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PNEUMONIA AS A CAUSE OF MORTALITY IN TWO DALL'S SHEEP IN THE MACKENZIE MOUNTAINS, NORTHWEST TERRITORIES, CANADA

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Abstract: Fresh, intact carcasses of 2 adult Dall's sheep (*Ovis dalli dalli*) ewes were recovered from the northern Mackenzie Mountains, Northwest Territories on 11 June and 25 July 1999. Both ewes were emaciated and post mortem examinations demonstrated severe, subacute to chronic, fibrino-purulent bronchopneumonia, with bacterial septicaemia in the first ewe. Gross and histological lesions along the dorso-caudal borders of the diaphragmatic lobes of both ewes were consistent with the lungworm *Protostrongylus stilesi*. Eggs consistent with those of *Parelaphostrongylus odocoilei* (muscleworm) were observed in lung histological sections of the first ewe. Bacterial culture of lung and other tissues from the first ewe yielded a pure growth of *Arcanobacterium* ("*Actinomyces*") *pyogenes*, and from the second ewe, a mixed population including *Escherichia coli*, *A. pyogenes*, and, from lung tissue only, a *Mannheimia* ("*Pasteurella*") species. Bacterial cultures of lung tissue collected previously from 6 healthy ewes from the same region yielded only an unusual *Corynebacterium* species. Fixed lung and tracheal tissues from both ewes that were found dead were examined by immunohistochemistry for *Mycobacteria* spp., *Haemophilus somnus*, and *Mannheimia haemolytica*; the bovine respiratory viruses parainfluenza type 3, bovine herpes type 1, and bovine respiratory syncytial virus; and bovine viral diarrhea. The second ewe was positive for *Mycobacterium* sp.; all other samples were negative. Fecal parasitology revealed coccidia and *Trichuris* in the first ewe, *Protostrongylus* sp. larvae in the second ewe, and, in both ewes, the gastrointestinal trichostrongyles *Marshallagia* and *Nematodirus*. These are the first confirmed reports of pneumonia as a primary cause of mortality in Dall's sheep. In addition to these 2 cases, in September 1999, we received reports of 2 rams with signs of respiratory distress and 3 dead adults (2 rams, 1 ewe) from 2 different sites in the northern Mackenzie Mountains. One of the ill rams was shot and culture of lung tissue was positive for *A. pyogenes*. The significance of these mortalities in Dall's sheep demographics in the Mackenzie Mountains is unknown. In light of the importance of pneumonia as a mortality factor in bighorn sheep (*Ovis canadensis*), we, in cooperation with outfitters and hunters, have begun a project to monitor and further investigate the health status of Dall's sheep in the Mackenzie Mountains.

The Mackenzie Mountains of the western Northwest Territories are inhabited by an estimated 14,000-26,000 Dall's sheep (*Ovis dalli dalli*) (Veitch et al. 2000). This population is relatively stable, and major mortality events ("die-offs") observed in bighorn sheep (*Ovis canadensis*) have never been reported in this population (Simmons et al. 1984; Veitch and Simmons 1999) or in Dall's sheep elsewhere in their range (Bowyer and Leslie 1992; Nichols and Bunnell 1999). Conversely, the stress-lungworm-pneumonia complex in bighorn sheep is the most important cause of mortality in some bighorn populations (Forrester 1971; Aguirre and Starkey 1994; Bunch et al. 1999).

In 1997, a parasitological investigation was initiated to determine if *Umingmakstrongylus pallikuukensis*, a lungworm of muskoxen (Hoberg et al. 1995) was present in Dall's sheep of the Mackenzie Mountains. *Umingmakstrongylus pallikuukensis* was not found, but 2 other protostrongylids were identified, *Protostrongylus stilesi*, the lungworm of bighorn sheep, and *Parelaphostrongylus odocoilei*, a muscleworm which had not previously been reported in wild sheep (Kutz et al. 2001). Both parasites are common and widespread in the Mackenzie Mountain sheep population, and have the potential to cause significant pulmonary damage (Kutz et al. 2001).

In 1999, the Thinhorn Health Investigation Network (THIN) launched the Dall's sheep Health and Parasitology Project to investigate the role of these protostrongylids and other potential pathogens in the health of Dall's sheep. This project includes passive surveillance and opportunistic carcass collection. This report summarizes our findings in 1999; monitoring will continue in 2000 and 2001. While on one occasion a carcass was discovered by the investigators and a field necropsy performed on site, the only people routinely present in sheep range within the Mackenzie Mountains are outfitters; guides; non-resident, resident, and subsistence hunters; and staff from the Department of Resources, Wildlife, and Economic Development.

METHODS

In total, 5 dead and 2 sick sheep from the northern Mackenzie Mountains, NT, were reported in 1999. We examined a small lung sample from 1 sick ram that was shot (Ram 1) and necropsied 2 of the 5 dead sheep (Ewe 1 and 2). Three samples were taken from most tissues, 1 fresh, 1 frozen, and 1 preserved in 10% neutral buffered formalin. Fixed tissues were sectioned, mounted in paraffin, and stained with hematoxylin and eosin for histology. Femurs were frozen and later marrows were collected to determine percent fat content.

Tissues were cultured on MacConkey's and blood agar. Fresh samples from Ewe 1 were refrigerated and cultured 4 days later, and paired frozen samples were cultured 3 months later. The carcass of Ewe 2 was kept cool for 48 hours, and fresh samples were then cultured. The lung sample from Ram 1 was kept frozen for 1 week, and cultured immediately upon thawing.

Fixed lung and tracheal tissues from both ewes were stained immunohistochemically for: infectious bovine rhinotracheitis virus (IBR), parainfluenza-3 virus (PI_3), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVD), *Mycobacteria spp.*, *Haemophilus somnus*, and *Mannheimia haemolytica* (Haines and Chelack 1991).

Right ears (previously frozen) of both ewes were curetted, and the scrapings placed in 10% KOH for 10 min in a boiling water bath. This mixture was centrifuged for 10 minutes, decanted, and the sediment re-

suspended in sugar solution. This was centrifuged again for 10 minutes with a cover slip on top of the test tube, which was removed, placed on a slide, and examined for ectoparasites.

The mucosal surface of the abomasa of each ewe was rinsed and lightly scrubbed 3 times with tap water. The rinse was collected and contents brought to 1 L final volume. Two 10% aliquots were fixed in 10% neutral buffered formalin. The remaining contents were preserved in 37% formalin. In Ewe 2, small intestinal and large intestinal/caecal contents were also retained, and abomasal nematodes were frozen in saline at -80°C for molecular work.

Fecal samples from both ewes were examined by Wisconsin quantitative flotation (Cox and Todd 1962) and a beaker Baermann larval sedimentation (Forrester and Lankester 1997). In Ewe 2, a lung wash was also performed. Five pieces of randomly selected lung tissue (from affected and unaffected areas) were placed in a plastic bag, and swirled in tap water for 1 minute. Sediment was examined in small glass Petri dishes at 25X on a dissecting scope. One hundred larvae were identified on a compound light microscope.

The remainder of the eviscerated carcass of Ewe 2 was skinned and selected skeletal muscle groups examined. The muscles of the right hind leg, right foreleg, right chest wall, left trapezius, and both longissimus dorsi were sectioned in 0.5 cm slices; muscles of the left hind proximal to the stifle and left fore proximal to the cubital joint were briefly examined. Linear hemorrhages were excised, placed in saline, then either dissected under 6.7X on a dissecting microscope or placed in a compressorium and examined under the microscope.

RESULTS

The first carcass (Ewe 1) was found during a census on 11 June 1999 at 65°03' N and 127°41' W. The second carcass (Ewe 2) was found with a live lamb in an abandoned building on the Canol Heritage Trail at 64°20' N and 128°00' W by an outfitter on 25 July 1999. On 1 September 1999, another outfitter reported 2 sick rams at 64°20' N and 129°30' W that had coughs and nasal discharges, and were ostracized by nearby healthy rams. One of the sick rams (11.5-years-old) was harvested (Ram 1) and a small lung sample containing an abscess was examined. In another northern outfitting zone in early September, 3 adult sheep were found dead with no sign of predation; these carcasses were not recovered. In total, five mortalities and 2 sick animals were reported in 1999 (Fig.1).

Gross pathology

In Ewe 1, little autolysis was present, the carcass was moderately dehydrated, and the estimated time elapsed since death was 2-6 hours. Age as determined by horn annuli was approximately 10 years, and the ewe was lactating. The animal had no back, renal, or omental fat, and the femoral marrow fat content was 45%. There was a bloody discharge from the left nostril and the vulva. Ecchymotic hemorrhages were present on conjunctiva, vulvar mucosa, subcutaneous and intradermal tissues, and rumen serosa.

The most significant findings were in the thoracic cavity. There was approximately 500 ml of free serosanguinous fluid. On the right side, the visceral pleura of the cranial and accessory lobes were adhered (both fibrinous and fibrous adhesions) to the thickened parietal pericardium and to the costal pleura. On the left, a thick fibrinous/fibrous sheet, extending from the

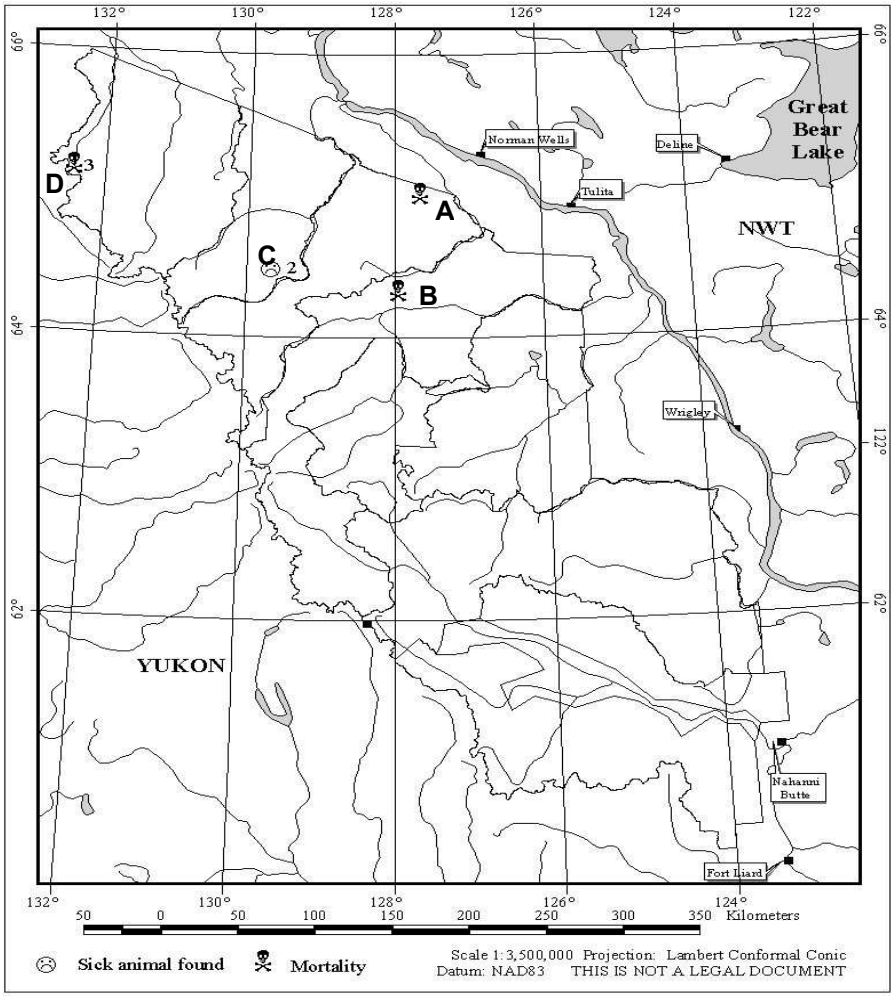

Figure 1: locations of known sick and dead Dall's sheep in the Mackenzie Mountains, Northwest Territories, June-September 1999. Numbers refer to the number of sick or dead animals at the site, while letters correspond to the animal in the text of the paper (Ewe 1 = A, Ewe 2 = B, Ram 1 = C, anecdotal report of 3 dead adults = D).

a cranial border of the cranial lobe to the middle of the diaphragmatic lobe, was dhered to the cranio-ventral costal pleura, displacing the cranial lobe ventrally and medially. Multifocal, coalescing foci of purulent material, ranging from liquid to caseous, were seen on the surface and upon cut section of both cranial lung lobes. The parenchyma at the dorso-caudal margins of both diaphragmatic lobes had lesions consistent with those described for *P. stilesi* by Spraker et al. (1984) and Kutz et al. (2001). There were multifocal petechial and ecchymotic hemorrhages throughout the epicardium of the heart, and a large hemorrhage ran along the paraconal interventricular branch of the left coronary artery.

The carcass of Ewe 2 was at least 48-hours-old at time of necropsy in Saskatoon. Age as estimated by horn annuli was 8 years, and the carcass weighed 34.4 kg, with a length of 146 cm and girth 93 cm. The ewe was in poor body condition, with no back, renal, or omental fat, and the femoral marrow fat content was 51%. The ewe was lactating.

The most significant findings were severe pneumonia and pleuritis, affecting approximately 75% of the left lung surface and 25% of the right. The visceral pleura of the right and left cranial lobes were adhered to the parietal pericardium. The visceral pleura of the right cranial and accessory lobes were adhered to the costal pleura, and that of the accessory and left diaphragmatic lobes to the diaphragmatic pleura. The visceral pleura of the left diaphragmatic lobe was also adhered to the parietal pericardium and to the costal pleura. Both cranial lobes and the cranio-ventral half of the left diaphragmatic lobe were completely consolidated, dark red to grey in colour, and had multiple, 2-30 mm in diameter, purulent foci on both surface and cut section. Contents ranged from liquid to caseous and, in some cases, had been replaced by an organized, pale, firm, spongy material that was well demarcated from surrounding lung tissue. The right middle and diaphragmatic lobes were voluminous and non-collapsing, and the caudo-dorsal border of the right diaphragmatic lobe contained lesions characteristic of *P. stilesi*.

The ventrum of the right mandible bore a 6x2 cm bony malformation rostral to the junction of ramus and body, associated with the impacted second molar of the right mandible. The first cheek teeth (the second premolars) of both mandibles were absent, and all cheek teeth had sharp projections on the lingual surface. The maxillary arrays of cheek teeth were complete, and the buccal surfaces had sharp projections.

Histopathology (Ewe 1 and 2 only)

In the cranial lung lobes, many bronchioles were obliterated by accumulations of degenerate neutrophils, while less affected bronchioles displayed epithelial hyperplasia and sub-epithelial mononuclear cell infiltrations. In Ewe 2, some bronchiolar borders were maintained and the neutrophils were less degenerate. There were massive quantities of fibrin and fluid in the alveoli, particularly in Ewe 1. Most blood vessels were congested, with some evidence of focal hemorrhage, and a few had marginating leukocytes. In both ewes, but more notably in Ewe 1, there were many "micro-" and "macro-abscesses" (ranging from the size of a small bronchiole to several centimeters in diameter) with necrotic centers and bacterial clumps. In Ewe 1, there were on average 4 protostrongylid eggs and/or larvae per field at 100X magnification (primarily eggs). Most of the eggs were consistent with those of *P. odocoilei* (Kutz et al. 2001), and were accompanied by mild granulomatous inflammation. Fewer eggs and larvae were present in lung sections from Ewe 2 (mean of 1.2 eggs/larvae – primarily larvae - per field at 100X magnification), and no *P. odocoilei* type eggs were observed.

Lung tissue in the caudal lobes of both ewes was generally less affected by the bronchopneumonia, although there was some congestion and hemorrhage. The alveoli in these sections were distended with air and many had ruptured. There were many nematode adults, larvae, and eggs typical of *P. stilesi*, and these were often associated with mononuclear inflammatory cell infiltrates in the alveolar interstitia. Larvae and mucous were frequently observed in the bronchioles, and, in Ewe 2, cross-sections of adult nematodes were present within bronchioles.

In the spleen of Ewe 1, there was evidence of moderate lymphocytolysis, severe congestion, and large numbers of macrophages, lymphocytes, and plasma cells in the interstitium. Ewe 1 also had mild, chronic lymphocytic/plasmacytic nephritis and granulomatous periacinar hepatitis; in Ewe 2 there was mild periacinar hepatic fibrosis. In both ewes there was renal and

hepatic congestion, and numerous *Sarcocystis* spp. tissue cysts were present in both cardiac and skeletal muscle; in the cardiac muscle, these were occasionally associated with fibrosis. The pericardium of Ewe 2 was thickened and mononuclear cell infiltrations were visible in places. In Ewe 1, but not Ewe 2, there were locally extensive hemorrhages running parallel to the muscle fibers, fiber necrosis, and sarcocysts, but no muscle nematodes were observed.

Morphological diagnoses included subacute to chronic and on-going fibrinopurulent bronchopneumonia involving approximately 60% (Ewe 1) and 50% (Ewe 2) of the entire surface area of the lung, as well as fibrinous and fibrous pleuritis and pericarditis. Both ewes had moderately severe, granulomatous, verminous pneumonia in the caudo-dorsal diaphragmatic lobes. The first ewe had lesions of septicaemia, while the second had chronic right mandibular osteomyelitis ("lumpy jaw") associated with an impacted second molar.

Bacteriology (Table 1)

There was pure growth of *Arcanobacterium pyogenes* from spleen, liver, kidney, and cranial and caudal lung lobes in fresh and frozen samples from Ewe 1. Frozen pericardial and pleural fluid also yielded *A. pyogenes*. In Ewe 2, *A. pyogenes* was cultured from liver, bone marrow, and

Table 1: Results of microbiological investigation – parasitology, bacteriology, and virology – of 3 Dall's sheep of the Mackenzie Mountains, NT.

	Protostrongylid larvae	**Other parasites** (eggs or oocysts per gram of feces)	**Bacteria** Culture & immunohistochemistry (IHC)[4]	**Virology** IHC[5]
Ewe 1	*Protostrongylus*[1] (gross and histology) and *Parelapho-strongylus odocoilei* eggs (histology)	*Marshallagia* sp. (11.8) *Nematodirus* sp. (1.4) *Trichuris* sp. (4.6) *Eimeria*[3] spp. (0.4) *Sarcocystis* sp. present No ectoparasites	*Arcanobacterium pyogenes* (culture of cranial and caudal lung, other organs, pericardial and pleural fluids)	Negative
Ewe 2	Only *Protostrongylus* (gross & histology, lung wash, and 1.2 LPG Baermann)	*Marshallagia* sp. (0.6) *Nematodirus* sp. (2.0) *Sarcocystis* sp. present No ectoparasites	*A. pyogenes* (culture of cranial and caudal lung, bone marrow, and liver); *Mannheimia* sp. (culture of cranial lung); *Mycobacterium* spp. (IHC)	Negative
Ram 1	Only DSL[2] recovered on lung wash of small sample	NA	*A. pyogenes* from small lung sample; IHC NA	NA

[1] most likely *Protostrongylus stilesi* (no adult *P. rushi* observed in airways)
[2] dorsal-spined first-stage larvae, most likely *Parelaphostrongylus odocoilei* in this population
[3] most likely *Eimeria dalli, E. ahsata*, and/or *E. crandaillis* – Clark and Colwell (1974)
[4] immunohistochemistry was performed for the following bacterial antigens: *Mycobacteria* spp., *Haemophilus somnus*, and *Mannheimia haemolytica*
[5] immunohistochemistry was performed for the following viral antigens: infectious bovine rhinotracheitis virus (IBR), parainfluenza-3 virus (PI3), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVD)

cranial and caudal lung lobes (but not kidney or adrenal gland). In addition, a *Mannheimia* sp. was cultured from the cranial lung lobes of Ewe 2 only; based on standard biochemical testing this organism was characterized as *Mannheimia haemolytica*-like. Only *A. pyogenes* was cultured from the lung sample from the Ram 1.

Immunohistochemistry (Table 1)

Ewe 2 was positive for *Mycobacteria* spp. (most likely *M. avium* although this was not confirmed). All other results were negative.

Parasitology (Table 1)

Ectoparasites were not recovered from either of the ewes. Based on fecal flotation, eggs of *Marshallagia* spp. and *Nematodirus* spp. were present in both ewes; *Trichuris* spp. and *Eimeria* spp. were only present in Ewe 1. Ewe 2 was positive for *Protostrongylus* spp. larvae, but no other protostrongylid species, on Baermann and lung wash. A few dorsal-spined larvae were recovered from the lung wash of Ram 1.

DISCUSSION

These are the first reported cases of pneumonia as a cause of mortality in wild Dall's sheep. While there is little published information about pneumonia and associated pathogens in Dall's sheep, the bighorn sheep pneumonia complex has been the focus of considerable research efforts. Therefore, our results are interpreted in the context of bighorn sheep pneumonia, a disease multifactorial in origin, often involving bacteria, parasites including *Protostrongylus* spp. lungworms, viruses, and a stress 'trigger'.

Pathology

The pathological picture of subacute to chronic fibrinopurulent bronchopneumonia, fibrinous pleuritis, and pulmonary abscessation seen in the 2 Dall's ewes found dead in 1999 is very similar to that reported in pneumonia in bighorn sheep (Spraker et al. 1984; Bunch et al. 1999). Pathological lesions associated with *Mannheimia haemolytica* in most animal species are generally more fibrinous than purulent, while hallmarks of *A. pyogenes* invasion include abundant purulent material in the bronchioles and abscessation (Schwantje 1988); both processes were present in the 2 Dall's ewes examined. Granulomatous inflammation associated with lungworm lesions in the caudo-dorsal lobes and eggs/larvae in the cranial lobes was consistent with that reported for *Protostrongylus* sp. in bighorn sheep (Spraker 1979; Spraker et al. 1984; Schwantje 1986), *Muellerius* sp. in bighorn sheep (Demartini and Davies 1977), and eggs and larvae of *Parelaphostrongylus odocoilei* in Dall's sheep of the Mackenzie Mountains (Kutz et al. 2001) and mule deer (Platt and Samuel 1978; Pybus and Samuel 1984a; Pybus and Samuel 1984b). The dental abnormalities and mandibular osteomyelitis in Ewe 2 were consistent with other descriptions of lumpy jaw in wild sheep (Glaze et al. 1982; Kutny and Stenhouse 1991; Hoefs and Bunch 2001); apparently lumpy jaw is more prevalent in thinhorn sheep than in bighorn sheep (Hoefs and Bunch 2001).

Bacteria

Pneumonia in bighorn sheep most commonly involves the bacterial species *Mannheimia* (*Pasteurella) haemolytica* (various biotypes) or *Pasteurella multocida,* often in conjunction with *Arcanobacterium (Actinomyces* or *Corynebacterium)*

pyogenes (Spraker et al. 1984; Schwantje 1988; Bunch et al. 1999). *Pasteurella/Mannheimia* spp. and *A. pyogenes* are commensals of the upper respiratory tract, tonsils, and skin (the last *A. pyogenes* only) of healthy domestic and bighorn sheep. These bacteria are opportunistic pathogens in the lungs when natural defenses are compromised by stressors or other pathogens (Queen et al. 1994; Brogden et al. 1998). One exception to this opportunistic role occurs when naïve bighorns are exposed to a foreign *Mannheimia* species from domestic sheep or biotypes from other bighorn sheep. In such instances, these bacteria may act as primary pathogens (no need for pre-disposing factors) in the naïve bighorns (Foreyt and Jessup 1982; Foreyt et al. 1994). The latter scenario is unlikely in Dall's sheep in the Northwest Territories, since there are no domestic sheep or goats within 50 km of sheep range in the Territory, nor have wild sheep or goat translocations occurred (Veitch 1996). Experimental infections of Dall's sheep have, however, illustrated that they are as susceptible to domestic animal bacteria as bighorns. In fact, *in vitro* studies show that Dall's sheep neutrophils may be more sensitive to cytotoxins of *Mannheimia haemolytica* biotype A serotype 2 (a domestic sheep strain) than neutrophils of bighorns (Foreyt et al. 1996).

We recovered *A. pyogenes* from all lung samples (Ram 1, Ewes 1 and 2) and some organs sampled in Ewes 1 and 2. An unusual *Mannheimia* sp. was isolated from the cranial lung of only 1 of the 3 Dall's sheep sampled (Ewe 2); this may, however, simply reflect the fragility of *Mannheimia* species. The positive result for *Mannheimia* sp. was from the only samples that had not been frozen or in transit for extended periods of time. Alternatively, *A. pyogenes* might have been the only bacterial species involved in the pneumonia/septicaemia, although *A. pyogenes* is more commonly found with concomitant *Pasteurella* or *Mannheimia* spp. in cases of bighorn sheep pneumonia (Marsh 1938; Buechner 1960; Demartini and Davies 1977; Spraker 1979; Foreyt and Jessup 1982).

Arcanobacterium pyogenes is likely part of the normal pharyngeal fauna of Dall's sheep. Recently, we recovered *A. pyogenes*-like bacteria from the tonsils of a healthy Dall's ewe (Jenkins and Chirino-Trejo, unpublished data), and *A. pyogenes* is the most frequently cultured bacteria from lumpy jaw in Dall's sheep (Neiland 1972; Glaze et al. 1982; Heimer et al. 1982). We have not recovered *A. pyogenes* or *Mannheimia* spp. bacteria from the lungs of any hunter-killed or collected healthy sheep, only an unusual *Corynebacterium* sp. (Kutz, Jenkins, and Chirino-Trejo, unpublished data); therefore, the presence of these bacteria in pneumonic lungs must be considered significant.

It is not surprising that the *Mannheimia* species recovered from Mackenzie Mountain Dall's sheep was distinct from those of bighorn or domestic sheep, as this Dall's sheep population has historically been isolated (Veitch and Simmons 1999) such that divergent evolution may have occurred. Further work has been undertaken to genetically characterize the *Mannheimia* sp., which was sufficiently distinct from *Mannheimia haemolytica* that the immunohistochemistry for this pathogen was negative. Immunohistochemistry was also performed for *Mycobacteria* spp. and *Haemophilus somnus*. The second ewe was positive for *Mycobacteria* sp. antigen, but due to lack of contact with humans and cattle, it was most likely *M . avium* rather than *M. bovis* or *M. tuberculosis*.

Parasites

The roles of the protostrongylid lungworms *Protostrongylus stilesi* and *Protostrongylus rushi* in the bighorn sheep pneumonia complex have been a source of controversy. Some feel that these lungworms may not play an immediate role in all-age die-offs but may instead act as a predisposing factor to fatal pneumonia (Samson et al. 1987). In the Mackenzie Mountains, wildlife biologist N. Simmons reported lesions of verminous pneumonia in harvested animals as long ago as 1967 (Simmons, Veitch, and Kutz, unpublished data), and more recent work suggests that *P. stilesi* is endemic in Dall's sheep of the Mackenzie Mountains (Kutz et al. 2001). *Protostrongylus rushi* has been recovered only from 1 Dall's sheep in the Yukon Territory (Kutz et al. 2001).

Parelaphostrongylus odocoilei has only recently been recognized in the Mackenzie Mountain Dall's sheep population (Kutz et al. 2001), and it may play a greater role than *P. stilesi* in pneumonia in Dall's sheep. While *P. stilesi* adults, larvae and eggs are usually localized to the caudal lobes, haematogenously delivered eggs and larvae of *P. odocoilei* create focal granulomas diffusely throughout the entire lung (Pybus and Samuel 1984a; Kutz et al. 2001).

Lungworm larvae can be aspirated into the cranial lobes (Schwantje 1988), where, in combination with *P. odocoilei* eggs and larvae, they cause inflammation. Such larvae may facilitate opportunistic bacterial invasion by obstructing airways, dispersing bacteria throughout the lungs, and/or causing immunosuppression (Demartini and Davies 1977; Spraker et al. 1984). Conversely, some feel that the nematodes or the tissues they damage may produce anti-bacterial substances (Vazquez 1975); it is possible, however, that bacteria are seldom recovered from the caudal lobes, where *P. stilesi* dwells, simply due to the cranio-ventral distribution of bacterial bronchopneumonia.

The lungworm, *Protostrongylus stilesi*, was present in both ewes, and *P. odocoilei* was likely present in Ewe 1 and Ram 1 (Table 1). It is unclear why Ewe 1, with many eggs and larvae present in lung histological sections, was negative for larvae on fecal parasitology. Fecal larval shedding may be decreased in chronically debilitated animals, although this has not been reported in the literature. On histology, these parasites were responsible for focal granulomatous inflammation, and in the cranial lobes, larvae and eggs were sometimes associated with bacterial abscesses. Therefore, these protostrongylids may have played a predisposing role in the pneumonia, but the proximate cause of death was no doubt the bacterial bronchopneumonia and septicaemia.

Gastro-intestinal parasites, adults of which have yet to be quantified, may have contributed to the poor body condition of the animals and thereby increased their susceptibility to pneumonia; 1 study on Montana bighorns suggests that gastro-intestinal parasites may act as a stress factor (Worley and Seesee 1992).

Viruses

Immunohistochemistry for bovine respiratory viruses was performed because they have been associated with bighorn pneumonia, and are thought to play a predisposing role in domestic sheep *Mannheimia haemolytica* pneumonia (Brogden et al. 1998). PI_3 virus has been recovered from captive and wild bighorn sheep dying from pneumonia in Wyoming and Colorado (Parks et al. 1972 and Spraker 1979, respectively), and nasal swabs of healthy desert bighorns (Clark et al. 1985). BRSV has also been implicated in the bighorn pneumonia complex (Spraker and

Collins 1986; Aguirre and Starkey 1994) and there are seropositive bighorn herds (Clark et al. 1985; Dunbar et al. 1985). IBR has been recovered from nasal swabs of healthy bighorn sheep (Clark et al. 1993), and some desert bighorn sheep were seropositive for this virus (Clark et al. 1985), as well as BVD. In Dall's sheep populations, only 1% of Alaskan Dall's sheep were seropositive for PI_3, but negative for IBR and BVD (Foreyt et al. 1983). The source of PI_3 for Alaskan Dall's sheep was most likely domestic sheep and goats in the Alaskan interior, which are also the likely source of contagious ecthyma in Alaskan Dall's sheep (Zarnke et al. 1983).

Both ewes were negative on immunohistochemistry for the bovine viruses, and it is unlikely that Mackenzie Mountain Dall's sheep have been exposed to any domestic cattle pathogens. We cannot completely rule out a viral component to the pneumonia, however, because immunohistochemistry for cattle viruses would probably not detect viruses specific to Dall's sheep. As well, it is conceivable that a virus present earlier in the disease process would not be detected in tissue in the terminal stages of the pneumonia. In future, when possible, we will do serological testing for viral pathogens.

Stress

Numerous stressors in bighorn sheep populations have been implicated in triggering die-offs, including overcrowding, decreased nutrition, loss of escape cover, harassment, noise, domestic animals, high snowfall, and high dust levels (Bunch et al. 1999). As well, some authors propose that loss of chase predators, which cull sheep heavily infected with lungworm, may lead to decreased sheep population quality and mass die-offs (Geist 1971; Uhazy et al. 1973). Most of the stressors present in bighorn populations are absent in the Northwest Territories, with the exception of some mineral exploration, aircraft intrusion, and light hunting pressure, and there has not been substantial attrition of predator (wolf and grizzly) populations (Poole and Graf 1985; Veitch and Simmons 1999; Veitch et al. 2000).

Both ewes were in poor body condition, and low bone marrow fat content (45% and 51%), particularly in midsummer, indicated severe nutritional stress (Mech and Delgiudice 1985). Both ewes were lactating, which may have increased energy demands. One of the consequences of reproduction in bighorn ewes is decreased resistance to parasites and pathogens (Festa-Bianchet 1989). The dental anomalies may well have contributed to the poor body condition of the second ewe by decreasing her foraging ability (Glaze et al. 1982).

The case reports described here are consistent with the classic pathological description of pneumonia in bighorn sheep. We observed a few differences in the microbiological fauna, namely an unusual *Mannheimia haemolytica*-like species that is probably specific to Dall's sheep, and a possible role for *P. odocoilei*. Mackenzie Mountain Dall's sheep harbor most of the necessary endemic microbiological ingredients for wild sheep pneumonia, including the newly recognized *P. odocoilei*, but the pattern of mortality is thus far one of sporadic, isolated cases and not a widespread, all-age die-off. This is most likely attributable to the lack of anthropogenic stressors (relative to bighorn sheep) and opportunities for transmission of domestic sheep and cattle pathogens within the Mackenzie Mountain Dall's sheep population. The current situation for Dall's sheep in the NWT may reflect that of bighorn sheep in western Canada and the United States before habitat loss, decreased suitability of available habitat, pathogen

introduction, translocations, and loss of chase predators weakened these wild sheep populations. Thinhorn sheep managers and researchers in northern North America should continue to strive to keep their populations free of man-made stressors and introduced pathogens. Even so, natural stressors and global climate change may shift the equilibrium of host-pathogen relationships in Mackenzie Mountain Dall's sheep, and thus continued population health monitoring and baseline parasitological data collection in the Northwest Territories is necessary.

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LITERATURE CITED

AGUIRRE, A. A., AND E. E. STARKEY. 1994. Wildlife disease in U.S. National Parks: historical and co-evolutionary perspectives. Conservation Biology 8:654-661.

BOWYER, R. T., AND D. M. LESLIE, JR. 1992. *Ovis dalli*. Mammalian Species 393:1-7.

BROGDEN, K. A., H. D. LEHMKUHL, AND R. C. CUTLIP. 1998. *Pasteurella haemolytica* complicated respiratory infections in sheep and goats. Veterinary Research 29:233-254.

BUECHNER, H. K.1960. The bighorn sheep in the United States, its past, present, and future. Wildlife Monograph No. 4:1-174.

BUNCH, T. D., W. M. BOYCE, C. P. HIBLER, W. R. LANCE, T. R. SPRAKER, AND E. S. WILLIAMS. 1999. Diseases of North American wild sheep. Pp. 209-238 *in* Mountain Sheep of North America, R. Valdez and P. R. Krausman, editors. University of Arizona Press, Tucson, Arizona, USA.

CLARK, G. W., AND D. A. COLWELL. 1974. *Eimeria dalli*: new species of protozoan (Eimeriidae) from Dall sheep, *Ovis dalli*. Journal of Protozoology 21:197-199.

CLARK, R. K., D. A. JESSUP, M. D. KOCK, AND R. A. WEAVER. 1985. Survey of desert bighorn sheep in California for exposure to selected infectious diseases. Journal of the American Veterinary Medical Association 11:1175-1179.

_____, C. A. WHETSTONE, A. E. CASTRO, M. M. JORGENSON, J. F. JENSEN, AND D. A. JESSUP. 1993. Restriction endocnuclease analysis of herpesviruses isolated from two peninsular bighorn sheep (*Ovis canadensis cremnobates*). Journal of Wildlife Diseases 29:50-56.

COX, D. D., AND A. C. TODD. 1962. Survey of gastrointestinal parasitism in Wisconsin dairy cattle. Journal of the American Veterinary Medical Association 141:706-709.

DEMARTINI, J. C., AND R. B. DAVIES. 1977. An epizootic of pneumonia in captive bighorn sheep infected with *Muellerius* sp. Journal of Wildlife Diseases 13:117-124.

DUNBAR, M. R., D. A. JESSUP, J. F. EVERMANN, AND W. J. FOREYT. 1985. Seroprevalence of respiratory syncytial virus in free-ranging bighorn sheep. Journal of the American Veterinary Medical Association 11:1173-1175.

FESTA-BIANCHET, M. 1989. Individual differences, parasites, and the costs of reproduction for bighorn ewes. Journal of Animal Ecology 58:785-795.

FOREYT, W. J., AND D. A. JESSUP. 1982. Fatal pneumonia of bighorn sheep following association with domestic sheep. Journal of Wildlife Diseases 18:163-168.

_____, R. M. SILFLOW AND J. E. LAGERQUIST. 1996. Susceptibility of Dall's sheep *(Ovis dalli dalli*) to pneumonia caused by *Pasteurella haemolytica*. Journal of Wildlife Diseases 32:586-593.

_____, T. C. SMITH, J. F. EVERMANN AND W. E. HEIMER. 1983. Hematologic, serum chemistry and serologic values of Dall's sheep (*Ovis dalli dalli*) in Alaska. Journal of Wildlife Diseases 19:136-139.

_____, K. P. SNIPES AND R. W. KASTEN. 1994. Fatal pneumonia following inoculation of healthy bighorn sheep with *Pasteurella haemolytica* from healthy domestic sheep. Journal of Wildlife Diseases 20:137-145.

FORRESTER, D. J. 1971. Bighorn sheep lungworm-pneumonia complex. Pp.158-173 *in* Parasitic diseases of wild mammals, J. W. Davis and R. C. Anderson, editors. Iowa State University Press, Ames, Iowa, USA,

FORRESTER, S. G., AND M. W. LANKESTER. 1997. Extracting protostrongylid nematode larvae from ungulate feces. Journal of Wildlife Diseases 33: 511-516.

GEIST, V. 1971. Mountain sheep: a study in behavior and evolution. University of Chicago Press, Chicago, Illinois, USA.

GLAZE, R. L., M. HOEFS, AND T. D. BUNCH. 1982. Aberrations of the tooth arcade and mandible in Dall's sheep from southwestern Yukon. Journal of Wildlife Diseases 18:305-309.

HAINES, D. M., AND B. J. CHELACK. 1991. Technical considerations for developing enzyme immunohistochemical staining procedures on formalin-fixed paraffin-embedded tissues for diagnostic pathology. Journal of Veterinary Diagnostic Investigation 3:101-112.

HEIMER, W. E., R. L ZARNKE, AND D. J PRESTON. 1982. Disease surveys in Dall's sheep in Alaska. Biennial Symposium of the Northern Wild Sheep and Goat Council 3:188-197.

HOBERG, E. P., L. POLLEY, A. GUNN, AND J. S. NISHI. 1995. *Umingmakstrongylus pallikuukensis* gen.nov. et sp.nov. (Nematoda: Protostrongylidae) from muskoxen, *Ovibos moschatus*, in the central Canadian Arctic, with comments on biology and biogeography. Canadian Journal of Zoology 73:2266-2282.

HOEFS, M., AND T. D. BUNCH. 2001. Lumpy jaw in wild sheep and its evolutionary implications. Journal of Wildlife Diseases 37:39-48.

KUTNY, L., AND G. STENHOUSE. 1991. Age determination and lumpy jaw evaluation of Dall's sheep harvested by non-residents in the Mackenzie Mountains, 1987. Department of Renewable Resources Manuscript Report No. 35, Inuvik, NT. 12 pp.

KUTZ, S. J., A. M. VEITCH, E. P. HOBERG, B. T. ELKIN, E. J. JENKINS, AND L. POLLEY. 2001. New host and geographic records for two protostrongylids in Dall's sheep. Journal of Wildlife Diseases, In Press.

MARSH, H. 1938. Pneumonia in Rocky Mountain bighorn sheep. Journal of Mammalogy 19:214-219.

MECH, L. D., AND G. D. DELGIUDICE. 1985. Limitations of the marrow-fat technique as an indicator of body condition. Wildlife Society Bulletin 13:204-206.

NEILAND, K. A. 1972. Sheep disease studies. Alaska Department of Fish and Game, Juneau, Alaska, Project W-17-3, 32 pp.

NICHOLS, L., AND F. BUNNELL.1999. Natural history of thinhorn sheep. Pp. 23-77 *in* Mountain Sheep of North America, R. Valdez and P. Krausman, editors. University of Arizona Press, Tucson Arizona, USA,

PARKS, J. B., G. POST AND E. T. THORNE. 1972. Isolation of parainfluenza virus from Rocky Mountain bighorn sheep. Journal of the American Veterinary Medical Association 161:669-672.

PLATT, T. R., AND W. M. SAMUEL. 1978. *Parelaphostrongylus odocoilei*: life cycle in experimentally infected cervids including the mule deer, *Odocoileus h. hemionus*. Experimental Parasitology 46:330-338.

POOLE, K. G., AND R. P. GRAF. 1985. Status of Dall's sheep in the Northwest Territories, Canada. Pp. 35-42 i*n* Wild sheep: distribution, abundance, management, and conservation of the sheep of the world and closely related mountain ungulates, ed. M. Hoefs. Special Report of Northern Wild Sheep and Goat Council, Whitehorse, Yukon, Canada.

PYBUS, M. J., AND W. M. SAMUEL.1984*a*. Lesions caused by *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea) in two cervid hosts. Veterinary Pathology 21:425-431.

_____, and _____. 1984*b*. *Parelaphostrongylus andersoni* (Nematoda: Protostrongylidae) and *P. odocoilei* in two cervid definitive hosts. The Journal of Parasitology 70:507-515.

QUEEN, C., A. C. S. WARD, AND D. L. HUNTER. 1994. Bacteria isolated from nasal and tonsillar samples of clinically healthy Rocky Mountain bighorn and domestic sheep. Journal of Wildlife Diseases 30:1-7.

SAMSON, J., J. C. HOLMES, J. T. JORGENSON, AND W. D. WISHART. 1987. Experimental infections of free-ranging Rocky Mountain bighorn sheep with lungworms (Protostrongylus spp,; Nematoda: Protostrongylidae). Journal of Wildlife Diseases 23:396-403.

SCHWANTJE, H. M. 1986. A comparative study of bighorn sheep herds in southeastern British Columbia. Northern Wild Sheep and Goat Council 5: 231-252.

_____. 1988. Evaluation of health status of Rocky Mountain sheep (*Ovis canadensis canadensis*) in southeastern British Columbia. MSc Thesis. University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

SIMMONS, N. M., N. B. BAYER, AND L. O. SINKEY. 1984. Demography of Dall's sheep in the Mackenzie Mountains, Northwest Territories. The Journal of Wildlife Management 48:156-162.

SPRAKER, T. R. 1979. The pathogenesis of pulmonary protostrongylosis in bighorn lambs. PhD Dissertation. Colorado State University, Fort Collins, Colorado, USA.

_____, AND J. K. COLLINS. 1986. Isolation and serologic evidence of a respiratory syncytial virus in bighorn sheep from Colorado. Journal of Wildlife Diseases 22:416-418.

_____, C. P. HIBLER, G. G. SCHOONVELD, AND W. S. ADNEY. 1984. Pathologic changes and microorganisms found in bighorn sheep during a stress-related die-off. Journal of Wildlife Diseases 20:319-327.

UHAZY, L. S., J. C. HOLMES, AND J. G. STELFOX. 1973. Lungworms in the Rocky Mountain bighorn sheep of western Canada. Canadian Journal of Zoology 51:817-824.

VAZQUEZ, R. J. 1975. English abstract of Spanish paper. The relationship between Protostrongylinae and aerobic bacteria in the lungs of sheep. Trabajos de la Estacion Agricola Experimental de Leon 12:29-79.

VEITCH, A. M. 1996. History of transplanting mountain goats and mountain sheep – Northwest Territories. Northern Wild Sheep and Goat Council 10:188-189.

_____, AND N. M. SIMMONS. 1999. Thinhorn sheep: Northwest territories. Pp. 54-58 *in* Return of Royalty; Wild Sheep of North America, D. E. Toweill and V. Geist, editors. Boone and Crockett Club and Foundation for North American Wild Sheep, Missoula, Montana, USA.

_____, E. SIMMONS, J. ADAMCZEWSKI, AND R. POPKO. 2000. Status, harvest, and co-management of Dall's sheep in the Mackenzie Mountains, NWT. Northern Wild Sheep and Goat Council 11:134-153.

WORLEY, D. E., AND F. M. SEESEE. 1992. Gastrointestinal parasites of bighorn sheep in western Montana and their relationship to herd health. Northern Wild Sheep and Goat Council 8:202-212.

ZARNKE, R. L., R. A. DIETERICH, K. A. NEILAND, AND G. RANGLACK. 1983. Serologic and experimental investigations of contagious ecthyma in Alaska. Journal of Wildlife Diseases 19:170-174.