MUELLERIUS CAPILLARIS ASSOCIATED PNEUMONIA IN CAPTIVE BIGHORN SHEEP

By
J. C. DeMartini and R. B. Davies
Wild Animal Disease Center
Colorado State University
Fort Collins

INTRODUCTION

The susceptibility of Rocky Mountain bighorn sheep to pneumonia of parasitic (Protostrongylus stilesi) (Forrester 1971, Hibler et al. 1972, Marsh 1938), bacterial (Forrester 1971, Harrington 1969, Marsh 1938, Woolf and Kradel 1973), and viral etiology (Parks et al. 1972) is well-known. Pneumonia occurs in wild (Marsh 1938) as well as captive sheep (Woolf and Kradel 1973), but physiological stress appears to be a common underlying factor. The present report describes the course of an epizootic of pneumonia in 20 captive bighorn sheep as well as etiologic and pathologic features of the disease process.

HISTORY AND CLINICAL SIGNS

Utilizing a drop net, personnel from the Colorado Division of Wildlife, in cooperation with the South Dakota Game and Fish Department, captured 30 bighorn sheep (Ovis canadensis canadensis) from Custer State Park, South Dakota in January 1974. The animals were transported to Fort Collins, Colorado, via an enclosed flat-bed truck and released into three 2 ha pens which were surrounded by a 2.5 m chain link fence.

Ten of the animals died during transport or shortly after arrival in Fort Collins due to a degenerative muscle syndrome (capture myopathy). Most of these animals suffered ruptured gastrocnemius muscles and died in spite of treatment.

The remaining sheep remained healthy and were fed free choice alfalfa hay and grain dairy ration in addition to the grasses and forbs growing in the pens. Salt and mineral blocks were also provided and the animals were observed several times each week.

The epizootic began on 26 August 1974 when one ewe was found dead and several other ewes had signs of dyspnea (including rapid, shallow, open-mouth breathing) and listlessness. Two more ewes died the next day. On the following day, the remaining sheep were trapped under a drop net, clinically examined, and injected with 250 mg of oxytetracycline and 2 ml of polyvalent Pasteurella spp. bacterin (obtained from Dr. John Parks, Diamond Laboratories, Ames, Iowa). Body temperatures were recorded

and blood samples were collected at that time. One lamb died the following day and only one ewe died during the following week. All the remaining animals including a ram, 2 lambs, and 12 ewes died during the next 10 days.

METHODS

Each dead sheep was examined at necropsy within 12-18 hours of death. When carcasses were fresh, appropriate tissues were collected for histopathology, bacteriology, and virology. Samples of various organs and multiple sections of lungs were labelled and fixed in 10 percent buffered neutral formalin, after which sections were paraffin-embedded, sectioned at 6 microns, and stained with hematoxylin and eosin. Other tissue samples were routinely cultured for bacteria.

Viral isolation was attempted on lungs and other tissues from 13 sheep. Supernatant fluid from ground tissues was placed on domestic lamb kidney or bighorn sheep kidney cells for at least three blind passages. Cultures were observed daily for development of cytopathic effects. Samples from the lungs of 11 sheep were plated on Modified Taylor-Robinson media in an attempt to isolate Mycoplasma.

RESULTS

Virology and Mycoplasmology

No cytopathogenic agents were detected on viral isolation attempts, nor was growth observed in mycoplasma media.

Bacteriology

Bacterial isolates from the tissues of seven sheep are presented in Table 1. *Pasteurella hemolytica* and *P. multocida* were each isolated from the respiratory tracts of 6/7 sheep, and *Corynebacterium pyogenes* and *Neisseria* sp. each from the tracts of 4/7 sheep. Other species were isolated in lower frequency or were contaminants.

Clincial Pathology

Body temperature and hematologic values of sheep trapped on 28 August 1974 are presented (Table 2). All animals but one were markedly febrile. Although there was no evidence of leukocytosis, relative neutrophilia and lymphopenia were observed in most animals. Erythrocyte, total protein, and blood urea nitrogen values were not significantly altered, but fibrinogen values were markedly elevated.

GROSS PATHOLOGICAL OBSERVATIONS

The sheep were in good flesh with abundant subcutaneous and retroperitoneal fat. The most frequent and significant lesions were observed in

Table 1

BACTERIOLOGICAL SUMMARY OF CULTURES
CAPTIVE SOUTH DAKOTA BIGHORN EPIZOOTIC

	Corynebacterium pyogenes	E. coli and coliforms	Hemophilus ovis	Herella sp.	Klebsiella sp.	Neisseria sp.	Mima polymorpha	Pasteurella hemolytica	Pasteurella multocida	Proteus vulgaris	Pseudomonas aeruginosa	Staphylococcus sp.	Streptococcus sp.
1. Upper resp. tract	2,4	2,6		5		5		2,4	5,7	2,5, 6		2,4, 5,6, 7	5,6
2. Right lung bronchus		1				6	1	1,6					
3. Right lung parenchyma	1,6		6			1,6		1,6	1,6			1,6	1
4. Left lung bronchus						3		2,3, 7	3,7			7	
5. Left lung parenchyma	2		2	3		2	3	2,3 7	2,7			3	3,7
6. Peripheral blood								2	2				
7. Spleen								2	2,7				
8. Mediastinal lymph node								2					
9. Peritoneum											3		
10. Pericardium								2	2				
11. Mammary tissue		2			2		2?	2?	2				

Code: 1. 74BHS-28
2. 74BHS-29
3. 74BHS-30
4. 74BHS-31
5. 74BHS-32
6. 74BHS-34
7. 74BHS-40

TABLE 2

CLINICAL PATHOLOGY IN BIGHORN SHEEP WITH PNEUMONIA

	Body Temp.	Packed Cell Volume	Total Protein (g/100ml)	Fibrinogen (mg/100ml)	Blood Urea Nitrogen (mg/100ml)	Blood Leukocytes (cells/mm^3)	Neut. (%)	Lymph. (%)	Mono. (%)	Eo. (%)
Normal Bighorn Sheep*	102.6 ± 1.3	53± 4	6.6 ±.6	241.7 ±121	14 ±3	4,954 ±1,939	64 ±17	29 ±14	2.5 ±2	4 ±4
Pneumonia - Colo. (N=17)	105.5 ± 1.7	51 ±3	7.8 ±7	800 ± 340	17 ±5	6,800 ± 2,200	66 ± 13	27 ±14	4 ±3	1 ±2
Pneumonia - Penn. (N=11)[1]	-	43 ±7	7.6 ±1.6			17,600 ±6,300	72 ± 9	22 ± 7	2 ±2	2 ±4

* Unpublished data

[1]Woolf and Kradel 1973

the respiratory tract. In most cases the mucosae of the nasal cavity, sinuses, pharynx, and trachea were deep red; the trachea occasionally contained some white to pink froth, but exudate was otherwise absent. There were scattered petechial hemorrhages in the costal pelura and excessive clear pleural fluid in several animals. The most frequent pleural lesion which was observed in 12/20 sheep was severe fibrinous pleuritis, especially in the ventral thorax. Two animals had pleural abscesses containing yellow-green purulent exudate. Fibrous pleural adhesions were present in 12/20 sheep. There was consolidation of ventral portions of the apical, cardiac, and diaphragmatic lobes of both lungs of all animals. The consolidated areas were red, firm, and, on cut surface, often studded with irregular grey foci 2-8 mm in diameter. Purulent exudate could occasionally be squeezed from these areas but only a clear red fluid or froth was present in larger airways. The right lung was often more severely involved than the left. There were scattered, slightly raised subpleural, firm nodules 0.5-1 cm in diameter in the mid- to caudal portions of the dorsal diaphragmatic lobes of both lungs of all the adult sheep. Bronchial and mediastinal lymph nodes were always enlarged and appeared moist and reddened on cut surface.

Miscellaneous lesions were found in several organ systems, some associated with terminal anoxia. Petechial and ecchymotic hemorrhages were present in the epicardium, adventitia of the pulmonary artery and aorta, and in the urinary bladder of several sheep. The liver was often congested and occasionally had a prominant lobular pattern and the gall bladder was usually engorged with bile, probably a result of anorexia. The gastrocnemius or gluteal musculature of three ewes had firm white streaks and pale areas, probably areas of fibrosis associated with previous (capture) myopathy. One ewe had a vaginitis. The synovial membranes of hock and stifle joints of three animals were thickened, and the synovial fluid of one ewe was watery and contained fibrin flecks.

Adrenal glands in the adult sheep were grossly enlarged weighing between 6 and 12 g total, which would represent about 0.015-0.03 percent of the body weight for animals weighing 80-120 pounds.

HISTOPATHOLOGY

The most significant histopathologic abnormalities were found in the respiratory system. Chronic verminous pneumonia was associated with the presence of adult lungworms (Muellerius capillaris), and subacute granulomatous (interstitial) pneumonia, and chronic bronchiolitis and bronchiectasis were associated with M. Capillaris larvae. Acute fibrinous pneumonia and bronchiolitis were associated with various bacteria.

Verminous nodules containing adult M. capillaris, embryonating ova, and larvae were histologically identified in random sections of apical lobes of lungs of 6/10 sheep examined, in 3/9 cardiac lobes examined, and in 11/11 diaphragmatic lobes examined. Although there was a definite tendency for subpleural localization, areas of verminous reproductive activity were also frequently found in deeper parenchyma. The adult nematodes were about

48-55 microns in diameter and were usually found in disrupted alveoli, although a few were present in bronchioles. Ova were usually numerous, but did not appear to elicit a significant inflammatory cell response. First stage larvae of M. capillaris, identified on the basis of a characteristic "corkscrew" tail, dorsal spine and lateral alae, were present in alveoli and concentrated in bronchioles. Within the larger airways they, in combination with mucus, sloughed epithelium and necrotic debris, often obstructed the lumen resulting in bronchiectasis. Other changes associated with areas of verminous reproduction were bronchiolar epithelial hyperplasia, smooth muscle hyperplasia, fibrosis, mononuclear phagocyte infiltration, proliferation of alveolar epithelial cells, and marked perivascular and peribronchiolar lymphoid cell accumulation. Neither degenerating parasites nor areas of mineralization were observed. No Protostrongylus spp. were identified in sections.

In all lobes examined from each lung, especially ventrally, there were areas of granulomatous pneumonia characterized by alveolar infiltration of macrophages with foamy cytoplasm, alveolar epithelial hyperplasia and peribronchiolar and perivascular lymphoid accumulation. Sections of first stage larvae were almost invariably scattered throughout such areas. In many lungs basophilic bacterial colonies, often containing a central larva, were observed in bronchioles and extending into alveolar ducts, but larger airways were clear. Bacterial colonies were usually surrounded by a zone of necrosis, fibrin-filled alveoli, and congested capillaries. Very few neutophils were observed. In a few lungs there were larger areas of bacteria-associated necrosis surrounded by fibrin and alveolar edema and congestion. Such areas were especially common in the lambs, in which larvae or adults were not observed, although areas of granulomatous pneumonia and occasional giant cells were present.

Fibrinous pleuritis was usually characterized by a thick layer of fibrin, often containing bacterial colonies, overlying a congested edematous pleural surface. Subpleural lymphatics were often dilated by mononuclear cells and proteinaceous fluid and occasional areas of organization (fibrosis) of the exudate were observed. The trachea and other upper respiratory surfaces usually had severe congestion of vessels in the lamina propria with areas of edema and hemorrhage, and foci of epithelial sloughing.

Bronchial and mediastinal lymph nodes were severely congested and often depleted of lymphoid cells. Lymph follicles were rare, and when present, had small inactive germinal centers containing amorphous eosinophilic hyalin material.

Histologic lesions in other organs were only of incidental significance. Mild hepatic lipidosis was found in three animals. Amyloidosis of the liver or spleen was not observed. Fibrosis and myofiber degeneration associated with "capture myopathy" was seen in the gastrocnemius and/or gluteal musculature of three sheep.

DISCUSSION

The point of most significance emerging from this study is the definite

association of M. capillaris with the pneumonia in all but 3 of the 20 animals involved. The three animals were lambs; one was not available for histopathologic study and the lungs were not thoroughly examined from the other two. It is certainly possible that the lambs died of an uncomplicated Pasteurella spp. pneumonia. Although M. capillaris is considered of minimal pathogenicity in domestic sheep (Rose 1959, Thomas et al. 1970), the finding of large numbers of first stage larvae throughout the lungs of affected animals in areas of granulomatous pneumonia and often in the center of bacterial colonies suggests that these larvae may predispose to the development of bacterial pneumonia by obstructing airways, disseminating bacteria or, possibly, by causing immunodepression of the host. Thus, it is probable that bighorn sheep respond quite differently to infection with M. capillaris than do domestic sheep. The location of bacteria primarily within bronchioles rather than in alveolar parenchyma as is usual in pasteurella pneumonia in domestic sheep (Jubb and Kennedy 1970), suggests that they may be secondary invaders, although undoubtedly of great importance in death of the host. The inflammatory cell reaction of the bighorn sheep host to adults and larvae of M. capillaris consisted mainly of lymphocytes and macrophages, whereas in domestic sheep, there is often marked eosinophilic infiltration with secondary necrosis, calcification, and fibrous encapsulation (Beresford-Jones 1967, Rose 1959).

The source of the lungworms found in this herd is unclear, although it is known that they were infected before transportation to Colorado. There were no snails found in the sheep pens which would be capable of transmitting M. capillaris. No Protostrongylus spp. adults or larvae were detected in histologic sections of any lung, but this does not exclude the possibility of their presence. If present in the ewes, it seems probable that the lambs would have been transplacentally infected (Hibler et al. 1972).

It is not clear what precipitated the epizootic of pneumonia in this herd since there was no inclement weather prior to the start of the die-off nor were any recent changes made which would have increased psychological stress. However, observation of the sheep by people not associated with the study, or a lack of escape cover in the pens may have stressed the animals resulting in elevated corticosteroid levels and decreased immunity to the parasites. Another possibility, in view of the very large number of M. capillaris larvae in the lungs of affected animals, is that there was a late summer rise in egg and larval production, as has been reported to occur in domestic sheep in England (Thomas et al. 1970), with a consequent detrimental hypersensitivity reaction which predisposed the host to development of Pasteurella spp. pneumonia. The pasteurellae, normal residents of the upper respiratory passages, would probably have increased in virulence by rapid serial passage in the sheep. Lambs may have succumbed solely to the Pasteurella spp. infection. It is noteworthy that viruses and mycoplasma did not appear to play a role in the etiology of the disease, although they were considered important in a previously reported epizootic of pneumonia in captive bighorn sheep (Woolf and Kradel 1973).

Despite a high fever, there was no indication of leukocytosis in the sheep 7-14 days before dying, although slight relative neutrophilia and lymphopenia may suggest physiologic stress (Franzmann and Thorne 1970, Woolf

Kradel 1970). Fibrinogen levels were very high, averaging 2-4 times normal values and may be the best clinicopathologic indicator of a severe disease process such as pneumonia in bighorn sheep.

Literature Cited

Beresford-Jones, W. P. 1967. Observations on Muellerius capillaris (Muller 1889) Cameron, 1927 III Experimental infection of sheep. Res. Vet. Sci. 8:272-279.

Forrester, D. J. 1971. Bighorn sheep lungworm-pneumonia complex. Pages 158-173 in J. W. Davis and R. C. Anderson, eds. Parasitic diseases of wild mammals. Iowa State University Press, Ames.

Franzmann, A. W., and E. T. Thorne. 1970. Physiologic values in wild bighorn sheep (Ovis canadensis canadensis) at capture, after handling, and after captivity. J. Am. Vet. Med. Assoc. 157:647-650.

Harrington, F. 1969. Pasteurellosis in bighorn sheep. Ph.D. Thesis. Colorado State Univ., Fort Collins.

Hibler, C. P., R. E. Lange, and C. J. Metzger. 1972. Transplacental transmission of Protostrongylus spp. in bighorn sheep. J. Wildl. Dis. 8(4):389.

Jubb, K. V. F., and P. C. Kennedy. 1970. Pathology of domestic animals. 2nd edition. Academic Press, New York and London.

Marsh, H. 1938. Pneumonia in Rocky Mountain bighorn sheep. J. Mammal. 19:214-219.

Parks, J. B., G. Post, T. Thorne, and P. Nash. 1972. Parainfluenza-3 virus infection in Rocky Mountain Bighorn Sheep. J. Am. Vet. Med. Assoc. 161:669.

Rose, J. H. 1959. Experimental infection of lambs with Muellerius capillaris. J. Comp. Path. 69:414-422.

Thomas, R. J., V. J. Nunns, and B. Boag. 1970. The incidence of lungworm infection in sheep in North-east England. Vet. Rec. 87:70-74.

Woolf, A., and D. C. Kradel. 1970. Hematological values of captive Rocky Mountain bighorns. J. Wildl. Dis. 6:67-68.

_____. 1973. Mortality in captive bighorn sheep - clinical, hematological, and pathological observations. J. Wildl. Dis. 9:12-20.