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## PATHOLOGIC CHANGES AND MICROORGANISMS FOUND IN BIGHORN SHEEP DURING A STRESS-RELATED DIE-OFF

T. R. Spraker,<sup>1</sup> C. P. Hibler,<sup>2</sup> G. G. Schoonveld,<sup>3</sup> and W. S. Adney<sup>1</sup>

**ABSTRACT:** An all-age die-off of Rocky Mountain bighorn sheep (*Ovis c. canadensis* Shaw) occurred from late October 1980 through March 1981 in Waterton Canyon, Colorado, with a loss of 75 to 85% of the sheep. The cause of death was a subacute to chronic bronchopneumonia and the primary etiologic agents isolated from the respiratory system were a *Pasteurella* sp., *P. multocida*, *Corynebacterium pyogenes*, and *Protostrongylus stilesi* Dikmans, 1931. The underlying predisposing factors that initiated this die-off were believed to be related to multiple chronic environmental stressors associated with the building of a dam which included human contact, vehicular traffic, atmospheric dust, noise and harassment. The die-off was succeeded by a 100% lamb mortality the following summer and a 67% lamb mortality the next two summers. The pneumonia found in these lambs was similar to that found in adult sheep during the previous die-off, except that mature lungworms were absent.

### INTRODUCTION

From the late 1800's to the present, a steady decline has been reported in many populations of bighorn sheep. Causes for these declines include market hunting (Seton, 1929; Honess, 1942; Buechner, 1960), loss of winter range (Honess, 1942), and disease (Marsh, 1938; Buechner, 1960). The two major diseases blamed for die-offs include mange or scabies (Seton, 1929; Lange et al., 1980), and pneumonia (Rush, 1927; Potts, 1937; Marsh, 1938).

The most important disease that is believed to limit the population is a subacute to chronic fibrinopurulent bronchopneumonia. Agents isolated from infected sheep include bacteria, viruses, and lungworms. The most common bacterial agents include species of *Pasteurella* (Potts, 1937; Marsh, 1938; Post, 1962; Spraker, 1977; Spraker and Hibler, 1977; Foreyt and Jessup, 1982) and *Corynebacterium pyogenes* (Marsh, 1938; Buechner, 1960; Spraker, 1979). Parainfluenza virus type-3

has been isolated from the nasal cavity during an outbreak of pneumonia in a single herd of captive bighorn sheep in Wyoming (Parks et al., 1972). The virus has been isolated also from free-ranging bighorn lambs dying from a verminous bronchopneumonia in Colorado (Spraker, 1979). Parasites found in the lungs of bighorn sheep during die-offs include *Protostrongylus stilesi* Dikmans, 1931 and *P. rushi* Dikmans, 1937 (Marsh, 1938; Buechner, 1960).

There are two different field manifestations of mortality in which pneumonia is the primary cause of death in bighorn sheep. The first is the classic all-age die-off that usually occurs in winter, but can occur at any time of the year; it has been seen in free-ranging as well as captive animals. Generally this is a stress-induced pneumonia. The second type of mortality, referred to as "summer-lamb mortality," usually occurs from July through September and affects lambs. There appear to be two different types of "summer-lamb mortality," one in which lungworms play a primary predisposing etiologic role, and the other in which lungworms do not play such a role. This second type of lamb mortality appears to affect stressed lambs (Spraker and Hibler, 1982).

From October 1980 through March 1981 an all-age die-off of bighorn sheep

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decimated 75 to 85% of the Waterton Canyon sheep herd. This herd is located 32 km southwest of Denver, Colorado. Prior to the die-off the population was estimated to be a minimum of 75–80 animals. This report describes and documents a subacute to chronic fibrinopurulent bronchopneumonia associated with stress, bacteria (a *Pasteurella* sp., *P. multocida*, and *Corynebacterium pyogenes*) and lungworms (*Protostrongylus stilesi*) that resulted in an all-age die-off and a bacterial pneumonia in lambs that resulted in high mortality during the following three summers.

#### MATERIALS AND METHODS

Necropsies were conducted on 18 sheep in the fall and winter of 1980–1981, on two lambs in the summer of 1981, and on one lamb during the summer of 1983. Sheep were found dead and taken to the Pathology Department, Colorado State University, or clinically ill animals were shot with a rifle and examined in the field. Bacterial samples were collected from the nasal cavity, trachea, lungs, mediastinal lymph nodes, and spleen from 18 animals. Bacterial samples were maintained on wet ice or frozen on dry ice and transported to the laboratory within 3–4 hr for routine bacterial cultures. Tissues were plated on blood agar and MacConkey's agar for primary isolation. Isolates were identified by using conventional techniques (Kilian, 1981). Samples to be tested for the presence of *Mycoplasma* were transported to the laboratory on dry ice and cultured using modified Hayflick's broth and media.

Samples for virus isolation and fluorescent antibody examination were obtained from the nasal cavity, trachea, and lungs of eight animals. These were transported to the laboratory on dry ice and were inoculated subsequently onto bovine embryonic lung and Modin-Darby bovine kidney (MDBK) cell cultures, passed four times, and then checked for cytopathic effect (CPE). Samples were tested for *Chlamydia* by inoculation into 8-day-old embryonating chicken eggs. The eggs were candled daily. If a dead embryo was found, a smear of its yolk sac was stained with Gimenez stain. If elementary bodies were observed, the sample was considered positive. Routine fluorescent antibody tests were examined for parainfluenza type-3 (PI-3) virus and infectious rhinotracheitis (IBR) virus. Sera from seven sheep were checked for titers to

PI-3 virus using a hemagglutination inhibition test, IBR virus and bovine virus diarrhea virus (BVD) using the serum neutralization test, bluetongue (BT) virus using the agar immunodiffusion test, and contagious ecthyma (CE) virus using the complement fixation test. Reagents were obtained from the National Veterinary Services Laboratories, Ames, Iowa 50010, USA. Serum from six sheep was evaluated for blood urea nitrogen (BUN), glutamic oxaloacetic transaminase (SGOT), and creatine phosphokinase (CPK). Tests were run on the Rotochem IIa Parallel Fast Analyzer made by Aminco, Travenol Laboratories, Inc., Deerfield, Illinois 60015, USA. Serum cortisol values for seven sheep were determined using a radioimmunoassay technique (Hasler et al., 1976). Tissues from major organs of all sheep were fixed in 10% buffered formalin, sectioned at 5–6  $\mu$ m, and stained with hematoxylin and eosin (H&E) for light microscopic examination.

#### RESULTS

##### Gross pathology

The major portion of the die-off occurred from October 1980 through February 1981. During this period 18 sheep were examined at necropsy; six had been shot and 12 were found dead. Ten were females and eight were males; ages ranged from 6 mo to 6 yr. Seven sheep were in excellent body condition, eight were in good body condition, and three were in poor body condition. All of the sheep had a mild degree of reddening and a small amount of exudate within the nasal cavity, trachea and bronchi. All sheep had a pneumonia characterized by a mild (5 to 10% lung involvement; Fig. 1), moderate (20 to 30% lung involvement) or severe (greater than 30% lung involvement; Fig. 2) consolidation of the anteroventral aspects of the lungs. These consolidated areas were dark red to grey and firm. A sharp line of demarcation was usually present between the pneumonic and normal areas of the lung. Cut surfaces of consolidated areas were dark red to grey and had a cobblestone surface characterized by clusters of three to four small (1–2-mm), white nodules (similar to a 3–4-leaf clover) sur-

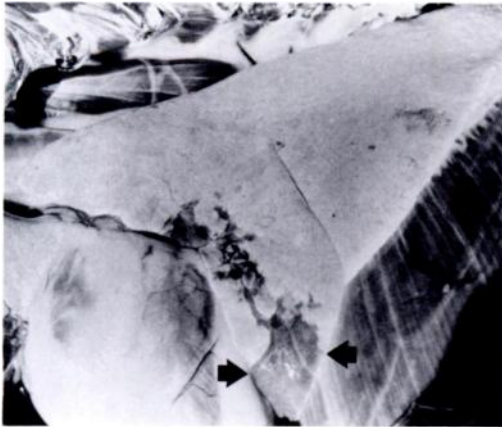


FIGURE 1. Left lung of a bighorn sheep from Waterton Canyon with mild acute pneumonia. Note the small amount of consolidation in the ventral aspect of the cardiac lobe (arrows).



FIGURE 2. Left lung of a bighorn sheep from Waterton Canyon with severe chronic suppurative bronchopneumonia with pyothorax. Note the extensive consolidation (C) of the lung and the small amount of normal lung (N) that remains within the posterior aspects of the diaphragmatic lobe.

rounded by a depressed, dark red to grey zone. A small to moderate amount of white, mucoid exudate could be expressed from cut bronchioles. Of the 18 animals with pneumonia, 14 also had fibrinous pleuritis characterized by a thin to thick coat of yellow, friable material, loosely attached to the parietal and visceral pleura. Four of the animals with pneumonia had pulmonary abscesses. Seventeen of the 18 sheep had firm, raised, yellow nodules located within the posterior dorsal aspects of the diaphragmatic lobes. These nodules were associated with infections of *P. stilesi*. The severity of these infections ranged from mild (3–4 nodules measuring 3–5 mm in diameter) to moderate (8–10 nodules measuring 5–10 mm in diameter). Two of the 18 sheep were infected with a few specimens of *P. rushi*. *Marshallagia marshalli* (Ransom, 1907) was found in small numbers in the abomasum of four sheep, several unidentified tapeworms were found in the gall bladder and bile ducts of four sheep, and a single third instar of *Oestrus ovis* (Linné, 1761) was found in the frontal sinus of an adult ewe.

Mediastinal and retropharyngeal lymph nodes and the lymphoid tissue located in

the posterior aspects of the nasal septum were moderately to markedly enlarged in all animals. The thymus was atrophic in all lambs and adrenal glands were either normal or slightly enlarged.

Seven lambs were born during the following lambing season. By mid-July all of them were lethargic and had rough hair coats. Lambs were also noticed coughing and would lag behind when the herd was moved. Two were examined at necropsy: one was shot (case 19) and the other was found dead (case 20). Both were small and in poor body condition. The major lesions found in these two lambs included chronic fibrinopurulent bronchopneumonia with pulmonary abscesses (similar to the appearance of the lungs in the adult sheep during the major all-age die-off), marked atrophy of the thymus, and hyperplasia of mediastinal lymph nodes. Lungworm nodules were not found in either lamb. By late fall all of the lambs were dead.

Six lambs were born during the spring of 1982. Two lambs survived the summer. Sick lambs were not collected nor were any found dead, thus no lambs were available for necropsy.

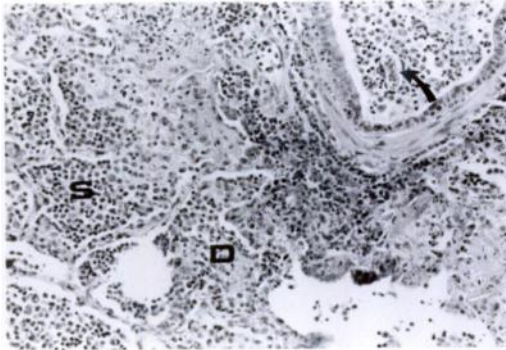


FIGURE 3. Photomicrograph of the consolidated portion of the lung of a bighorn sheep from Waterton Canyon. Note the exudate and first-stage lungworm larva (arrow) within a bronchiole and the cellular infiltrate within alveolar ducts (D) and alveolar spaces (S). H&E,  $\times 100$ .

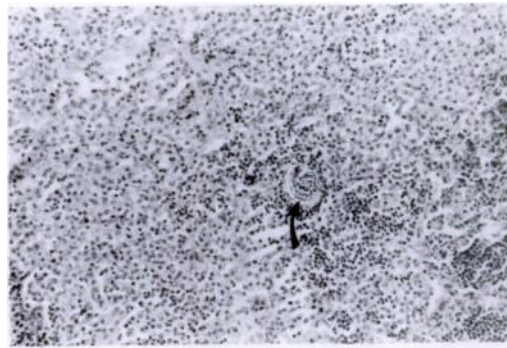


FIGURE 4. Photomicrograph of the consolidated portion of the ventral aspects of the right apical lobe of the lung of a bighorn sheep from Waterton Canyon. Note the partially collapsed alveolar spaces filled with an admixture of neutrophils and macrophages and the first-stage lungworm larva surrounded by neutrophils (arrow). H&E,  $\times 100$ .

Six lambs were born during the spring of 1983. Several were noted sick by mid-summer, and only two survived the summer and fall. One lamb was examined at necropsy (case 21) in July 1983. A similar pneumonia was found in this lamb as described for the lambs examined in 1981.

#### Histopathology

Histopathologic examination of the lungs (including the lambs examined during the summers of 1981 and 1983) revealed a fibrinopurulent bronchopneumonia. Subacute cases were characterized by mild rhinitis, tracheitis and bronchitis. There was a mild degree of hyperplasia of bronchiolar epithelium with exudate commonly occurring within bronchioles (Fig. 3). Many air passages and vessels were cuffed by a narrow zone of lymphocytes and plasma cells. Alveoli were usually open and filled with edema, neutrophils, and fibrin admixed with a few macrophages (Fig. 4). A few macrophages disseminated throughout the lung parenchyma contained minute anisotropic crystalloid anthraco-silicotic pigment compatible with dust particles. Fibrin admixed with inflammatory cells often was found

on the surface of the pleura. Lungworm larvae were found occasionally in the consolidated anteroventral aspects of the lungs (Fig. 4). Lungworm nodules contained adult male and female nematodes, eggs, and larvae. These parasites, especially the eggs and larvae, were surrounded by lymphoid cells, macrophages, plasma cells and a few neutrophils and eosinophils. Suppurative bronchitis and bronchiolitis with neutrophils within alveolar ducts and adjacent alveoli were common within these lungworm nodules. Lymphoid cells also were found cuffing bronchioles and vessels within the lungworm nodules.

The chronic form of the pneumonia was similar to the subacute form except for more extensive atrophy of tracheal epithelium and hyperplasia of bronchiolar and alveolar duct epithelium. These air passages, with adjacent vessels, were often cuffed by lymphoid cells and plasma cells. In some areas fibrosis surrounded bronchioles (Fig. 5). Exudate (mostly neutrophils and desquamated epithelial cells) usually filled the lumen of bronchioles. Alveolar ducts often were filled with neutrophils. The alveoli were partially collapsed



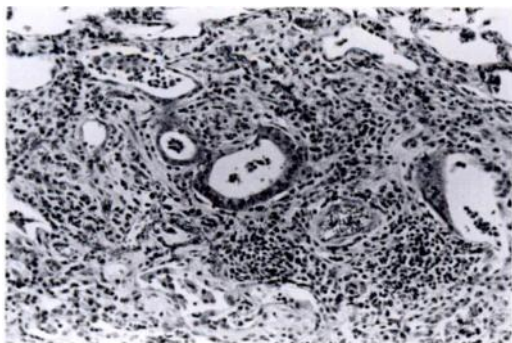


FIGURE 5. Photomicrograph of a portion of lung with chronic bronchopneumonia dorsal to the consolidated area in a bighorn sheep from Waterton Canyon. Note the extensive fibrosis and infiltration of plasma cells and lymphoid cells around a bronchiole and the hyperplasia of bronchiolar epithelium. H&E,  $\times 100$ .

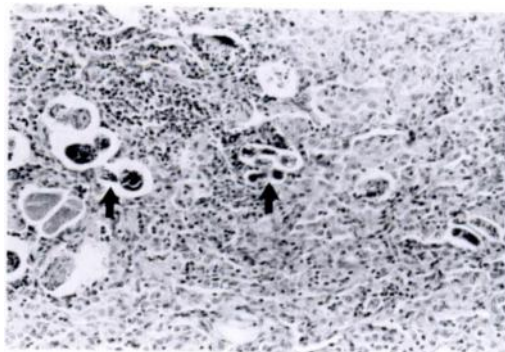


FIGURE 6. Photomicrograph of a lungworm nodule from the dorsal posterior aspects of the diaphragmatic lobe of the lung from a bighorn sheep from Waterton Canyon. Note the eggs and larvae of *Prostrongylus stilesi* (arrows), and suppurative inflammation composed primarily of macrophages, neutrophils, lymphocytes, and plasma cells. H&E,  $\times 100$ .

and contained macrophages with an admixture of neutrophils. A few areas of pulmonary parenchyma were characterized by atelectasis. Degenerated or viable protostrongylid larvae could be observed occasionally in the ventral aspects of the apical or cardiac lobes. The pleura was covered often with granulation tissue overlaid by fibrin admixed with neutrophils and macrophages. Micro- to macroabscesses usually were found within the lung parenchyma. Lungworm nodules were composed of parasites, lymphoid cells cuffing bronchioles and vessels, and alveoli containing lymphoid cells, plasma cells, macrophages, and a few neutrophils and eosinophils. The lungworm nodules of several sheep contained bronchioles that were characterized by suppurative bronchiolitis similar to the ventral consolidated portions of the lungs (Fig. 6).

Mediastinal lymph nodes were often hyperplastic and sometimes contained first-stage lungworm larvae. The thymus was characterized by lymphoid depletion of the cortex in the lambs and yearlings. A mild degree of centrilobular lipidosis was found in the liver of eight sheep. Bile

duct dilatation with hyperplasia and hypertrophy of bile duct epithelial cells was found in the liver of the four sheep that had infections of tapeworms.

#### Microbiology

Bacterial cultures were done on 18 of the 21 sheep and mycoplasmal, chlamydial, and viral cultures were done on nine. The three major microorganisms isolated were an oxidase positive, catalase positive, gram negative diplococcus identified as a *Pasteurella* sp., *Pasteurella multocida*, and *Corynebacterium pyogenes* (Table 1). *Mycoplasma*, *Chlamydia*, and virus cultures were negative in all sheep.

Fluorescent antibody tests for PI-3 virus and IBR virus in six of the collected animals were negative. Serum antibody titers for PI-3 virus were insignificant in five of seven sheep and significant ( $>1:256$ ) in two of seven. Titers for IBR virus, BVD virus, and BT virus antibody were insignificant in these seven animals. Contagious ecthyma virus antibody titers of 1:10 and 1:20 were found in two sheep; the remainder were negative.

TABLE 1. Bacteria isolated from bighorn sheep during the die-off at Waterton Canyon, Colorado.

Sheep number	<i>Pasteurella</i> sp.	<i>Corynebacterium pyogenes</i>	<i>Pasteurella multocida</i>
1	NG <sup>a</sup>	L	NG
3	NG	L	NG
4	NG	L	NG
5	N <sup>b</sup> , T <sup>c</sup> , L <sup>d</sup> , S <sup>e</sup>	N, L	NG
6	NG	T, L	NG
7	N, T	N	NG
8	T, L	NG	NG
9	T, L	T, L	NG
10	NG	L	NG
11	N, T	N, T, L, M	NG
12	T, L, M <sup>f</sup>	T, L, M	NG
13	T, L	T, L	NG
14	NG	L	NG
17	NG	L	NG
18	L	NG	L
19	T, L	NG	NG
20	NG	L	L
21	NG	NG	L

<sup>a</sup> NG = no growth.

<sup>b</sup> N = nasal cavity.

<sup>c</sup> T = trachea.

<sup>d</sup> L = lungs

<sup>e</sup> S = spleen.

<sup>f</sup> M = mediastinal lymph node.

### Clinical pathology

Blood urea nitrogen, serum glutamic oxaloacetic transaminase, creatine phosphokinase and cortisol values are reported in tabular form (Table 2).

### DISCUSSION

The pathogenesis of this all-age die-off appears to be a complex of factors that resulted in death of 75 to 85% of the population and also resulted in significant mortality of lambs born the following three springs. Sheep in Waterton Canyon had been unduly stressed over a relatively short period of time (1 yr). Stress factors for sheep of Waterton Canyon were related to increased disturbance due to construction of a high level dam within the Canyon. Other added stress factors included: 1) increased human activity; 2)

extreme car and truck traffic (one vehicle every 3 min) which caused high levels of dust within the canyon atmosphere; 3) increased noise—blasting, trucks etc.; and 4) the increased traffic may have discouraged sheep from using the river for water. Other contributing stress factors not related to construction activities were a closed-in range of approximately 25 to 27 km<sup>2</sup> and plant succession resulting in undesirable range conditions primarily due to invasion of gambel oak (*Quercus gambelii*).

These environmental factors could have caused chronic stress within the animals, which would result in an increase of steroid secretion from the adrenal cortex. The higher levels of steroids inhibit the inflammatory processes of the animal and result in an increased susceptibility to bacterial pathogens (Feldman, 1983). Cortisol levels in seven sheep were examined. While normal cortisol levels have not been defined for resting bighorn sheep, it appears to be less than 5 ng/ml in bighorn sheep, mule deer (*Odocoileus h. hemionus* (Rafinesque)) and pronghorn (*Antilocapra a. americana* (Ord)) (Spraker, unpubl. data). Four of the collected sheep were considered to be minimally stressed; their cortisol values ranged from 7.3 to 13.6 ng/ml. This increase from the suggested normal may indicate that the sheep were, in fact, undergoing adrenal cortical hyperfunction, but more data are needed before an accurate interpretation of these values can be made. Low CPK values for sheep 5, 7, 8, 9, and 12 also indicated little muscle activity prior to collection. The CPK level in sheep 6 was slightly elevated which indicated mild muscular activity; however, this animal had run slowly for about 1 km prior to collection. These data support the assumption that sheep were not acutely stressed before they were collected. Sheep had normal SGOT levels. Five of the six sheep had normal BUN levels. The one sheep (case 12) with the elevated BUN, when found, ran approximately 30

TABLE 2. Serum isoenzyme levels in bighorn sheep collected during the die-off at Waterton Canyon, Colorado.

	Bighorn sheep no.							Normals <sup>d</sup>
	5	6	7	8	9	12	19	
BUN <sup>a</sup> (mg/dl)	13	11	8	8	12	56	ND <sup>c</sup>	20–30
SGOT <sup>b</sup> (IU/liter)	329	78	31	36	40	159	ND	55–170
CPK <sup>c</sup> (IU/liter)	40	287	54	134	92	80	ND	50–300
Cortisol (ng/ml)	13.6	18.9	12.1	7.3	8.1	50.6	34.2	<5

<sup>a</sup> BUN = blood urea nitrogen.

<sup>b</sup> SGOT = serum glutamic oxaloacetic transaminase.

<sup>c</sup> CPK = creatine phosphokinase.

<sup>d</sup> Blood values from healthy bighorn sheep in Colorado (Spraker, unpubl. data).

<sup>e</sup> ND = not done.

m and died. This elevation of BUN may have prerenal causes, for no evidence of any renal problem was found in this sheep.

A common factor in previously reported die-offs (Marsh, 1938; Buechner, 1960) and in the Waterton Canyon die-off was the presence of bacteria of low pathogenicity and lungworms within the respiratory system. The primary organisms isolated from the upper and lower respiratory tract was a *Pasteurella* sp., *P. multocida* and *C. pyogenes*. The gram negative diplococcus identified as a *Pasteurella* sp. was isolated in highest numbers in the lungs during the acute to subacute stages of the disease. High numbers of *P. multocida* and *C. pyogenes* were isolated when the disease was more chronic. This suggested that the *Pasteurella* sp., a normal inhabitant of the upper respiratory mucosa, may have played a role in the pathogenesis of the pneumonia, followed by a proliferation of *P. multocida* and *C. pyogenes* as the disease progressively became chronic. These organisms have limited pathogenicity and have difficulty invading the lung and producing pneumonia in normal, unstressed animals (Adney, 1980).

Viruses, *Chlamydia*, and *Mycoplasma* were not isolated from the sheep. Parainfluenza virus type-3 antibody titers of 1:8 in five of seven sheep were not considered significant. Only two of seven sheep

had titers of >1:256. Paired serum samples were not available to show whether these titers represented an ongoing PI-3 virus infection or a previous exposure to PI-3 virus; the titers only indicated that infection with PI-3 virus had occurred in the herd. Failure to isolate PI-3 virus and to demonstrate PI-3 antigen within the lung and trachea in any of the five sheep with early pneumonia suggests that PI-3 virus did not play a role in the pathogenesis.

Lungworms could have played a minor role in the pathogenesis of this pneumonia. Adult lungworms and their larvae aspirated into the ventral aspects of the lungs could cause tissue damage and, possibly, carry bacteria into the lower lobes. However, most of the sheep were considered to have low to medium lungworm loads.

The increased amount of atmospheric dust inhaled by the sheep also was considered to be a factor of low pathogenicity. Inhalation of dust would not alone cause any problem; however, it could have become an added factor that predisposed the lungs to bacterial invasion by reducing the number of functional pulmonary macrophages. Phagocytosis of dust particles by macrophages can result in fewer macrophages available for phagocytosis of bacteria. It has been demonstrated that hamsters exposed to aerosolized *P. multocida*



had minimal pneumonic foci, whereas hamsters exposed to aerosolized *P. multocida* and dust resulted in severe pneumonia (Adney, 1980).

Any of these factors alone if severe enough could predispose the lungs to bacterial invasion; however, the history of the die-off, the clinical signs, the length of time that sheep were observed to be sick, and the subacute to chronic nature of the pneumonia suggested that all factors were probably important in predisposing the lungs to the overwhelming proliferation of bacteria. The overall degree of stress in the sheep could not be measured, but probably resulted from the factors previously mentioned and was the underlying cause predisposing to the pneumonia. While the predisposing causes were multiple, together they allowed an overwhelming proliferation of bacteria of low pathogenicity, resulting in fibrinopurulent bronchopneumonia and death.

Lamb mortality associated with thymic atrophy and bacterial pneumonia without lungworms or with only immature lungworms, has been noted among lambs in captivity (Spraker, unpubl. data), and in a single, free-ranging lamb on Pikes Peak, near Colorado Springs, Colorado (Spraker, 1979). The pathogenesis of this pneumonia was undefined; however, several factors should be considered. The stress that precipitated the all-age die-off in the adults may have caused some type of decreased immunity in the surviving pregnant ewes which resulted in a retardation of the development of the fetal immune system. Also the stressed ewes would produce less colostral antibody. Thus, the lambs, during the first month of life would have decreased maternal protection. Another possibility is that the surviving ewes were carrying high levels of *Pasteurella* in their upper respiratory system. The ewes could possibly transfer large numbers of bacteria to their lambs and the lambs would not be able to fight this bac-

terial infection because of their immunological immaturity. The lambs then would be overwhelmed by severe pulmonary infections that could progress to a fatal pneumonia. These factors, and probably others, may be present and account for this lamb mortality following an all-age die-off. This "stress"-related or bacterial pneumonia of lambs following all-age die-offs may account for the slow recovery of some herds after such die-offs.

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