level, 031 succumbed following signs of encephalopathy, and at the high dose level, 032 died with similar signs. Signs were apparent for 12 hours in 031 and 19 hours in 032.

Necropsy of rabbits in this group revealed the following gross lesions: 031 - hepatomegaly and splenomegaly; 032 - hepatomegaly, splenomegaly, pulmonary edema and frothy fluid in the trachea.

Experiment II

Optimal Inoculation Method Study

Preliminary Trial

Rabbit A in this trial exhibited no abnormal signs following inoculation and remained normal until termination of the study 7 days P.I. Necropsy, histopathologic, and bacteriologic examinations were negative. Rabbit B exhibited dyspnea, lethargy, anorexia, and a white purulent naso-ocular discharge within 24 hours P.I. The nasal and ocular discharges increased in quantity until 48 hours P.I. and were absent at 72 hours when the rabbit appeared clinically normal. Necropsy revealed thickened meninges at the site of

inoculation and a 0.5 cm. circular, yellow foci in the cerebrum. Microscopic and bacteriologic studies were negative. Rabbit C became lethargic within 12 hours P.I. Lethargy and anorexia persisted until 36 hours P.I. when the rabbit appeared clinically normal. Necropsy, histopathologic, and bacteriologic examinations, however, were negative.

Group I

All rabbits developed an increased respiratory rate within 1 hour of inoculation. The control rabbits (110, 111, and 112) were clinically normal within 2 hours of inoculation. The test rabbits developed signs of depression and lethargy during the first 4 hours P.I. By 6 hours P.I. 1 rabbit (101) had a copious bilateral ocular discharge of white, tenacious material, and 2 rabbits (108 and 109) were incoordinated, severely depressed, had injected scleras and cyanotic ears, the latter were dead at 10 hours P.I. No changes were observed in the clinical condition of the other test rabbits. At 20 hours P.I., 3 more rabbits (105, 106, and 107) were dead, 3 (101, 103, and 104) had a white copious and tenacious nasal discharge and 4 (101, 102, 103, and 104) had a similar ocular discharge. The condition of the affected rabbits remained unchanged for 7 days. Rectal temperatures increased to a maximum of 1.5 degrees (105.0 F) above normal within 12 hours. Feed and water consumption was greatly reduced.

Necropsy of the rabbits failed to reveal any gross lesions other than the nasal and/or ocular discharges. Histopathologic studies revealed meningoencephalitis in rabbit 103 similar to that described in experiment I of this study. Microbiologic examination revealed H. somnus (n. sp.) in the brain of this rabbit.

Group II

The conjunctiva was reddened in all rabbits within the first hour P.I. but no other signs were noted. The eyes of the control rabbits appeared normal within 2 hours P.I. At 2 hours P.I., the maximum reddening was noted in rabbits 201, 202, 203, 204, 205, 206, and 209. At 4 hours P.I., all rabbits appeared normal. A thin, white, ocular discharge was observed in 205, 206, and 208 at 20 hours P.I. and was accompanied by swelling of the palpebrum. This condition persisted in 2 rabbits (205 and 208) for 5 days. The other test rabbits were normal.

There were no apparent gross lesions observed at necropsy. Microscopically, a meningoencephalitis was observed in rabbit 201. Bacteriologic examinations were negative.

Group III

No clinical signs were observed following inoculation other than an immediate transient dyspnea for 1 to 2 minutes. Necropsy, histopathologic, and bacteriologic examinations

were negative.

Group IV

There were no clinical signs observed following inoculation other than a profuse, fetid diarrhea in rabbits 405, 406 and 409 at 10 hours P.I. which persisted for 72 hours. Necropsy, histopathologic, and bacteriologic examinations were negative.

Experiment III

Endotoxin Study

Localized Shwartzman Reaction

The intradermal inoculation site revealed no changes in the control rabbits at 24 hours. The test rabbits revealed an elevated 2 cm. circumscribed red area in 602, 603, 605, and 606; an elevated 1 cm. slightly red circumscribed area in 608 and 609; and no elevation but a slightly reddened area in 611 and 612.

Examination of the intradermal site 24 hours after the intravenous provoking injection revealed the following: no change in the control animals or in 608, 611, and 612; necrosis of the entire 2 cm. site in 602, 603, 606, and 609; and necrosis of one-fourth of the 2 cm. site in 605.

Rectal temperatures 24 hours after the intravenous injection varied from normal to 105.3 F., the highest rise occurred in rabbit 606 which received unheated and unfiltered

standardized inoculum.

Other than the dermal lesions, the necropsy, histopathologic, and bacteriologic examinations were negative.

Generalized Shwartzman Reaction

Two rabbits (705 and 709) died within one hour following the second intravenous injection.

Necropsy examination revealed the kidneys of rabbits 702, 706, and 708 to be slightly yellow with numerous, white, irregular foci, 1-2 mm. in diameter. Additional lesions were not observed in these or the other rabbits. Histopathologic and bacteriologic examinations were negative.

Experiment IV

Serial Passage Effect on Virulence Study

The rabbits were observed for 4 days following inoculation. During the first 24 hours P.I., dyspnea, lethargy, and anorexia developed in 2 rabbits (802 and 806) and remained constant until 96 hours P.I. Rectal temperatures rose within the first 2 to 4 hours and ranged from 105.9 to 107.0 F. At 8 hours P.I., temperatures were 105.5 to 106.8 F. and all returned to normal within 18 to 24 hours P.I.

Necropsy and histopathologic examinations of this group were negative. H. somnus (n. sp.) was isolated from the brain of rabbit 802 and the spleen of 806.

Signs of lethargy and anorexia developed in rabbits 808, 811, and 812 within 8 hours P.I. and rectal temperatures ranged from 104.6 to 106.2 F. Body temperatures were returned to normal by 18 hours P.I. Necropsy, histopathologic, and bacteriologic examinations were negative at 96 hours P.I.

DISCUSSION

The investigation of the optimal infective dosage of Haemophilus somnus (n. sp.) administered intravenously revealed a dosage of standardized inoculum could produce microscopic lesions of a nonsuppurative meningoencephalitis in 100% of the test rabbits. The brain lesions were characterized by a mononuclear call infiltration of the meninges and perivascular areas, glial nodule formation, necrosis, satellitosis, neuronophagia, and hyperplasia of the reticular cells of the vascular adventitia. For a 10 to 12 week-old New Zealand white rabbit, the dosage was calculated to be in the range of 300,000 to 900,000 organisms per kilogram of body weight. The dosage did not consistently produce clinical evidence of encephalitis and macroscopic lesions were absent in all rabbits. Microscopic lesions of the brain were present by 96 hours P.I.

A bacteremia occurred during the P.I. period. Bacteria were isolated from the blood from 24 hours to 96 hours P.I. in 33.3% of the test rabbits. In other rabbits, bacteria were isolated only at or during specific P.I. times.

Rectal temperature responses varied considerably, but alterations closely parallelled the leukocytic responses.

Alterations in the specific hemogram parameters were uniform for all rabbits. An initial slight leukopenia was followed by a slight to marked leukocytosis with a duration of 72 to 96 hours. The leukocytosis was initially

pseudoeosinophilic in the early P.I. period, and later, lymphocytic.

Higher dosage levels administered intravenously resulted in extremely variable clinical effects and did not produce macroscopic or microscopic lesions.

Investigations of various methods of inoculation revealed that the conjunctival route produced microscopic lesions of meningoencephalitis without clinical signs in 11.1% of the test rabbits. Attempts to produce signs or lesions of encephalitis following oral or nasal inoculation were unsuccessful. Production of a characteristic meningoencephalitis following exposure of the conjunctiva to H. somnus (n. sp.) suggests that it is a possible route of infection in field cases of bovine thromboembolic meningoencephalitis.

A significant number of animals inoculated by various methods developed a copious, white tenacious nasal and/or ocular exudate. The relationship of this sign to infection produced by H. somnus (n. sp.) could not be determined. Microbiologic examination of the exudative material did not reveal any significant microorganisms.

Studies to evaluate the production of endotoxin were partially successful. Necrosis of the intradermal injection site was produced following an intravenous injection of filtered standardized inoculum, standardized inoculum, and heated standardized inoculum. Necrosis was not produced,

however, by heated and filtered standardized inoculum. The heated preparation retained the toxic properties which is in agreement with the thermostable characteristic of endotoxins (Thomas, 1954). Other evidence of endotoxin-like properties was revealed by the studies in Experiment I. An initial leukopenia followed by leukocytosis occurred in all rabbits inoculated in this experiment. Clinical signs noted throughout the research, e.g. incoordination, recumbancy, hyperthermia, dyspnea, fetid diarrhea and sudden death of some animals, also are in agreement with those signs reported to occur as a result of endotoxin (Thomas, 1954). Rectal temperatures and clinical responses during the endotoxin studies were similar to those observed in previous Experiments in this study. The absence of gross and microscopic lesions in these animals at 48 hours P.I. suggests that brain lesions developed between 48 hours and 96 hours P.I. in experimentally-infected rabbits.

Studies to evaluate the generalized Shwartzman response were inconclusive. Inoculation of animals with standardized inoculum, heated standardized inoculum, and filtered, heated standardized inoculum produced gross lesions in the kidneys suggestive of multiple focal necrosis. Histopathologic evaluation of tissue sections from the kidneys failed to confirm the etiology of the macroscopic lesions. This could be explained by the time interval as the microscopic lesions are more distinct by 7 days (Shwartzman and Schneierson, 1953;

and Thomas, 1954).

Studies of the effect of serial passage of H. somnus (n. sp.) in rabbits resulted in more clinical response, but no increase in the incidence of encephalitis.

CONCLUSIONS

The microorganism isolated from the brain tissues of cattle with a condition termed "thromboembolic meningoencephalitis" was experimentally infective for 10 to 12 weeks old New Zealand white rabbits. Intravenous inoculation of 300,000 to 900,000 organisms per kilogram of body weight produced microscopic lesions of nonsuppurative meningoencephalitis within 96 hours P.I. without clinical evidence. Similar microscopic lesions resulted from conjunctival inoculation in one rabbit in a group of 9 inoculated by this method. Nasal and oral routes of inoculation were not successful.

Specific hemogram alterations occurred following experimental inoculation. An initial leukopenia of short duration was followed by a leukocytosis. The leukocytosis was at first pseudoeosinophilic and later, lymphocytic.

Endotoxin-like properties of the microorganism were evident throughout this study namely, local and generalized Shwartzman reaction, leukopenia followed by leukocytosis, hyperthermia, diarrhea and death.

The results of the effects of serial passage on the virulence of H. somnus (n. sp.) for rabbits were inconclusive.

The results of this investigation indicate that the rabbit is of limited use as an experimental animal for studies with H.somnus (n. sp.) because of erratic response. The rabbit could be utilized for studies involving the pathogenesis

of the microscopic lesions of the brain, and for evaluation of endotoxic properties of H. somnus (n. sp.).

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APPENDIX

TABLE I

AVERAGE PREINOCULATION (PRE. INOC.) AND POST-INOCULATION (POST INOC.) VALUES OF SPECIFIC HEMOGRAM PARAMETERS OF RABBITS INOCULATED WITH HAEMOPHILUS SOMNUS (n. sp.) IN EXPERIMENT I.

RABBIT GROUP AND NUMBER	RBC mill./cu. mm.	WBC mill./cu. mm.	Pseudoeosin. %	LG. Lymph. %	SM. Lymph. %	Eosinophil %	Basophil %	Hb. gm./100 ml.	Hmt. %
Group I Pre. Inoc. 001 - 008	3.01	5.4	47	34	15	1	1	7.3	22
Post Inoc. 8 hours									
CONTROL	3.18	5.2	47	32	17	0	2	7.4	21
LD	3.41	4.9	76	16	8	0	0	8.1	24
MD	3.30	5.9	73	17	9	1	0	7.7	21
HD	3.18	4.8	68	27	5	0	0	7.7	23
32 hours									
CONTROL	3.12	5.3	45	31	19	2	3	7.4	21
LD	3.31	6.3	20	58	22	0	0	7.1	21
MD	3.20	6.4	21	62	16	1	0	7.5	22
HD	3.30	6.6	23	62	15	0	0	7.0	22
48 hours									
CONTROL	3.15	5.2	48	35	15	0	2	7.4	21
LD	3.33	8.8	22	58	20	0	0	7.1	21
MD	3.30	8.8	23	54	22	1	0	7.3	21
HD	3.27	10.1	26	60	14	0	0	7.0	22
96 hours									
CONTROL	3.30	5.4	47	32	17	0	2	7.4	21
LD	3.27	8.4	37	50	13	0	0	7.0	22
MD	3.32	8.4	28	61	10	1	0	7.1	20
HD	3.00	9.3	31	53	15	0	1	7.1	20

TABLE I (Continued)

AVERAGE PREINOCULATION (PRE. INOC.) AND POST-INOCULATION (POST INOC.) VALUES OF SPECIFIC HEMOGRAM PARAMETERS OF RABBITS INOCULATED WITH HAEMOPHILUS SOMNUS (n. sp.) IN EXPERIMENT I.

RABBIT GROUP AND NUMBER	RBC mill./cu. mm.	WBC mill./cu. mm.	Pseudoeosin. %	LG. Lymph. %	SM. Lymph. %	Eosinophil %	Basophil %	Hb. gm./100 ml.	Hmt. %
Group I Pre. Inoc. 009 - 016	3.40	4.9	42	43	13	1	1	8.4	28
Post Inoc. 8 hours									
CONTROL	3.38	4.9	46	42	12	0	0	7.9	28
LD	3.27	4.7	54	38	10	0	0	7.9	27
MD	3.09	4.7	57	36	7	0	0	7.6	28
HD	3.15	4.6	63	27	9	1	0	7.1	20
32 hours									
CONTROL (1)	3.30	5.7	46	43	11	0	0	7.7	26
LD	3.28	5.9	61	29	8	0	2	7.7	28
MD (2)	3.29	5.6	59	28	12	1	0	7.3	23
HD (3)	3.30	6.3	60	28	12	0	0	7.4	23
48 hours									
CONTROL	3.27	5.2	45	31	20	1	3	7.4	22
LD (4)	3.31	9.4	31	58	11	0	0	7.2	21
MD	3.18	7.9	27	60	13	0	0	7.2	21
HD	3.30	9.5	29	61	10	0	0	7.2	22
96 hours									
CONTROL	3.30	5.2	45	33	20	0	2	7.2	21
LD	3.32	8.7	27	59	10	1	3	7.4	21
MD	3.00	7.8	22	68	9	1	0	7.4	22
HD	3.33	7.2	24	57	16	0	3	7.4	21

(1) 009 - Death, cardiac tamponade follwoing withdrawal of 32 hour specimen.
(2) 011 - Death, cardiac tamponade following withdrawal of 32 hour specimen.
(3) 016 - Euthantized due to broken leg.
(4) 014 - Dead at 48 hours.

TABLE I (Continued)

AVERAGE PREINOCULATION (PRE. INOC.) AND POST-INOCULATION (POST INOC.) VALUES OF SPECIFIC HEMOGRAM PARAMETERS OF RABBITS INOCULATED WITH HAEMOPHILUS SOMNUS (n. sp.) IN EXPERIMENT I.

RABBIT GROUP AND NUMBER	RBC mill./cu. mm.	WBC mill./cu. mm.	Pseudoeosin. %	Lg. Lymph. %	Sm. Lymph. %	Eosinophil %	Basophil %	Hb. gm./100 ml.	Hmt. %
Group I									
Pre. Inoc.									
017 - 024	3.24	5.1	48	31	19	1	1	7.3	21
Post Inoc.									
8 hours									
CONTROL	3.26	5.4	46	31	21	0	2	7.0	22
LD	3.31	4.8	63	21	16	0	0	7.3	21
MD	3.27	4.8	68	18	14	0	0	7.4	20
HD	3.00	4.7	61	19	18	0	2	7.0	20
32 hours									
CONTROL	3.18	5.6	45	30	20	2	3	7.4	21
LD (1)	3.30	6.4	31	58	11	0	0	7.4	23
MD (2)	3.15	6.6	28	59	13	0	0	7.1	22
HD (3)	3.27	6.7	23	60	17	0	0	7.0	22
48 hours									
CONTROL	3.13	5.4	49	34	14	1	2	7.1	21
LD	3.40	9.6	22	61	17	0	0	7.0	21
MD	3.10	11.3	24	59	17	1	1	7.2	23
HD	3.25	10.2	22	60	18	0	0	7.0	22
96 hours									
CONTROL	3.27	5.1	46	35	17	1	1	7.4	21
LD	3.00	8.9	36	50	14	0	0	7.0	23
MD	3.30	9.3	24	64	12	0	0	7.1	21
HD	3.30	9.0	30	55	15	0	0	7.1	20

(1) 018 - Dead at 24 hours.
(2) 023 - Dead at 24 hours.
(3) 024 - Dead at 24 hours.

TABLE I (Continued)

AVERAGE PREINOCULATION (PRE. INOC.) AND POST-INOCULATION (POST INOC.) VALUES OF SPECIFIC HEMOGRAM PARAMETERS OF RABBITS INOCULATED WITH HAEMOPHILUS SOMNUS (n. sp.) IN EXPERIMENT I.

RABBIT GROUP AND NUMBER	RBC mill./cu. mm.	WBC mill./cu. mm.	Pseudoeosin. %	Lg. Lymph. %	SM. Lymph. %	Eosinophil %	Basophil %	Hb. gm./100 ml.	Hmt. %
Group I									
Pre. Inoc.									
025 - 032	3.08	5.4	46	35	15	0	2	7.3	22
Post Inoc.									
8 hours									
CONTROL	3.40	5.3	47	36	17	0	0	7.4	24
LD	3.23	4.6	69	16	15	0	0	7.4	21
MD	3.31	4.5	71	16	13	0	0	7.2	23
HD	3.38	4.7	72	17	10	0	1	7.3	23
32 hours									
CONTROL	3.33	5.4	45	34	20	0	1	7.4	24
LD	3.31	6.1	31	60	9	0	0	7.2	24
MD	3.33	5.9	20	63	15	1	1	7.4	21
HD	3.27	6.3	23	62	14	0	1	7.3	22
48 hours									
CONTROL	3.35	5.2	48	31	17	1	3	7.3	22
LD	3.31	9.4	26	60	13	0	1	7.0	21
MD (1)	3.20	9.4	24	61	14	1	0	7.0	23
HD (2)	3.30	10.8	27	61	12	0	0	7.1	21
96 hours									
CONTROL	3.34	5.2	47	32	18	0	3	7.4	22
LD	3.30	9.0	31	56	13	0	0	7.3	21
MD	3.20	9.2	30	56	13	0	1	7.2	22
HD	3.32	9.4	33	53	14	0	0	7.2	21

(1) 031 - Dead at 36 hours.
(2) 032 - Dead at 36 hours.

TABLE II

RECTAL TEMPERATURES OF RABBITS INOCULATED WITH *HAEMOPHILUS SOMNUS* (n. sp.) IN EXPERIMENT I.

Rabbit Group and Number	Average Preinoculation Temperature	Post Inoculation Temperature (hours post inoculation) 2	24	48	60
Group I					
001	102.0	103.1	103.0	102.2	102.2
002	102.1	102.8	104.6	103.8	102.8
003	102.0	103.8	104.0	103.6	103.0
004	102.4	104.2	104.8	103.6	102.4
005	101.8	102.5	102.8	102.0	102.5
006	102.8	103.8	104.0	103.6	103.0
007	103.0	104.1	104.6	103.8	103.0
008	102.5	104.0	104.4	104.0	103.2
Group II					
009	102.6	103.6	103.0	(1)	-
010	102.6	103.8	103.8	103.6	105.0
011	101.8	102.8	103.6	(2)	-
012	101.0	102.2	102.8	102.8	102.8
013	101.5	102.4	102.8	102.6	102.6
014	101.8	102.0	102.8	(3)	-
015	102.0	102.8	102.6	103.0	103.5
016	102.0	103.2	103.8	(4)	-
Group III					
017	102.0	103.4	102.9	102.6	102.7
018	102.1	103.8	(5)	-	-
019	101.8	102.8	103.6	103.6	103.0
020	102.0	103.2	103.8	104.0	103.6
021	102.1	102.8	102.8	102.5	102.5
022	101.8	103.0	103.4	103.5	103.2
023	102.4	103.8	(6)	-	-
024	102.4	103.8	(7)	-	-
Group IV					
025	102.0	103.0	103.0	102.8	102.8
026	102.4	103.6	103.0	102.8	102.8
027	101.8	102.6	102.8	103.0	102.6
028	102.0	102.3	103.1	102.4	102.8
029	101.8	102.4	102.8	102.0	102.0
030	102.0	102.8	103.2	102.8	103.0
031	101.8	102.8	104.0	(8)	-
032	102.2	103.2	103.8	(9)	-

(1) Dead at 32 hours (cardiac tamponade).
(2) Dead at 32 hours (cardiac tamponade).
(3) Dead at 48 hours.
(4) Euthantized at 32 hours (broken leg).
(5) Dead at 24 hours.
(6) Dead at 24 hours.
(7) Dead at 24 hours.
(8) Dead at 36 hours.
(9) Dead at 36 hours.

TABLE III

CLINICAL MANIFESTATIONS OF RABBITS INOCULATED WITH *HAEMOPHILUS SOMNUS* (n. sp.) IN EXPERIMENT I.

Rabbit Group and Number	Post Inoculation Respiration 15 min	30 min	1 hr	Feed and Water Consumption 2 hr	18 hr	24 hr	48 hr	72 hr	96 hr
Group I									
001	I	N	N	N	D	D	N	N	N
002	I	N	N	N	D	D	N	N	N
003	I	N	N	N	D	D	D	N	N
004	I	I	I	N	D	D	D	D	D
005	I	N	N	N	D	D	N	N	N
006	I	N	N	N	D	D	D	N	N
007	I	I	I	I	D	D	D	D	D
008	I	N	N	N	D	D	N	N	N
Group II									
009	I	N	N	N	D	N	(1)	-	-
010	I	I	N	N	D	D	D	N	N
011	I	N	N	N	D	N	(2)	-	-
012	I	I	I	N	D	D	D	D	D
013	I	N	N	N	D	N	N	N	N
014	I	I	I	I	D	D	(3)	-	-
015	I	I	N	N	D	D	D	N	N
016	I	N	N	N	D	N	(4)	-	-
Group III									
017	I	N	N	N	D	D	N	N	N
018	I	I	I	I	D	(5)	-	-	-
019	I	I	N	N	D	D	D	D	D
020	I	I	I	I	D	D	D	D	D
021	I	N	N	N	D	D	N	N	N
022	I	I	I	N	D	D	D	D	D
023	I	I	I	I	D	(6)	-	-	-
024	I	I	I	I	D	(7)	-	-	-
Group IV									
025	I	N	N	N	D	N	N	N	N
026	I	I	N	N	D	D	D	N	N
027	I	I	N	N	D	D	D	D	N
028	I	N	N	N	D	D	N	N	N
029	I	N	N	N	D	N	N	N	N
030	I	N	N	N	D	D	N	N	N
031	I	I	I	I	D	D	(8)	-	-
032	I	I	I	I	D	D	(9)	-	-

I = Increased.
N = Normal.
D = Decreased.

(1) Dead at 32 hours.
(2) Dead at 32 hours.
(3) Dead at 48 hours.
(4) Euthanitized at 32 hours.
(5) Dead at 24 hours.
(6) Dead at 24 hours.
(7) Dead at 24 hours.
(8) Dead at 36 hours.
(9) Dead at 36 hours.

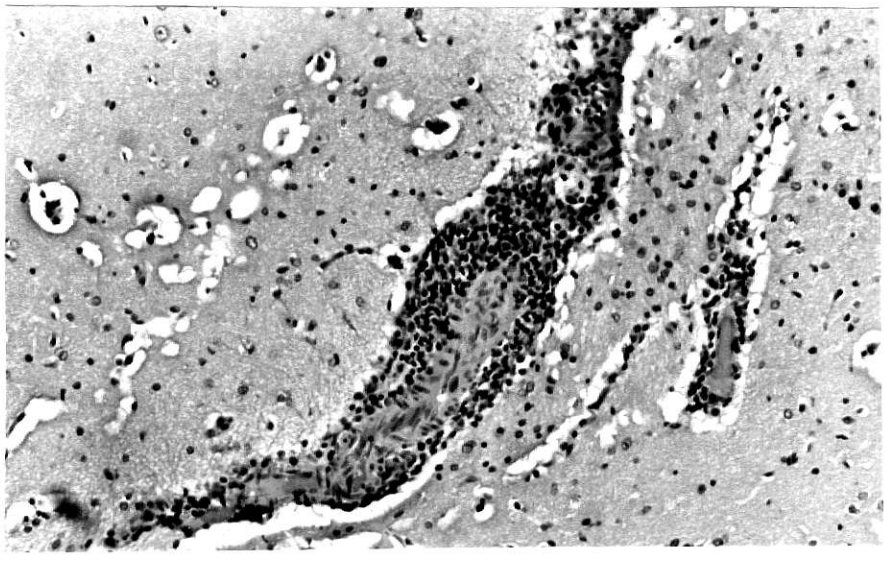
EXPLANATION OF PLATE I

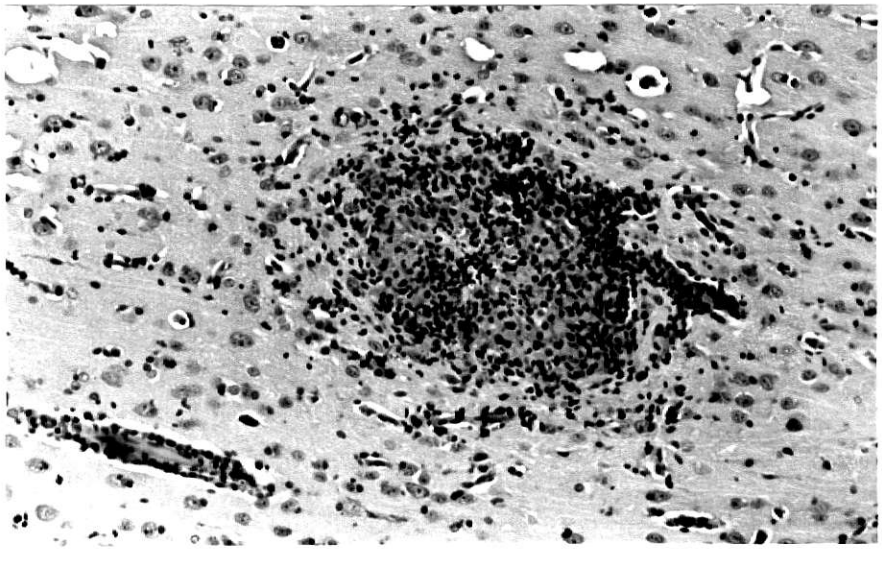
Photomicrographs (x 100) of brain lesions in the rabbit as a result of intravenous inoculation of *Haemophilus somnus* (n. sp.)

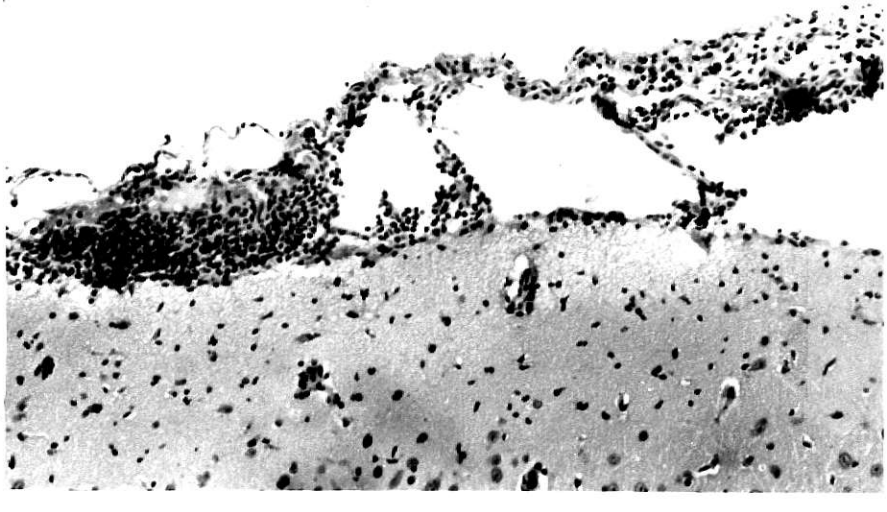
Fig. 1. Perivascular cuffing of mononuclear cells and hyperplasia of the reticular cells of the vascular adventitia.

Fig. 2. Necrotic foci.

Fig. 3. Meningitis characterized by a mononuclear cellular infiltration.







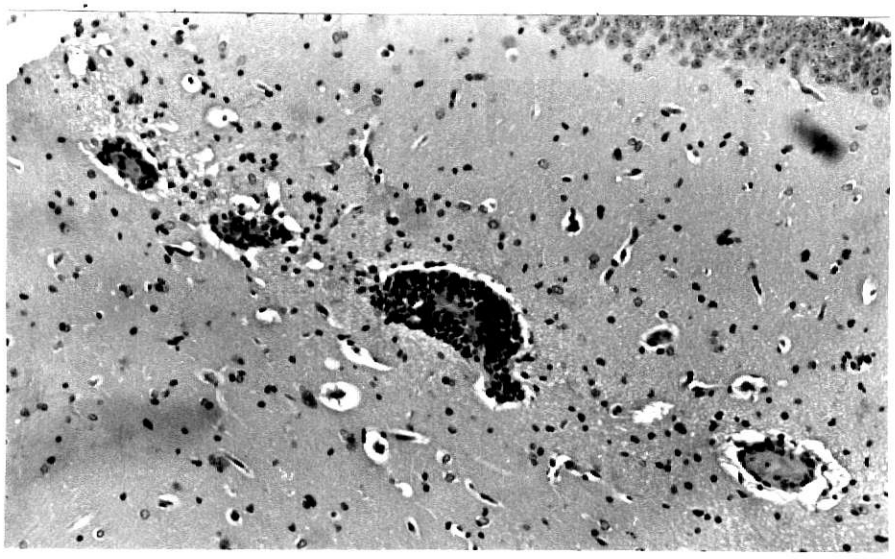
EXPLANATION OF PLATE II

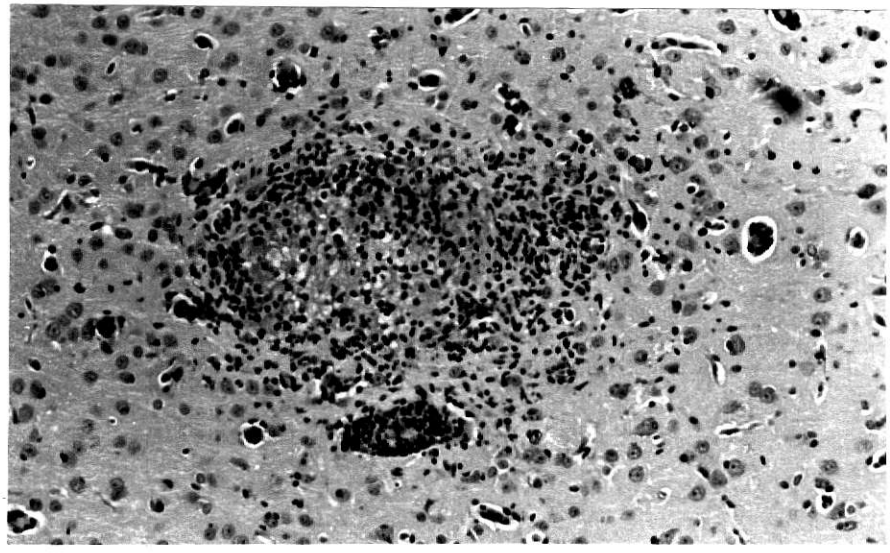
Photomicrographs (x 100) of brain lesions in the rabbit as a result of conjunctiva inoculation of *Haemophilus somnus* (n. sp.).

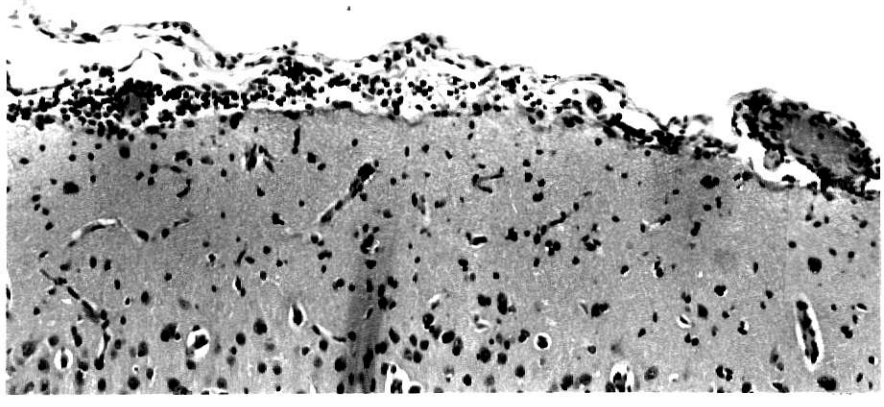
Fig. 1. Perivascular cuffing of mononuclear cells and hyperplasia of the reticular cells of the vascular adventitia.

Fig. 2. Necrotic foci.

Fig. 3. Meningitis characterized by a mononuclear cellular infiltration.







EXPERIMENTAL INFECTION OF RABBITS WITH *HAEMOPHILUS SOMNUS* (NEW SPECIES)

by

WILLIAM RODGER RAPP

B.S., Kansas State University, 1964
D.V.M., Kansas State University, 1966

AN ABSTRACT OF A MASTER'S THESIS

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MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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ABSTRACT

Reports of an encephalopathy in feedlot cattle, in which neuropathologic studies suggested a morphologic diagnosis of thromboembolic meningoencephalitis, indicated a widespread occurrence of the disease. The disease had also been reported in cattle grazing pastures. Microbiologic examinations of brain tissues from affected animals revealed a small, Gram-negative, pleomorphic bacterium which has been named Haemophilus somnus (n. sp.).

This research was designed to test the reliability of the rabbit as a laboratory animal for pathogenic studies of this organism. The research objectives were: (1) to determine and characterize the gross and microscopic lesions of infection in rabbits; (2) to determine the method of inoculation and the infective dose necessary to reliably reproduce signs and lesions of encephalitis; (3) to determine alterations of specific hemogram and clinical parameters; (4) to test for endotoxic manifestations of the microorganism; and (5) to investigate the effects of serial passage of the microorganism in rabbits.

Microscopic lesions of meningoencephalitis were produced in the rabbit by intravenous and by conjunctive inoculation of the bacteria. The lesions were characterized by a mononuclear cell infiltration of the meninges and perivascular areas, glial nodule formation, necrosis, satellitosis, neuronophagia, and hyperplasia of the reticular cells of the

vascular adventitia. Microscopic lesions in the brain were not produced by oral or by nasal inoculation. Gross lesions of the brain were absent in all test rabbits. Significant non-neural lesions were not observed either grossly or microscopically.

The dosage of a standardized inoculum required to produce microscopic lesions by intravenous inoculation of H. somnus (n. sp.) was determined to be 300,000 to 900,000 organisms per kilogram of body weight for a 10 - 12 week old New Zealand white rabbit. Higher dosages produced widely variable results. The dosage required for the conjunctiva method was not specific.

Hemogram alterations following intravenous inoculation were consistently uniform. An initial slight leukopenia was followed by a marked leukocytosis. The leukocytosis was initially pseudoesinophilic and later, lymphocytic.

Clinical manifestations in test rabbits varied considerably and anorexia and dyspnea were the only consistent signs.

Endotoxic-like properties of H. somnus (n. sp.) were confirmed by production of the localized and generalized Shwartzman phenomena utilizing a bacterial suspension, a heated bacterial suspension, and a filtrate of a bacterial suspension.

The effect of serial passage of H. somnus (n. sp.) in rabbits resulted in a greater clinical response, but no increase in the incidence of encephalitis.

It is concluded that the rabbit has limited use as an experimental animal for studies with H. somnus (n. sp.) because of erratic response. The rabbit could be utilized for studies involving pathogenesis of the microscopic lesions in the brain, and for evaluation of endotoxic properties of H. somnus (n. sp.).