

HAEMOPHILUS SOMNIFER (N. SP.) STUDIES IN SHEEP

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Abstract

INTRODUCTION

A disease syndrome described primarily as an encephalitis was first reported in Colorado by Griner et al., (1956). The disease involved both feedlot and pastured cattle. This was followed by similar reports involving only feedlot cattle in several states (California, Kennedy et al., 1960; Kansas, Weide et al., 1964 and Bailie et al., 1966; and Illinois, Case et al., 1965). Panciera et al., (1968) stated the disease occurred in both feedlot and pastured cattle in Oklahoma. Lesions involving the respiratory tract and the cardiovascular system were reported by Griner et al., (1956); Kennedy et al., (1960); Weide et al., (1964) and Bailie et al., (1966). Panciera et al., (1968) described the disease as a septicemia and not primarily an encephalitis.

Bacteriologic findings of involved animals have varied considerably. Griner et al., (1956) stated a gram-positive cocci was the predominant organism. Kennedy et al., (1960) isolated a Haemophilus-like organism as did Case et al., (1965), and Panciera et al., (1968). Bailie et al., (1966) isolated a similar organism and called it an Actinobacillus actinoides-like organism. Bailie (1969) has since proposed the name Haemophilus somnifer (n. sp.).

Experimental reproduction of the disease has been difficult but it has been reproduced in cattle by Kennedy et al., (1960); Panciera et al., (1968) and Young and Hoerlein (1970). Panciera et al., (1968) reproduced the disease by an intra-carotid inoculation of the Haemophilus-like organism. Young and Hoerlein (1970) found the disease could be reproduced by inoculation of septic brain suspensions or of cultures of the Haemophilus-like organism administered with suspensions of brain tissue. Kennedy et al., (1958) and Young and Hoerlein (1970) stated sheep were not susceptible.

Diseases caused by a similar organism have been reported in sheep. Kennedy et al., (1958) isolated Haemophilus agni from a septicemic disease in lambs. Roberts (1956) isolated an organism from a ewe which resembled Haemophilus but named it Histophilus ovis. Kater et al., (1962) isolated an organism indistinguishable from Histophilus ovis which caused suppurative synovitis and pyemia in lambs. Hughes et al., (1964) isolated a similar organism from 4 cases of fibrinous synovitis. Hartley and Kater (1964) recorded findings similar to Kater et al., (1962). Dennis (1966) isolated a similar organism from 7 neonatal lamb deaths.

The purpose of this study was to establish the susceptibility of sheep to Haemophilus somnifer (n. sp.) administered

by various routes and to endeavor to enhance the susceptibility of sheep to this organism.

Paper 1: Haemophilus somnifer (n. sp.) Studies in Sheep

INTRODUCTION

A disease syndrome described primarily as an encephalitis of feedlot cattle was reported in Colorado in 1956⁶, California in 1960¹⁰, Kansas in 1964¹⁸ and 1966², Illinois in 1965³, and Oklahoma in 1968¹². Numerous authors reported isolating a gram-negative, pleomorphic bacteria which was described as a Haemophilus-like organism.^{2,3,10,12} The name Haemophilus somnifer (n. sp.) was proposed in 1969.¹

Claims of experimental reproduction of the disease in cattle have been reported.^{10,12,19} Investigators have stated that sheep were apparently not susceptible but gave no details.^{10,19} Similar organisms, however, have been isolated from sheep.^{4,7,8,9,11,13}

This paper reports studies with Haemophilus somnifer (n. sp.) in sheep.

MATERIALS AND METHODS

A total of 46 Western and Southdown ewes from 1 to 7 years of age were used in this study. Temperatures were taken and recorded twice a day. The general appearance, appetite and eliminations were observed.

Preparation of Inoculum

Cultures for inoculation were prepared from brain tissue* from which Haemophilus somnifer (n. sp.) had previously been isolated. The brain tissue was cultured on 10% sheep blood agar** and incubated at 37 C in a 10% CO₂ atmosphere. The cultures used had the following characteristics: gram-negative, growth under microaerophilic conditions (10% CO₂), negative aerobic growth, catalase-negative, production of acid fermentation in serum enriched oxidation-fermentation media, oxidase positive and growth negative on MacConkeys agar. Plates were incubated for 72 hours and the growth washed off with 1% nutrient broth. The bacterial concentration was diluted to $6-8 \times 10^9$ cells per ml calculated by a Bausch and Lomb Spec 20.***

Bordetella bronchiseptica, tranquilizer, para-influenza-3, infectious bovine rhinotracheitis, and endotoxin were used to enhance the susceptibility of sheep to H. somnifer (n. sp.) The controls were given sterile 1% nutrient broth. Inoculation procedures are given in Table 1.

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

** Difco Laboratories, Detroit, Michigan.

*** Bausch and Lomb, Rochester, New York.

TABLE 1 -- Inoculation Procedure for Studies with Haemophilus somnifer (n. sp.) in Sheep

GROUP NO.	SHEEP NO.	INOCULATION ROUTE AND DOSAGE
I	01,02	2 ml inoculum IV
	04,05	2 ml inoculum IV -- Repeated in 24 hr
	06,07	2 ml inoculum intratracheally
	09,10	1 ml inoculum into conjunctival sac of each eye
	03	Control -- 2 ml broth IV
	08	Control -- 2 ml broth intratracheally
II	11,13,14	2 ml inoculum intracarotidally
	12	Control -- 2 ml broth intracarotidally
III	16,17,18	2 ml inoculum intratracheally followed 24 hr later by 2 ml inoculum IV
	15	Control -- 2 ml broth intratracheally followed 24 hr later by 2 ml broth IV
IV	19,20,21	2 mg Thorazine [*] /lb IV followed 2 hr later by 2 ml inoculum IV
	22	Control -- 2 mg Thorazine [*] /lb IV followed 2 hr later by 2 ml broth IV
V	23,24,26	25 doses PI ₃ vaccine ^{**} intratracheally followed 72 hr later by 2 ml inoculum intratracheally
	25	Control -- 25 doses PI ₃ vaccine ^{**} intratracheally followed 72 hr later by 2 ml broth intratracheally

* Pitman-Moore, Inc., Fort Washington, Pennsylvania.

** Dellen Laboratories, Omaha, Nebraska.

TABLE 1 -- Continued

GROUP SHEEP NO. NO.	INOCULATION ROUTE AND DOSAGE
VI 27,28,29	500 micrograms endotoxin* IV followed 30 min later by 1 ml inoculum IV
30	Control -- 500 micrograms endotoxin* IV followed 30 min later by 1 ml broth IV
VII 31,32,34	1 ml inoculum intracarotidally
33	Control -- 1 ml broth intracarotidally
VIII 36,37,38	10 doses IBR vaccine** IM followed 24 hr later by 2 ml inoculum IV
35	Control -- 10 doses IBR vaccine** IM followed 24 hr later by 2 ml broth IV
IX 40,41,42	5 ml <u>Bordetella bronchiseptica</u> *** intranasally (19.2×10^9 cells/ml) followed one week later by 2 ml inoculum intranasally
39	Control -- 5 ml <u>Bordetella bronchiseptica</u> *** intranasally (19.2×10^9 cells/ml) followed one week later by 2 ml broth intranasally
X 43,44	.5 ml inoculum into carpus and .5 ml broth into opposite carpus
45,46	.5 ml inoculum into stifle and .5 ml broth into opposite stifle

* Bacto Lipopolysaccharide Salmonella typhimurium, Difco Laboratories, Detroit, Michigan.

** Dellen Laboratories, Omaha, Nebraska.

*** American Type Tissue Collection, Rockville, Maryland.

Sheep surviving the 10 day post-inoculation (PI) period were euthanatized with Barb-Euthol*, except those in Group X which were euthanatized 28 days PI. Brain, liver, lung and stifle joint were cultured on 10% sheep blood agar. The plates were incubated in a 10% CO₂ atmosphere at 37 C for 5 days before being discarded as negative. Sections of cerebrum, thalamus, pons, cerebellum, medulla, lung, liver, kidney, adrenal and stifle joint were fixed in 10% buffered neutral formalin, sectioned at 5 microns and stained with hematoxylin and eosin. When indicated sections were stained with Brown and Brenn modification of the Gram stain (Thompson and Hunt, 1966).

RESULTS

Clinical signs, significant pathological changes and bacteriological findings are summarized in Table 2.

* Haver-Lockhart Laboratories, Shawnee Mission, Kansas.

TABLE 2 -- Results of Haemophilus somnifer (n. sp.) Studies in Sheep

Group No.	Sheep No.	Clinical Signs	<u>Significant Pathological Changes</u>		Bacteriological Findings
			Gross	Microscopic	
I	01, 02	Depressed, anorexic for 48 hr.	None	None	Negative
	04, 05	Depressed, weak, dyspneic; died 8 hr following 2nd inoculation.	Hydrothorax, pul- monary edema. Subcutaneous, sub- endocardial, sub- epicardial, peri- renal ecchymotic & suffusion hemorrhages.	Subepicardial & subendocardial hemorrhages, pul- monary edema. Necrosis of epi- thelium of proxi- mal tubules of kidneys.	Negative
	06, 07	Depressed, anorexic for 12 hr.	None	None	Negative
	09, 10	None	None	None	Negative
	03, 08 (Controls)	None	None	None	Negative
II	11, 13, 14	Diarrhea, dyspnea; died 13, 15 & 12 hr post inoculation (PI) respectively.	Pulmonary edema, hydrothorax. Subendocardial hemorrhages.	Pulmonary conges- tion & intersti- tial edema. Focal necrosis & 2 throm- bosed arterioles in pons of 11.	Contaminated. <u>H. somnifer</u> (n. sp.) re- covered from brain of 13 & 14.
	12 (Control)	None	None	None	Negative

TABLE 2 -- Continued

Group Sheep No.	Clinical Signs	Significant Pathological Changes		Bacteriological Findings
		Gross	Microscopic	
III 16, 17, 18	Diarrhea, dyspnea; died 5, 8 & 9 hr PI, respectively.	Pulmonary edema, hydrothorax. Petechial hemor- rhages in abo- masal mucosa.	Pulmonary conges- tion & intersti- tial edema. Necro- sis of epithelium of proximal tubules of kidneys in 18.	<u>H. somnifer</u> (n. sp.) re- covered from lung of 18.
15 (Control)	None	None	None	Negative
IV 19, 20, 21	Sternal recumbency for 24 hr. Sheep 20 died 24 hr PI.	Hydrothorax, Sub- epicardial, sub- endocardial, ecchymotic & suffusion hemor- rhages in 20.	Congestion of brain & subendo- cardial hemor- rhages in 20.	<u>H. somnifer</u> (n. sp.) re- covered from lung of 20.
22 (Control)	Sternal recumbency for 24 hr.	None	None	Negative
V 23, 24, 26	Non-productive cough in 26 for 48 hr PI.	None	None	Negative
25 (Control)	None	None	None	Negative
VI 27, 28, 29	Depressed, anorexic for 48 hr. Blood from right nostril of 29 for 48 hr PI.	Thrombosed left jugular veins with adjacent abscesses in 28 & 29.	Granulation tissue necrotic debris & neutrophils in thrombosed jugulars & adjacent abscesses. 28 & 29.	<u>H. somnifer</u> (n. sp.) re- covered from abscesses in 28 & 29.

TABLE 2 -- Continued

Group Sheep No.	Clinical Signs	Significant Pathological Changes	Bacteriological Findings
No.		Gross	Microscopic
VI (Continued)			
30 (Control)	Depressed, anorexic for 24 hr PI.	None	None
			Negative
VII 31, 32, 34			
33 (Control)	Depressed, anorexic for 24 hr PI.	None	None
			Negative
	None	None	Negative
VIII 36, 37, 38			
35 (Control)	Depressed, anorexic for 36 hr PI.	None	None
			Negative
	None	None	Negative
IX 40, 41, 42			
39 (Control)	None	None	None
			Negative
	None	None	Negative
X 43, 44, 45, 46			
	1-30 Temperature elevation for 24 hr. 45 & 46 lame for 24 hr, 44 for 10 days & 43 for 16 days in inoculated legs.	None	Fibrous capsule of joint in 43 infiltrated with plasma cells & neutrophils.
			Negative

DISCUSSION

These experiments indicated that H. somnifer (n. sp.) elaborates a toxin. Sheep inoculated intravenously, intratracheally or intracarotidally developed signs compatible with those described for endotoxins derived from gram-negative bacteria.¹⁶ Sheep dying following two inoculations 24 hours apart (04, 05, 16, 17, 18) had lesions similar to those described for the generalized Shwartzman reaction.¹⁵ The findings in sheep in group II, which were inoculated intracarotidally, should be disregarded due to contaminants. Sheep in group VII, inoculated intracarotidally with 1 ml ($6-8 \times 10^9$ cells), developed signs compatible with endotoxin shock but did not die. The death of sheep 20 which received 3.3 gm thorazine may have resulted from a combination of endotoxin shock and a Thorazine^R toxicity.

The various agents administered to enhance the susceptibility of sheep to H. somnifer (n. sp.) were ineffective. A phenothiazine derivative (Thorazine^R) was given to lower the blood pressure and to slow the circulation. Endotoxin was administered to lower the blood-brain barrier and to partially block the reticulo-endothelial system. Infectious bovine rhinotracheitis (IBR) vaccine was given as previous observations indicated that IBR may be a predisposing factor in H. somnifer

(n. sp.) infection. Para-influenza 3 vaccine was given to create a pneumonic condition and to encourage growth of H. somnifer (n. sp.) in the affected lungs. Bordetella bronchiseptica was administered in an attempt to knock out the receptors in the nasal mucosa and thus allow entry of H. somnifer (n. sp.) to the respiratory and/or central nervous system.

The fact H. somnifer (n. sp.) was isolated inconsistently from sheep dying acutely raises the question whether sheep have a mechanism or an enzyme which quickly destroys the organisms after inoculation or a highly efficient reticulo-endothelial system. The only isolation of H. somnifer (n. sp.) 10 days PI was from the perijugular abscesses that developed in sheep 28 and 29. This probably resulted from the inoculum being partially injected perivascularly. H. somnifer (n. sp.) was not recovered from tissues of any sheep living longer than 24 hours except for sheep 28 and 29.

There is a possibility the organism used in this study¹ is not the same as described by other authors^{3,5,10,12,14}, although most of the characteristics and biochemical reactions were similar. The pathogenicity of H. somnifer (n. sp.) for cattle is debatable. One group of researchers could only reproduce the characteristic lesions of thromboembolic

meningoencephalitis (TEME) when 50 ml of blood from febrile animals was collected in 100 ml of brain-heart-infusion agar, incubated, and inoculated into test animals.¹⁰ A later report of experimental lesions in two cattle given H. somnifer (n. sp.) intracarotidally did not state whether other routes of inoculation were attempted.¹² Other workers reproduced the disease by the inoculation of septic brain suspensions or of cultures administered together with suspensions of brain tissue.¹⁹ Inoculation of pure cultures of H. somnifer (n. sp.) alone did not produce the intracranial infarctive lesion. All these reports leave doubt as to whether or not H. somnifer (n. sp.) is a primary pathogen of cattle.

Due to the number of sheep used, the various routes of inoculation and the attempts to enhance the susceptibility by various means, it appears that H. somnifer (n. sp.) alone is not pathogenic for sheep. It is entirely possible that H. somnifer (n. sp.) may participate in the natural disease process in concert with some other agents or chain of events not yet recognized.

SUMMARY

Forty-six adult sheep were inoculated by various routes with Haemophilus somnifer (n. sp.), which is considered to be

responsible for an encephalitic and septicemic disease in cattle. Various agents were used to enhance the susceptibility of sheep to the affects of H. somnifer (n. sp.). Deaths were apparently due to endotoxicity or the generalized Shwartzman reaction. Sheep which survived 24 hours or longer PI, with the exception of two sheep which developed abscesses at the inoculation site, had no significant gross or microscopic lesions and H. somnifer (n. sp.) was not recovered. It is concluded from these studies that H. somnifer (n. sp.) is not pathogenic for sheep.

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APPENDIX

REVIEW OF LITERATURE

Infectious embolic meningoencephalitis (TEME) was first reported by Griner et al. in 1956 when they reported on the disease in 36 cattle. The age of the affected animals varied from under 1 year to over 3 years with the majority being 1 - 2 years of age. Thirty of the cattle were from feedlots and 6 were on pasture. Typical necropsy findings were multiple reddish-brown foci of necrosis and inflammation in the brain. Similar lesions were found in all areas of the brain with the cerebral hemispheres most frequently affected. Eleven out of 21 head had lesions in the respiratory tract which included necrotic laryngitis, tracheitis, pharyngitis, rhinitis, broncho-pneumonia and pleuritis. Ten of the 21 had cardiovascular lesions and 5 had lesions of the urinary system. The histopathological lesions were widely distributed in the gray and white matter of the brain and the meninges. Gram-positive cocci were the predominate organisms found. Thrombosed veins, lymphatics and capillaries were consistently observed.

Kennedy et al., (1960) reported on an outbreak of infectious meningoencephalitis in a feedlot caused by Haemophilus-like organisms. The mortality rate was approximately 2.5%. The characteristic lesions consisted of multiple or single hemorrhagic foci in any part of the brain. These

lesions were associated with thrombosed blood vessels. Also less conspicuous lesions such as excess pericardial fluid, synovial fluid and hemorrhages in the serosa of the heart were recorded. Histologically there was a bacterial vasculitis which led to thrombosis and subsequent septic areas of infarction involving the brain. Numerous capillaries and venules were occluded by masses of bacteria.

The bacterial isolate of Kennedy et al., (1960) killed mice when injected intraperitoneally and intracerebrally. Two out of 3 guinea pigs were killed by the inoculum and 1 of 2 rabbits inoculated intravenously (IV) also died. The disease was produced experimentally in 5 calves by collecting 50 ml of blood from a naturally occurring case in 100 ml of BHI media. The animals died 24 - 96 hours after the IV injection of this culture with the characteristic signs and lesions observed. Later 2 calves were resistant to a similar intravenous injection. Two other calves were found resistant to intranasal and intraocular administration and a subsequent IV injection. Two lambs challenged intravenously failed to sicken. Approximately three times as many subclinical as clinical infections were estimated in this particular outbreak.

Haemophilus spp was suggested for the organisms with a statement that it resembled Haemophilus agni (Kennedy et al., 1958).

Weide et al., (1964) reported infectious embolic meningo-encephalitis as the encephalitic condition most commonly encountered in Kansas. Cattle which developed the disease had usually been on feed 40 - 110 days. These workers reported the disease had not been observed in adult cattle or non-feedlot calves. The gross and histological lesions reported were similar to those reported by Griner et al., (1956) and Kennedy et al., (1960). Gross lesions of previous or concurrent respiratory problems were observed in approximately 75% of the cases. The lesions ranged from well healed, repaired pneumonia with pleural adhesions, to tracheitis, pleuritis, pneumonitis and consolidation of large portions of the lungs. These findings are similar to those of Griner et al., (1956).

In Illinois, Case et al., (1965) reported 5 cases in a lot of 120 head. Necropsy findings consisted of numerous foci of encephalomalacia in the cerebral hemispheres and subendocardial ecchymotic hemorrhages. Microscopically thrombosed vessels, usually veins, were found in the meninges. During March, 1965, 3 additional cases were diagnosed by the same workers and a Haemophilus-like organism similar to the one described by Kennedy et al., (1960) was isolated from the brain and other internal organs.

Pancier et al., (1968) described a septicemia of cattle caused by a Haemophilus-like organism. The disease occurred in both feedlot cattle and cattle on pasture which concurred with the findings of Griner et al., (1956). In their description the disease was separated into three clinical syndromes encompassing involvement of the central nervous system, the respiratory system, and the joints. The peracute syndrome was of short duration which primarily involved the central nervous system. The acute syndrome involved primarily the respiratory system and the subacute or chronic syndrome involved primarily the joints. The three syndromes commonly overlapped. The gross lesions observed were similar to those described by other investigators (Griner et al., 1956; Kennedy et al., 1960; Weide et al., 1964; Case et al., 1965; and Bailie et al., 1966). Gross and histologic lesions occurred throughout the body and all appeared related to vascular injury. A polyarthrititis consistently involved nearly all diarthrodial joints. Four of 12 animals necropsied in the laboratory had laryngitis which concurred with the findings of Griner et al., (1956). Classical infarcts were observed occasionally in the kidney. Histologically there was an intense vasculitis with or without infarction and a cellular response of neutrophils. Congestion and edema of the synovial

membranes with aggregation and margination of intravascular neutrophils were also reported. A gram-negative organism ranging in morphology from coccoid to diplobacillary was irregularly isolated from brain homogenates, cerebrospinal, pericardial, and synovial fluids. The organism was most successfully isolated by inoculating the infectious material into 5 - 7 day chick embryos followed by transfer to bovine blood agar plates which were incubated at 37 C in a 5-8% CO₂ atmosphere. Organisms were isolated from a cow by inoculation of brain tissue into chick embryos and transferred twice on blood agar. An 18-hour culture was washed off the plates with BHI broth and diluted to a concentration of 8.0×10^7 /ml. Intraperitoneal injection of 1 ml of this inoculum did not produce disease in guinea pigs. A 0.5 ml intravenous injection did not produce disease in mature rabbits. Mice given 0.1 ml intracranially developed signs within 24 hours. The organism was reisolated and histologically a severe suppurative meningoencephalitis was present. Two cattle were inoculated into the carotid with 5 ml of the same suspension. The animals were killed in extremis 50 and 52 hours post exposure. The gross and histological lesions closely resembled those of the spontaneous disease. One of 2 cattle exposed intra-tracheally after scarification of the mucosa developed a

transient elevation of body temperature 3 and 5 days post exposure. Panciera et al., (1968) concluded the disease was a septicemic one and the term infectious embolic meningoencephalitis was obsolete and inaccurate especially since there was no evidence of true emboli.

Young and Hoerlein (1970) described attempts of experimental reproduction of thromboembolic meningoencephalitis by using suspensions of septic brain lesions and pure cultures of bacterial isolates with and without certain tissue adjuvants. Calves, sheep, rabbits, guinea pigs and mice were exposed by various routes. The typical lesions were reproduced only in calves and only by the inoculation of septic brain suspensions or of culture administered together with suspensions of brain tissues. Inoculation of pure cultures of the organisms alone caused a septicemic disease in which the classical intracranial infarctive lesions were minimal or absent.

In 1966, Bailie et al., reported on infectious thromboembolic meningoencephalomyelitis or sleeper syndrome in Kansas. The necropsy lesions reported were consistent with those reported by previous investigators (Griner et al., 1956; Kennedy et al., 1960; Weide et al., 1964; and Case et al., 1965). An additional consistent finding reported was increased synovial fluid in the major limb joints. A small

gram-negative bacillus was isolated from approximately one-fourth of the 78 cases diagnosed as infectious thromboembolic meningoencephalomyelitis. The organism was similar to the Haemophilus-like organism previously reported (Kennedy et al., 1960 and Case et al., 1965). The organism was reported to be similar but not identical to Actinobacillus actinoides. Bailie et al., (1966) stated "Because of the distinct differences as well as similarities, the organism was tentatively called an 'Actinobacillus actinoides-like organism' until such time as a definite classification can be made."

Gossling (1966) described the bacteria isolated from lesions of embolic meningoencephalitis in cattle. Small, clear, non-hemolytic, shiny, convex colonies which later became lemon-yellow were described. The organism would not grow in an aerobic environment. Gram staining revealed a small gram-negative bacilli of varying length and constant width. The organism had the ability to produce cytochrome oxidase and failed to produce catalase.

Shigidi and Hoerlein (1970) also characterized the Haemophilus-like organism of infectious thromboembolic meningoencephalitis. Cystine heart agar enriched with 10% bovine blood and 0.5% yeast extract was found to be the best culture medium. Defibrinated bovine blood was superior to lapine

blood as a culture medium supplement and much superior to ovine blood. The Haemophilus-like organism would not grow in liquid medium on primary isolation but could be adapted to them by serial passage on blood agar plates. These investigators also observed a cross agglutination between Haemophilus agni, Bordetella bronchiseptica and the Haemophilus-like organism of TEME.

A total of 345 suspect cases of bovine encephalitis were examined by Bailie (1969) and 193 were TEME. One type of microorganism was isolated from 47 of 121 cases in which a histopathologic diagnosis of thromboembolic meningoencephalitis was made. This organism was similar to the isolate of Kennedy et al., (1960), Bailie et al., (1966) and Gossling (1966). Bailie (1969) proposed the name Haemophilus somnus (n. sp.) for the organism and this was later changed to H. somnifer (n. sp.).

Possible Related Diseases Occurring in Sheep

Kennedy et al., (1958) reported a disease syndrome in sheep which closely resembles infectious thromboembolic meningoencephalitis of cattle and is caused by a similar organism. The disease affected 6 - 7 month old lambs. Necropsy revealed multiple hemorrhages throughout the carcasses. Pale foci 0.5 - 1 mm in diameter were observed in the liver of

acute cases. Lambs that survived 24 - 36 hours had larger foci surrounded by a hemorrhagic ring. A fibrino-purulent arthritis was a frequent finding. Lymph nodes were edematous and hemorrhagic. Pulmonary congestion and edema were observed. A basilar meningitis was noticed in some cases. Histologically there was a disseminated bacterial thrombosis which lead to a severe focal vasculitis. These lesions were best seen in the liver. A saline suspension was prepared from 24-hour cultures on blood agar plates and from broth cultures. This suspension killed lambs when given intraperitoneally and intravenously. No disease was produced when this suspension was given intraconjunctivally, intranasally or orally. Intratracheal injections produced disease in 1 out of 2 lambs in 72 hours. The other lamb failed to sicken.

Roberts (1956) isolated a small gram-negative bacterium from the udder of a ewe with severe mastitis. The organisms would produce acute mastitis when injected into the udder of dry ewes. He stated the organism was similar to Haemophilus but suggested the name Histophilus ovis.

Kater et al., (1962) reported a gram-negative pleomorphic organism indistinguishable from Histophilus ovis (Roberts, 1956) which caused a suppurative synovitis and pyemia in lambs. The affected lambs, 1 - 6 weeks of age, had swollen joints

which contained large quantities of firm, rubbery, greenish-grey fibrin like material. The articular surfaces were never involved, the synovial membranes being the diseased tissue. A few lambs had foci in the liver and kidneys. The disease was experimentally reproduced by intravenous injection of cultures. Hartley and Kater (1964) recorded similar findings of Kater et al., (1962).

Hughes et al., (1964) isolated a small gram-negative pleomorphic coccobacillus from 4 cases of fibrinous synovitis. The bacterium had features in common with Histophilus ovis (Roberts, 1956), Haemophilus agni (Kennedy et al., 1958) and the organism described by Kater et al., (1962).

Dennis (1966) recovered a gram-negative pleomorphic bacillus from an aborted fetus and an unidentified gram-negative pleomorphic rod from 7 neonatal lamb deaths. The organism appeared to conform to the description of Kater et al., (1962) and Hartley and Kater (1964).

Endotoxins

Thomas (1954) described endotoxin as phosphorous-containing, polysacchride-protein-lipid complexes in the intact cells of a wide variety of gram-negative microorganisms, or liberated into culture media during autolysis of bacteria. Endotoxins isolated from numerous unrelated species of

gram-negative organisms appear to be similar in chemical structure and properties. Endotoxins from varying sources produce a syndrome of stereotyped physiological and pathological reactions.

Thomas (1958) listed the following as responses to a single injection of endotoxins:

- a. Fever with hypothermia in some species.
- b. Leukopenia followed by leukocytosis.
- c. Peripheral vasoconstriction and vasomotion.
- d. Shock.
- e. Depletion of liver glycogen.
- f. Hyperglycemia.
- g. Increased adhesiveness of polymorphonuclear leukocytes.
- h. Heparin-precipitability of fibrinogen.
- i. Impeded phagocytosis by reticuloendothelial cells.
- j. Augmentation of antibody response to protein antigens.
- k. Abortion.
- l. Hemorrhagic necrosis in malignant tumors.
- m. Disturbances of the reactivity of terminal blood vessels to epinephrine.

He also reported the range of susceptible host varied from 10-day-old chick embryos to man.

In his 1954 review Thomas discussed the local and generalized Shwartzman reaction. The localized Shwartzman reaction was produced by giving a "preparing" injection of endotoxin intradermally followed in 18 - 24 hours by a "provoking" injection intravenously. Within 3 hours after the latter injection, hemorrhagic necrosis occurred at the prepared skin site. With the generalized Shwartzman reaction, both preparing and provoking injections of endotoxin were given intravenously. The resultant lesions consist of extensive hemorrhagic necrosis in many internal organs. The most characteristic lesion and the one which identifies the reaction was bilateral cortical necrosis of the kidneys.

Kuida et al., (1958) reported an increase in total pulmonary vascular resistance due to endotoxins. The increased resistance was found to be due to venous constriction and subsequent pulmonary edema from the resultant increase in hydrostatic pressure.

MATERIALS AND METHODS

Sheep

A total of 46 Western and Southdown ewes ranging from 1 to 7 years of age were used in this experiment. Forty-two of the sheep were purchased as 1 unit and kept together in the same facility until transit to indoor pens for pretesting and inoculation. The remaining 4 sheep were purchased separately. The sheep were placed in air-conditioned quarters and the body temperature checked twice daily for 3 days prior to inoculation. The general appearance, appetite, and eliminations were also observed. The sheep were fed pelleted alfalfa ad libitum with access to water at all times. Following inoculation temperatures and signs were recorded twice a day until death or euthanasia of the animals. Sheep that did not die were euthanatized and necropsied 10 days post inoculation (PI) except group X which was euthanatized 28 days PI. The temperatures were recorded twice a day for 10 days in group X and the animals observed daily for the remaining 18 days.

Preparation of the Inoculum

The cultures used for inoculation were prepared from brain tissue* from which Haemophilus somnifer (n. sp.) had

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

previously been isolated. The brain tissue was cultured on blood agar base* containing 10% sheep blood. The cultures used had the following characteristics: gram-negative, growth under microaerophilic conditions (10% CO₂), negative growth in an aerobic atmosphere, catalase-negative, production of acid fermentation in OF media with serum, oxidase-positive, and negative MacConkeys agar growth. The colonies were washed from the medium with 1% nutrient broth after incubating for 72 hours at 37 C. The concentration of organisms was diluted to $6-8 \times 10^9$ cells per ml calculated by the use of a Bausch and Lomb Spec 20.** The inoculum was used within 1 hour of harvesting.

Inoculation Procedure

The sheep were randomly divided into 10 groups with group I containing 10 sheep and the remaining groups containing 4 each.

GROUP I:

Three inoculation routes were used. Sheep 01, 02, 04 and 05 received 2 ml of inoculum IV followed by a second IV inoculation in sheep 04 and 05 24 hours later. Sheep 06 and 07

* Difco Laboratories, Detroit, Michigan.

** Bausch and Lomb, Rochester, New York.

received 2 ml of inoculum intratracheally by injection directly into the trachea at the thoracic inlet. Sheep 09 and 10 received 1 ml in the conjunctival sac of each eye. Sheep 03 and 08 served as in contact controls. Control sheep 03 received 2 ml of 1% sterile nutrient broth IV and 08 received 2 ml of 1% sterile nutrient broth intratracheally.

GROUP II:

The right carotid artery was exposed. Sheep 11, 13 and 14 were given 2 ml of inoculum and 12 received 2 ml of 1% sterile nutrient broth in the exposed carotid.

GROUP III:

Sheep in this group were inoculated directly into the trachea at the level of the thoracic inlet. Sheep 15 received 2 ml of 1% sterile nutrient broth and 16, 17 and 18 received 2 ml of inoculum. Twenty-four hours following the intratracheal inoculation, sheep 16, 17 and 18 were given 2 ml of culture IV and sheep 15 received 2 ml of 1% sterile nutrient broth IV.

GROUP IV:

Two mg of Thorazine* per pound of body weight was given IV followed in 2 hours by the inoculum. Sheep 19, 20 and 21

* Pitman-Moore, Inc., Fort Washington, Pennsylvania.

were given 2 ml of inoculum IV and sheep 22 received 2 ml of 1% sterile nutrient broth.

GROUP V:

Each animal was given intratracheally 25 doses of Para-Influenza-3 (PI₃) vaccine* in 5 ml of sterile diluent.

Seventy-two hours later sheep 23, 24 and 26 received 2 ml of inoculum intratracheally and 25 received 2 ml of 1% sterile nutrient broth intratracheally.

GROUP VI:

Five hundred micrograms of endotoxin** was given in the right jugular vein. Sheep 27, 28 and 29 received 1 ml of inoculum in the left jugular vein and 30 received 1 ml of 1% sterile nutrient broth 30 minutes after the administration of the endotoxin.

GROUP VII:

Sheep 31, 32 and 34 were given 1 ml of inoculum and 33 was given 1 ml of 1% sterile nutrient broth into the carotid artery.

* Dellen Laboratories, Omaha, Nebraska.

** Bacto Lipopolysaccharide Salmonella typhimurium, Difco Laboratories, Detroit, Michigan.

GROUP VIII:

Each sheep was given intramuscularly 10 doses of Infectious Bovine Rhinotracheitis (IBR) vaccine* in 5 ml of sterile diluent. Twenty-four hours later 2 ml of inoculum was administered IV to sheep 36, 37 and 38 and 35 received 2 ml of 1% sterile nutrient broth IV.

GROUP IX:

Bordetella bronchiseptica** was administered intranasally with an atomizer. The sheep were each given 5 ml of an inoculum containing 19.2×10^9 cells/ml. One week later sheep 40, 41 and 42 received 2 ml of inoculum intranasally and 39 received 2 ml of 1% sterile nutrient broth intranasally.

GROUP X:

Sheep 43 and 44 received 0.5 ml of inoculum intrarticularly in the right carpus and 0.5 ml of 1% sterile nutrient broth in the left carpus. Sheep 45 and 46 were given 0.5 ml inoculum in the right stifle and 0.5 ml of 1% sterile nutrient broth in the left stifle.

* Dellen Laboratories, Omaha, Nebraska.

** American Type Culture Collection, Rockville, Maryland.

At the termination of each experiment surviving sheep were euthanatized with Barb-Euthol^{*}. All animals were necropsied within 2 hours of death. The brain, liver, lungs and stiffl joints were cultured on 10% sheep blood agar. The plates were incubated in a 10% CO₂ atmosphere at 37 C for 5 days. The brain and portions of lung, liver, kidney, adrenal, cardiac muscle and synovial membrane of the stiffl joint were placed in 10% buffered neutral formalin for histopathological examination. The brains were serially sectioned every 1 cm and examined grossly. Sections of the cerebrum, thalamus, pons, cerebellum and medulla were fixed in 10% BNF. All tissues for histopathological examination were sectioned at 5 microns and routinely stained with hematoxylin and eosin. Sections were stained with the Brown and Brenn modification of the Gram stain for bacteria when indicated (Thompson and Hunt, 1966).

* Haver-Lockhart Laboratories, Shawnee Mission, Kansas.

RESULTS

GROUP I:

Six hours PI a temperature elevation of 1 to 4 degrees was present in sheep 01, 02, 04, 05, 06 and 07. These sheep were depressed, lethargic, stood with their ears drooped and were anorexic. Eighteen hours PI all temperatures were normal but 01, 02, 04 and 05 were depressed and anorexic. Six hours following the second inoculation 04 and 05 were depressed, weak, dyspneic and in sternal recumbency. Sheep 04 and 05 died 8 hours following the second inoculation. Sheep 01 and 02 remained anorexic and depressed for 48 hours PI and then returned to normal and remained normal throughout the remainder of the experiment as did the others.

Gross Findings

Sheep 04 and 05 died 8 hours after the second inoculation (32 hours PI) and were necropsied within 2 hours of death. Subcutaneous ecchymotic and suffusion hemorrhages were present especially in the ventral portion of the neck. Approximately 150-200 ml of serous fluid was present in the thoracic cavity. The lungs were extremely congested and edematous. Edema fluid flowed from the cut surface and a sanguineous froth was present in the trachea. Subendocardial and subepicardial

ecchymotic and suffusion hemorrhages were present. Suffusion hemorrhages were present in the perirenal fat. The cut surface of the kidneys had a mottled appearance.

The remaining sheep in group I were euthanatized and necropsied 10 days PI. Sheep 01 and 02 had Oestrus ovis larvae in the frontal sinuses and numerous Oesophagostomum spp nodules in the large and small intestines but no other significant gross lesions. There was consolidation of the ventral one-fourth of the right apical lobe of the lung in sheep 06 with adhesions to the parietal pleura. Old fibrous adhesion of the right diaphragmatic lobe of the lung to the parietal pleura were also present. A few Haemonchus spp were present in the abomasum.

No significant gross lesions were present in 03, 07, 08, 09 and 10 except for a few Haemonchus spp in 07 and Oesophagostomum spp nodules in 08.

Microscopic Findings

In sheep 04 and 05 subendocardial and subepicardial hemorrhages were present in the myocardium together with sarcosporidia cysts. Sections of kidney had necrosis of the epithelium of the proximal convoluted tubules. Eosinophilic material was present in Bowman's space. Microcalculi were present in distal tubules. Severe congestion and edema was

present in sections of lung. Erythrocytes and edema fluid filled the alveoli and small bronchioles. No significant lesions were present in sections of brain, liver, synovial membrane and adrenal.

In sheep 06 foci of necrosis surrounded by neutrophils and fibrous tissue were present in the lungs. Adjacent to the necrotic areas the alveoli were filled with sloughed septal cells and neutrophils. A fibrous pleuritis was present. Microcalculi were present in distal tubules of the kidney. No other significant lesions were present in H & E sections. Gram-negative bacteria were present in the lungs.

In the remainder of group I no significant lesions were observed. Sarcosporidia cysts were present in the myocardium of sheep 01, 02, 03, 07, 08 and 10 as well as numerous microcalculi in distal tubules of the kidney. Sheep 09 had numerous microcalculi in distal tubules of the kidneys.

Bacteriological Findings

No bacteria were isolated from cultures of brain, lung, liver and joints of sheep in group I.

GROUP II:

No temperature elevation was noted in sheep 11, 13 and 14 11 hours PI although the 3 sheep were comatose and had severe

diarrhea and dyspnea. Sheep 11, 13 and 14 died 13, 15 and 12 hours respectively PI.

Gross Findings

Sheep 11, 13 and 14 were necropsied within 2 hours of death. Approximately 75-100 ml of serous fluid was present in the thoracic cavity of sheep 11. A small amount of froth was present in the bronchial tree and the lungs were extremely edematous. There were subpleural ecchymotic and suffusion hemorrhages and subendocardial suffusion hemorrhages.

In sheep 13 75-100 ml of serous fluid was present in the thoracic cavity and there was severe pulmonary edema. Subendocardial hemorrhages were evident. Numerous Oesophagostomum spp nodules were found in the large and small intestines.

In sheep 14 there was 100-150 ml of serous fluid in the thoracic cavity. The lungs were extremely edematous and sero-sanguineous fluid was present in the bronchial tree. There were subendocardial hemorrhages and hemorrhages in the adrenal cortex.

Control sheep 12 was euthanatized 6 days PI because all of the test sheep had died. No significant gross lesions were found although numerous Oestrus ovis larvae were present in the frontal sinuses.

Microscopic Findings

Two small foci of necrosis associated with small arterioles containing apparent bacterial emboli were present in sections of the pons of sheep 13. No lesions were observed in other sections of brain, liver, or synovial membrane. The lungs were congested and interstitial edema was present. Sarcosporidia cysts were observed in the myocardium and numerous microcalculi in distal tubules of the kidneys. There was hemorrhage in the adrenal cortex. Sections of pons were stained with Brown and Brenn stain but the sections were apparently taken anterior or posterior to the thrombus since no thrombosed vessels were noted.

Subendocardial hemorrhages were present in the myocardium of sheep 11 and 14. Sarcosporidia cysts were present in the myocardium of sheep 11, 12 and 14 and microcalculi in distal tubules of the kidneys. Interstitial edema was a feature of the lungs from sheep 11 and 14. No other significant lesions were present.

Bacteriological Findings

Numerous unidentified bacteria were cultured from brain of sheep 11. Many unidentified contaminants were cultured from brains of sheep 13 and 14 together with Haemophilus somnifer (n. sp.). Tissues from sheep 12 were negative as were other tissues of sheep 11, 13 and 14.

GROUP III:

No temperature elevation was observed in group III 11 hours after intratracheal inoculations. Within 2 hours of IV inoculation sheep 16, 17 and 18 had diarrhea, severe dyspnea and were extremely weak. The animals became comatose and sheep 16, 17 and 18 died 5, 8 and 9 hours respectively following IV inoculation.

Gross Findings

Sheep 16 had 50-75 ml of serous fluid within the thoracic cavity. The lungs were severely edematous and froth was present in the bronchial tree. Numerous petechial hemorrhages were present in the mucosa of the abomasum. Oestrus ovis larvae were found in the frontal sinuses and Oesophagostomum spp nodules in the large and small intestines.

Sheep 17 had pulmonary edema and hemorrhage with froth in the bronchial tree. Approximately 150-200 ml of serous fluid was present in the thoracic cavity. There was petechial hemorrhage in the mucosa of the abomasum.

Sheep 18 had severe pulmonary edema and hemorrhage with froth in the bronchial tree. One-hundred to 150 ml of serous fluid was present in the thoracic cavity. Petechial hemorrhages were present in the mucosa of the abomasum and

subendocardially. There were a few Haemonchus spp in the abomasum and numerous Oesophagostomum spp nodules in the large and small intestines.

Sheep 15 was euthanatized and necropsied 7 days following intratracheal inoculation. No significant lesions were observed. Oestrus ovis larvae were present in the frontal sinuses and Oesophagostomum spp nodules were observed in the large and small intestines.

Microscopic Findings

Sections of kidney from sheep 16 had hyaline droplets in some proximal tubules. There was severe congestion of the lungs together with interstitial edema which contained eosinophils, erythrocytes and mast cells. Sections of liver and adrenal were congested. Sarcosporidia cysts were observed in the myocardium. All other tissues appeared normal.

Sheep 17 had subendocardial hemorrhages and sarcosporidia in the myocardium. There was interstitial edema in the lungs which contained some erythrocytes and proteinaceous fluid in alveoli. Hemorrhage was present in the adrenal cortex. No other lesions were observed.

There was necrosis of epithelium of proximal tubules of the kidneys in sheep 18. Proteinaceous fluid was present in some tubules and microcalculi in distal tubules. There was

moderate interstitial edema of the lungs. The liver was congested and there was hemorrhage in the adrenal cortex. Sarcosporidia cysts were present in the myocardium. No other significant lesions were observed.

The myocardium of sheep 15 contained sarcosporidia cysts and the kidneys had microcalculi in distal tubules. No other lesions were observed.

Bacteriological Findings

Haemophilus somnifer (n. sp.) was isolated from the lung of sheep 18. All other tissues from sheep of group III were negative.

GROUP IV:

No temperature elevation was observed in sheep of group IV 11 hours PI. All 4 sheep were down in sternal recumbency for 24 hours due to the level of tranquilization. Sheep 20 remained down, became comatose and died 24 hours PI. The other 3 sheep returned to normal in approximately 30 hours.

Gross Findings

Sheep 20 had 100-150 ml of serous fluid in the thoracic cavity. The lungs had 2-4 mm foci of hemorrhage scattered diffusely throughout the parenchyma. The cardiac muscle was

pale with subepicardial and subendocardial ecchymotic and suffusion hemorrhages. Numerous Oesophagostomum spp nodules were present in the large and small intestines.

Sheep 19, 21 and 22 were euthanatized 10 days PI and necropsied. The 3 sheep had numerous Oesophagostomum spp nodules in the large and small intestines but no other gross lesions.

Microscopic Findings

All vessels in sections of brain in sheep 20 were congested. There was subendocardial hemorrhage and sarcosporidia cysts in the myocardium. Numerous microcalculi were present in distal tubules of the kidneys. No other lesions were observed. Sheep 19, 21 and 22 had sarcosporidia cysts in the myocardium and numerous microcalculi in distal tubules of the kidneys. No other lesions were observed.

Bacteriological Findings

Haemophilus somnifer (n. sp.) was isolated from the lung of sheep 20. All other tissues from group IV were negative.

GROUP V:

No temperature response was noted PI in group V. Sheep 26 coughed for 48 hours PI and then returned to normal. No other signs were observed during the experiment.

Gross Findings

Sheep 23, 24, 25 and 26 were euthanatized 10 days PI. Sheep 23, 24 and 25 had numerous Oesophagostomum spp nodules in the large and small intestines. A few Haemonchus spp were in the abomasum of sheep 25.

Sheep 26 had a fibrous pleuritis on the right side with adhesions of the cardiac and apical lobes to the parietal pleura. The entire right apical lobe and ventral one-third of the cardiac lobe of the lung were consolidated. A greenish pus was evident from the cut surface of affected portions of lung. Numerous Oesophagostomum spp nodules were present in the large and small intestines.

Microscopic Findings

Sarcosporidia cysts were present in the myocardium of sheep 23, 24 and 26 and microcalculi were present in distal tubules of kidneys of sheep 23, 24, 25 and 26. No other lesions were present in tissues of sheep 23, 24 and 25.

Sheep 26 had a suppurative bronchopneumonia. There was granulation tissue infiltrated with neutrophils together with foci of necrosis and bacterial colonies. The alveoli contained sloughed septal cells and bronchioles were filled with neutrophils in affected areas. Brown and Brenn stains of

affected lung tissue demonstrated large gram-positive and small gram-negative rods.

Bacteriological Findings

Pasteurella multocida and a Staphylococcus spp were isolated from lung tissue of sheep 26. All other tissues from animals in group V were negative.

GROUP VI:

Sheep in group VI did not show any elevation of temperature 10 hours PI. Sheep 29 had a blood stained fluid from the right nostril for 48 hours PI. Sheep 27, 28 and 29 were depressed and anorexic for 48 hours PI and then returned to normal.

Gross Findings

The sheep in group VI were euthanatized and necropsied 10 days PI.

No gross lesions were observed in sheep 27 and 30. Sheep 28 and 30 had a few Haemonchus spp in the abomasum and 28 had Oesophagostomum spp in the large and small intestines. The left jugular veins were thrombosed in sheep 28 and 29. There was an abscess 5 cm in diameter in 28 and one 3 cm in diameter in 29 adjacent to the thrombosed jugular veins. The abscesses contained a yellowish pus. No other gross lesions were observed.

Microscopic Findings

The myocardium in all 4 sheep in group VI contained sarcosporidia. Microcalculi were present in distal tubules of sheep 28, 29 and 30. No other lesions were observed in sheep 27 and 30.

The thrombosed jugular veins and adjacent abscesses in sheep 28 and 29 consisted of granulation tissue and necrotic foci infiltrated with neutrophils. Brown and Brenn stained sections of the tissue did not reveal any bacteria.

Bacteriological Findings

Haemophilus somnifer (n. sp.) was isolated from the abscesses present in the jugular furrow of sheep 28 and 29.

GROUP VII:

No temperature response was observed in group VII 10 hours PI. Sheep 31, 32 and 34 were depressed and anorexic for 24 hours and then returned to normal.

Gross Findings

The sheep in group VII were euthanatized and necropsied 10 days PI.

Sheep 31 had no gross lesions. Sheep 32, 33 and 34 had numerous Oesophagostomum spp nodules in the large and small

intestines and 32 and 33 had Oestrus ovis larvae in the frontal sinuses.

Microscopic Findings

All 4 sheep had sarcosporidia cysts in the myocardium and microcalculi in distal tubules of the kidneys. No other lesions were observed.

Bacteriological Findings

All cultures from sheep in group VII were negative.

GROUP VIII:

No temperature response was observed in group VIII 10 hours PI. Sheep 36, 37 and 38 became depressed and anorexic for 36 hours and then returned to normal. Sheep 36 developed a bloody diarrhea 6 hours PI which continued for 48 hours and then stopped.

Gross Findings

The sheep in group VIII were euthanatized and necropsied 10 days PI.

Sheep 35, 36 and 37 had numerous Oesophagostomum spp nodules in the small and large intestines and 35 had a few Haemonchus spp in the abomasum. No other gross lesions were observed in animals of group VIII.

Microscopic Findings

All sheep in group VIII had sarcosporidia cysts in the myocardium and microcalculi in distal tubules of the kidneys. No other lesions were observed.

Bacteriological Findings

All cultures from sheep in group VIII were negative.

GROUP IX:

No temperature response or clinical signs were observed in group IX PI.

Gross Findings

The animals in group IX were euthanatized and necropsied 10 days PI. All 4 animals had numerous Oesophagostomum spp nodules in the large and small intestines. Sheep 39, 41 and 42 had Oestus ovis larvae in the frontal sinuses. Sheep 41 and 42 had Haemonchus spp in the abomasum. No other gross lesions were observed.

Microscopic Findings

Sheep in group IX had sarcosporidia cysts in the myocardium and numerous microcalculi in distal tubules of the kidneys. No other lesions were observed.

Bacteriological Findings

All cultures from sheep in group IX were negative.

GROUP X:

Sheep 44, 45 and 46 had an elevated temperature for 24 hours PI and then returned to normal. All 4 sheep were lame on the inoculated joints for 24 hours. After 24 hours the sheep inoculated in the stifle joints resumed a natural gait. Sheep 43 and 44 had swollen, warm carpal joints for 10 days and were lame on the affected legs. After 10 days 44 resumed a normal gait and after 16 days 43 resumed a normal gait. No other signs were observed throughout the experiment.

Gross Findings

Sheep in group X were euthanatized and necropsied 28 days PI. All 4 sheep had numerous Haemonchus spp in the abomasum. Sheep 46 had a few Trichuris spp in the cecum. No other gross lesions were observed.

Microscopic Findings

All 4 sheep in group X had numerous microcalculi in distal tubules of the kidneys. The fibrous tissue capsule of the carpus of sheep 43 was infiltrated with a few plasma cells and neutrophils. No other lesions were observed in the group.

Bacteriological Findings

Cultures from sheep in group X were all negative.

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HAEMOPHILUS SOMNIFER (N. SP.) STUDIES IN SHEEP

by

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Experiments were designed to study the pathogenicity of Haemophilus somnifer (n. sp.) in sheep. H. somnifer (n. sp.) has been incriminated as an agent responsible for encephalitic and septicemic conditions in cattle called thromboembolic meningoencephalitis. Forty-six sheep were used in these experiments. Test sheep were given one or two ml of inoculum which contained $6-8 \times 10^9$ cells per ml. Control sheep were given a comparable amount of sterile 1% nutrient broth. Various routes of inoculation were used including: intravenous, intracarotid, intratracheal, intranasal and intra-articular. Various agents were used in attempts to enhance the susceptibility of sheep to H. somnifer (n. sp.). Purified endotoxin and infectious bovine rhinotracheitis virus was given to partially block the reticuloendothelial system. Para-influenza-3 virus was given in an attempt to establish a pneumonic condition. Tranquilization was used to lower the blood pressure and encourage the formation of thrombi.

Sheep which received two inoculations 24 hours apart either intravenously or intratracheally died with lesions typical of the generalized Shwartzman reaction. No significant gross or microscopic findings were observed in sheep surviving longer than 24 hours except two sheep which developed peri-vascular abscesses at the intravenous injection site.

H. somnifer (n. sp.) was not recovered from sheep living longer than 24 hours except for the two sheep that developed the perivascular abscesses. H. somnifer was isolated from these two abscesses 10 days post inoculation. Two sheep inoculated into the carpus were lame for 10 and 16 days but no significant lesions were found.

It was concluded from these studies H. somnifer (n. sp.) is not pathogenic for sheep.