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AN EPIZOOTIC OF PNEUMONIA IN CAPTIVE BIGHORN SHEEP INFECTED WITH *Muellerius* SP.

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Abstract: An epizootic of pneumonia in 20 captive Rocky Mountain bighorn sheep (*Ovis canadensis*) is described. The sheep were maintained in large paddocks for about 9 months after which, in the late summer, the entire herd died within a three week period.

Large numbers of *Muellerius* adults, eggs and larvae were in the lungs of all sheep. First stage larvae were widely disseminated throughout the lungs and apparently elicited a granulomatous pneumonia. *Pasteurella* sp. and other bacteria were isolated from the lungs of several sheep but no *Chlamydia, Mycoplasma* or viral agents were recovered.

Elevated serum fibrinogen levels and normal leukocyte values were found in blood samples taken from several sheep 7-14 days prior to death.

INTRODUCTION

The susceptibility of Rocky Mountain bighorn sheep (*Ovis canadensis*) to pneumonia of parasitic (*Protostrongylus stilesi*)[3,7,8] bacterial[3,5,8,11] and viral etiology[9] is well-known, although the role of each of the implicated agents in the pathogenesis of the disease is unclear. Pneumonia occurs in free-ranging[8] as well as captive sheep,[11] but physiologic stress appears to be a common underlying factor. The present report describes an epizootic of pneumonia in 20 captive bighorn sheep as well as etiologic and pathologic features of the disease process.

HISTORY AND CLINICAL SIGNS

In January, 1974, 30 bighorn sheep were captured by drop net from Custer State Park by personnel from the Colorado Division of Wildlife, in cooperation with the South Dakota Game and Fish Department. The animals were transported to Fort Collins, Colorado, and released into three 2 ha pens enclosed by a 2.5 m chain-link fence.

Ten 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 surviving 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 provided and the animals were observed several times each week.

Fecal samples were obtained from the rectum of each sheep at the time of capture and allowed to air dry. First stage larvae of *Muellerius* sp. were recovered by the Baermann technique[1] in all animals. Fecal samples were collected from the pens and a special effort was made to obtain feces from all lambs born. Adults continued to shed *Muellerius* larvae but no larvae were recovered from the lambs.

On 26 August 1974, one ewe was found dead and several other ewes had signs of dyspnea and listlessness. Two ewes died on 27 August and on the following day, the remaining sheep were trapped with a drop net, clinically examined, and injected intramuscularly with 250 mg of oxytetracycline and 2 ml of

polyvalent *Pasteurella* sp. bacteria (prepared from bighorn sheep *Pasteurella* sp. by Dr. John Parks, Diamond Laboratories, Ames, Iowa). Body temperatures were recorded and blood samples were collected at that time. One lamb died on 29 August and only one ewe died during the following week. All of the remaining animals, including a ram, 2 lambs, and 12 ewes died during the next 10 days (Fig. 1). Our reluctance to enhance stress in the sheep by frequent observation coupled with the rapidity with which they were dying precluded careful analysis of the duration and nature of clinical signs during the epizootic.

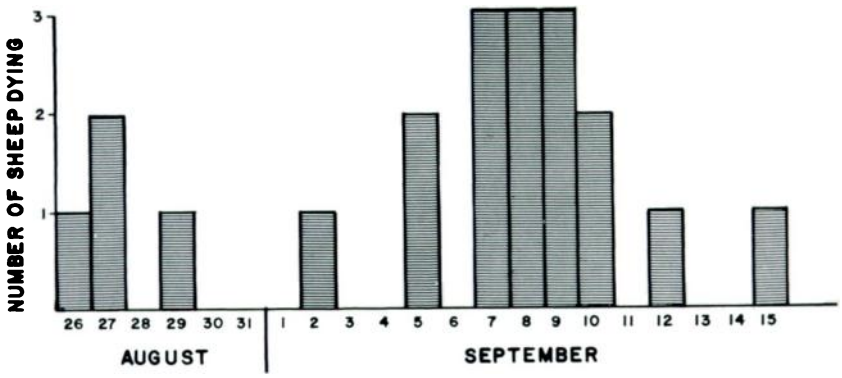

FIGURE 1. Temporal relationships in the pneumonia epizootic.

METHODS

Necropsy examinations were performed immediately after death (7 sheep), within 3 to 6 hrs. (5 sheep), or in 12 to 18 hrs. after death (8 sheep). Tissues were collected for microbiologic study from sheep dead less than 6 hrs. Samples of various organs and at least 2 sections of each lobe of each lung were labelled and fixed in 10% buffered neutral formalin, after which sections were paraffin-embedded, sectioned at 6 μm and stained with hematoxylin and eosin.

Lung tissue not used for isolation of etiologic agents or histopathology was examined for pulmonary nematodes by cutting the tissue into small pieces and placing them into gallon jars filled with physiologic saline solution (PSS) for 24 hrs. The tissues were then digested in a hydrochloric acid-pepsin solution until dissolved. The sediment from the PSS and digest was examined for first and third stage lungworm larvae. Since HCl-pepsin solution also digests the adult nematodes, papain was used to digest lungworm nodules for recovery of the adult lungworms. Fecal samples taken at necropsy were allowed to air-dry and were examined by the Baermann funnel method[1] for recovery of lungworm larvae. A small portion of the feces was examined for parasite ova by fecal flotation using magnesium sulfate.

Swabs taken from tissue examined for bacteria were streaked onto three blood agar plates and inoculated into thioglycolate enrichment broth. One plate was incubated aerobically, another plate incubated in a 10% CO_2 atmosphere and the third plate anaerobically; all plates were incubated at 37 C for 24 hrs. The thioglycolate enrichment broth was incubated aerobically at 37 C for 24 hrs. and subcultured.

Virus and *Chlamydia* isolation was attempted on lungs and other tissues from 12 sheep. Supernatant fluid from ground tissues was placed on domestic lamb kidney or bighorn sheep kidney cells for

at least 3 blind passages. Cultures were observed daily for development of cytopathic effects. Chlamydial isolation was attempted by injection of three dilutions of ground tissue supernatant fluid into 7 day egg embryos. The embryos were daily examined for viability for 14 days. Samples from the lungs of 12 sheep were plated on Taylor-Robinson media[11] in an attempt to isolate *Mycoplasma*.

RESULTS

Virus, Chlamydia, and Mycoplasma Studies

No cytopathogenic agents were detected on viral isolation attempts, nor were deaths of egg embryos observed in Chlamydial isolation attempts. Growth was not observed in *Mycoplasma* media inoculated with tissue samples from 12 sheep.

Bacteriology

Bacterial isolates from the tissues of 7 sheep are presented in Table 1. Both *Pasteurella hemolytica* and *P. multocida* were isolated from the lungs of 5/7 sheep, and *Corynebacterium pyogenes* and *Neisseria sp.* each from the lungs of 3/7 and 4/7 sheep, respectively. Other species were isolated in lower frequency or were contaminants.

Parasitology

Muellerius sp. first stage larvae were recovered from the lungs and feces of all adult sheep. These larvae were identified on the basis of a long "corkscrew" tail and a thin dorsal spine. First stage larvae of *Protostrongylus* sp. have long tapered straight tails and lack a dorsal spine. The differentiation from *Parelaphostrongylus* sp. is more difficult, but is based on greater width, a blunter tail, and a larger dorsal spine. Papain-digested lungworm nodules contained adult *Muellerius* sp. (probably *M. capillaris*). No larvae or adult lungworms were recovered from any of the lambs nor were any third-stage larvae found in the adult lungs. No significant numbers of gastrointestinal parasite ova were recovered.

Clinical Pathology

Body temperature and hematologic values of sheep trapped on 28 August 1974 are presented (Table 2). All animals but one were markedly febrile, Hemograms revealed slight evidence of leukocytosis with a suggestion of mild neutrophilia and lymphopenia. Erythrocyte, total protein, and blood urea nitrogen values were not significantly altered, but fibrinogen values were markedly elevated.

TABLE 1. Bacterial Isolates from the Lungs of Bighorn Sheep with Pneumonia.

Organism	Frequency
Pasteurella hemolytica	5/7 (71%)
Pasteurella multocida	5/7 (71%)
Corynebacterium pyogenes	3/7 (43%)
Neisseria sp.	4/7 (57%)
Hemophilus ovis	2/7 (29%)
Staphylococcus sp.	4/7 (57%)
Streptococcus sp.	3/7 (43%)
Mimo polymorpha	2/7 (29%)
Escherichia coli	1/7 (14%)
Herella sp.	1/7 (14%)

TABLE 2. Clinical Pathology in Bighorn Sheep with Pneumonia.

	Body Temp.	Packed Cell Volume	Total Protein (g/100 ml	Fibrinogen (mg/100 ml)	Blood Urea Nitrogen (mg/100 ml)	Leukocytes (cells/mm³)	Neut. (%)	Lymph. (%)	Mono. (%)	Eo. (%)
Normal Bighorn Sheep* (N=24)	39°C	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)	41°C	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)[12]	—	43±7	7.6±1.6			17,600 ±6,300	72± 9	22 ± 7	2±2	2±4

*Unpublished data

Gross Pathology

The sheep were in good flesh with abundant subcutaneous and retroperitoneal fat. The most frequent and significant lesions were observed in 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. Petechial hemorrhages were scattered in the costal pleura and excessive clear pleural fluid was present in several animals, but the most frequent pleural lesion was severe fibrinous pleuritis, especially in the ventral thorax, which was observed in 12/20 sheep. Two animals had pleural abscesses containing yellow-green purulent exudate. Fibrous pleural adhesions were present in 12/20 sheep. Consolidation of the ventral portions of the apical, cardiac, and diaphragmatic lobes of both lungs was present in all animals. Consolidated areas were red, firm and on cut surface, often studded with irregular grey foci 2-8 mm in diameter. Purulent exudate occasionally could be expressed from these areas but only a clear red fluid or froth was present in larger airways. The right lung often was more severely involved than the left. Scattered, slightly raised subpleural, firm nodules 0.5-1 cm in diameter were present in the mid- to caudal portions of the dorsal diaphragmatic lobes of both lungs of all the adult sheep. Nodules of similar consistency and appearance also were present in the parenchyma of apical and cardiac lobes. 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. Often the liver was congested and occasionally had a prominent lobular pattern. 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 3 animals were thickened, and the synovial fluid of one ewe was watery and contained flecks of fibrin.

Adrenal glands in the adult sheep were grossly enlarged, weighing between 6 and 12 g total, which would represent about 0.015-0.03% 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* sp.), and subacute granulomatous pneumonia, chronic bronchiolitis and bronchiectasis were associated with *Muellerius* larvae. Acute fibrinous pneumonia and bronchiolitis were associated with bacterial colonies.

Verminous nodules containing adult *Muellerius*, embryonating ova, and larvae were histologically identified (Fig. 2) in sections of apical lobes of lungs in about one-half of the sheep examined, in cardiac lobes of about one-third of the sheep, and in all of the diaphragmatic lobes of the animals. Although there was

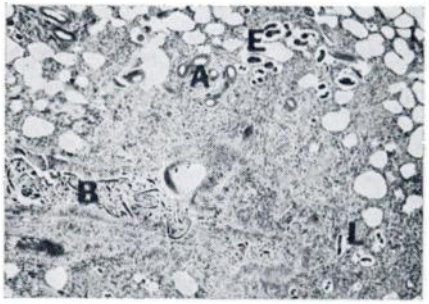

FIGURE 2. **Muellerius** sp. lungworm nodule. Observe adults (A) and eggs (E) in alveoli, and larvae (L) in alveoli and concentrated in bronchiole (B). The inflammatory cell reaction consists of lymphocytes and macrophages. H & E x 70.

a definite tendency for subpleural localization, areas of verminous reproductive activity were frequently found deeper in the parenchyma. Adult nematodes were 48-55 μm in diameter and usually were found in disrupted alveoli, although a few were present in bronchioles. Ova usually were numerous, but did not appear to induce a significant inflammatory cell response. First stage larvae of *Muellerius* sp., 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 the larvae, 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 (Fig. 3). Neither degenerating parasites nor areas of mineralization were observed. No *Protostrongylus sp.* were identified in tissue sections.

In all lobes examined from each lung, areas of granulomatous pneumonia were present, especially ventrally. These were characterized by alveolar infiltration of macrophages with foamy cytoplasm, alveolar epithelial hyperplasia and peribronchiolar and perivascular lymphoid accumulations. Invariably, sections of first-stage larvae were scattered throughout such areas, often in densities approaching 3 per high power field (Fig. 4). 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 usually were surrounded by a zone of necrosis, fibrin-filled alveoli, and congested capillaries. Very few neutrophils were observed. In a few lungs larger areas of bacteria-associated necrosis were 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. The fibrinous pleuritis was characterized by a thick layer of fibrin, often containing bacterial colonies, overlying a congested edematous pleural surface. Subpleural lymphatics often were dilated by mononuclear cells and proteinaceous fluid. Occasional areas of organization (fibrosis) of the exudate were observed. The trachea and other upper respiratory surfaces usually had severe congestion of vessels

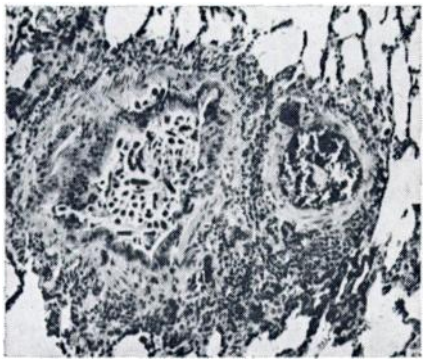

FIGURE 3. Bronchiole filled with larvae of **Muellerius** sp. Note bronchiolar epithelial and smooth muscle hyperplasia and the peribronchiolar and perivascular lymphocytic infiltration. H & E x 140.

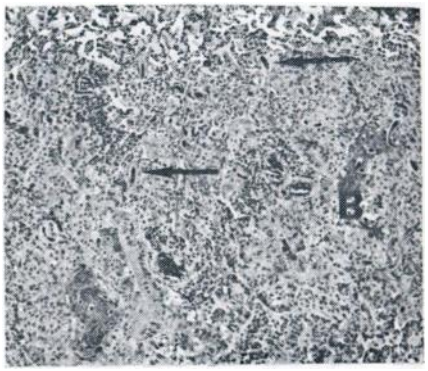

FIGURE 4. Granulomatous pneumonia associated with numerous larvae (arrows) and containing areas of bacterial proliferation (B) in alveolar ducts. H & E x 88.

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 3 animals. Amyloidosis of the liver or spleen was not observed. Fibrosis and myofiber degeneration associated with "capture myopathy" were seen in the gastrocnemius and/or gluteal musculature of 3 sheep.

DISCUSSION

Muellerius sp. lungworms, not previously reported in bighorn sheep, were found 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 histologically from the other two. It is possible that the lambs died of an uncomplicated *Pasteurella* spp. pneumonia. Although *M. capillaris* is considered of minimal pathogenicity in domestic sheep,[10,12] the presence 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 possible that captive bighorn sheep respond quite differently to infection with *Muellerius* sp. than do domestic sheep. The location of bacteria primarily within larger bronchioles rather than in respiratory bronchioles and alveolar parenchyma, as is usual in *Pasteurella*-induced pneumonia in domestic sheep,[6] 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 to adults and larvae of *Muellerius* consisted mainly of lymphocytes and macrophages, whereas in domestic sheep, there is often marked eosinophilic infiltration with secondary necrosis, calcification, and fibrous encapsulation.[2,10] In the present report, *Muellerius* sp. nematodes may have played the same role in the pathogenesis of pneumonia as did *Prostostrongylus* sp. in previous reports,[3] although the pathogenetic role of any nematode in the pneumonia complex of bighorn sheep is poorly understood.

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 in the sheep pens capable of transmitting *Muellerius* sp. No *Protostrongylus sp.* adults or larvae were detected in histologic sections of any lung, nor in tissue samples, but this does not completely exclude the possibility of their presence.

It is not clear what precipitated the epizootic of pneumonia in this herd. 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 *Muellerius* sp. larvae in the lungs of affected animals, is a later-summer rise in egg production, as has been reported to occur in domestic sheep in England,[12] with a consequent detrimental hypersensitivity reaction which may predispose the host to a bacterial pneumonia. The pasteurellae, normal residents of the upper respiratory passages, may have increased in virulence by rapid serial passage in the sheep; thus the three lambs may have succumbed solely to the *Pasteurella spp.* infection. Although viruses and mycoplasma were considered important in a previously-reported epizootic of pneumonia in captive bighorn sheep,[14] we did not recover such agents in the present study.

Despite a high fever, there was not a significant leukocytosis in the sheep 7-14 days before dying (although slight relative neutrophillia and lymphopenia may suggest physiologic stress).[4,13] Fibrinogen levels were very high, averaging 2 to 4 times normal values and may be the best clinicopathologic indicator of a severe disease process such as pneumonia in bighorn sheep.

Amyloidosis, a condition previously reported in bighorn sheep with pneumonia[14] was not observed in these sheep. This may have been a reflection of the relative acuteness of the pneumonia in the present report.

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