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PROTOSTRONGYLID PARASITES AND PNEUMONIA IN CAPTIVE AND WILD THINHORN SHEEP (*OVIS DALLI*)

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ABSTRACT: We describe health significance of protostrongylid parasites (*Parelaphostrongylus odocoilei* and *Protostrongylus stilesi*) and other respiratory pathogens in more than 50 naturally infected Dall's sheep (*Ovis dalli dalli*) from the Mackenzie Mountains, Northwest Territories (1998–2002) as well as in three Stone's sheep (*O. d. stonei*) experimentally infected with *P. odocoilei* (2000–2002). Histological lesions in the brain and distribution of *P. odocoilei* in the muscles of experimentally and naturally infected sheep were consistent with a previously hypothesized "central nervous system to muscle" pattern of migration for *P. odocoilei*. Dimensions of granulomas associated with eggs of *P. odocoilei* and density of protostrongylid eggs and larvae in the cranial lung correlated with intensity of larvae in feces, and all varied with season of collection. Prevalence of *P. stilesi* based on the presence of larvae in feces underestimated true prevalence (based on examination of lungs) in wild Dall's sheep collected in summer and fall. Similarly, counts of both types of protostrongylid larvae in feces were unreliable indicators of parasitic infection in wild Dall's sheep with concomitant bacterial pneumonia associated with *Arcanobacterium pyogenes*, *Pasteurella* sp., and *Mannheimia* sp. Diffuse, interstitial pneumonia due to *P. odocoilei* led to fatal pulmonary hemorrhage and edema after exertion in one experimentally infected Stone's sheep and one naturally infected Dall's sheep. Bacterial and verminous pneumonia associated with pathogens endemic in wild Dall's sheep in the Mackenzie Mountains caused sporadic mortalities. There was no evidence of respiratory viruses or bacterial strains associated with domestic ruminants, from which this population of wild sheep has been historically isolated.

Key words: Bacterial, neural, Northwest Territories, *Ovis dalli*, population health, protostrongylid, thinhorn sheep, verminous pneumonia.

INTRODUCTION

In 1998, the protostrongylids *Parelaphostrongylus odocoilei* and *Protostrongylus stilesi* were discovered in Dall's sheep (*Ovis d. dalli*) in the Mackenzie Mountains (64°N, 128°W), Northwest Territories (NT), Canada (Kutz et al., 2001). Subsequent widespread survey of Dall's sheep within the Mackenzie Mountains and elsewhere in Subarctic North America revealed a prevalence of infection with these parasites approaching 100% (Jenkins et al., 2005a). In 1999, the first cases of fatal bacterial pneumonia were observed in Dall's sheep in the Mackenzie Mountains (Jenkins et al., 2000). Despite the value of this resource (14,000–26,000 Dall's sheep in a 130,000-km^2 area) for subsistence harvest and trophy hunting (Veitch et al., 1998), little is known about disease-related mortalities in this or any other population of thinhorn sheep (Dall's and Stone's sheep, *O. d. stonei*) across their range in northwestern North America.

Protostrongylid lungworms (*P. stilesi*, *P. rushi*, and, less commonly, *Muellerius capillaris*) play a controversial role in a multifactorial pneumonia complex that causes "die-offs" in bighorn sheep (*Ovis*

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canadensis) throughout North America (Demartini and Davies, 1977; Spraker et al., 1984; Samson et al., 1987; Monello et al., 2001). The combination of lungworms, bacteria, viruses, and natural or anthropogenic stressors is likely of greater importance in pneumonia in wild sheep than any single pathogen alone, other than introduced bacterial strains, which may act as primary pathogens in naïve populations. *Parelaphostrongylus odocoilei* may cause respiratory disease in ungulate hosts (e.g., cervids and mountain goats), because adults in the muscles produce eggs that travel through the vasculature to the lungs (Brunetti, 1969; Platt and Samuel, 1978; Pybus and Samuel, 1984; Pybus et al., 1984). In Dall's sheep in the Mackenzie Mountains, *P. odocoilei* and *P. stilesi* may cause additive or even synergistic pulmonary damage (Kutz et al., 2001).

To isolate the effects of *P. odocoilei*, we experimentally infected thinhorn sheep and described the first neurological syndrome associated with *P. odocoilei* in any definitive host (Jenkins et al., 2005b). This led us to hypothesize a neural migration for this "muscleworm," but confirmation awaited demonstration of parasites and characteristic lesions in the central nervous system. Based on respiratory and neural pathogenicity in thinhorn sheep, *P. odocoilei* has the potential to contribute to fatal pneumonia and impact health at the population level.

In the current study, we describe parasite localization and associated pathology in three captive Stone's sheep experimentally infected with *P. odocoilei*, and in >50 wild Dall's sheep collected from the Mackenzie Mountains between 1998 and 2002. This is the first investigation of the pathology and seasonal patterns of prevalence and intensity of protostrongylid parasites in the lungs of thinhorn sheep, and it complements descriptions of seasonal patterns of larval shedding in feces of Dall's sheep in the Mackenzie Mountains (Jenkins et al., 2006). In addition, we investigated mortality due to disease in this population of Dall's sheep, with targeted sampling for pathogens that have been implicated in respiratory disease in domestic, bighorn, and captive Dall's sheep (for review, see Garde et al., 2005), or for pathogens that have been identified in wild Dall's sheep in Alaska through serologic surveys (Foreyt et al., 1983; Zarnke, 2000). To our knowledge, this is the first investigation of population health in wild Dall's sheep.

MATERIALS AND METHODS

Experimentally infected Stone's sheep

Three adult Stone's sheep (SS1, SS2, and SS3) were each infected with 200 third-stage larvae of *P. odocoilei* originating from first-stage larvae (L1) in feces of Dall's sheep in the Mackenzie Mountains (Jenkins et al., 2005b). Before infection with *P. odocoilei*, larvae of *Protostrongylus* spp. were present at low densities in pooled feces from SS2 and SS3. Each sheep was examined using bronchoscopy and bronchoalveolar lavage (BAL) on at least two occasions before infection. Sheep were chemically immobilized, the trachea and larger bronchi were visually examined with a fiberoptic endoscope, and 3 × 50 ml of sterile saline was inserted rapidly into the lower lung via a plastic tube and immediately aspirated (adapted from Begin et al., 1981; Silflow and Foreyt, 1988; Meyer et al., 1998). After infection, SS1 was examined using bronchoscopy and BAL 7 times at 2- to 4-wk intervals from 28 to 161 days postinfection (dpi), whereas SS2 and SS3 were examined on 35, 63, and, for SS3 only, 91 dpi.

Analyses of BAL fluid included determination of total protein levels, a differential count of 100 (SS1) or 200 (SS2 and SS3) nucleated cells stained with Diff-Quick® (Dade Behring, Newark, Delaware, USA), and, in SS2 and SS3, the proportion of 100 macrophages that stained with Perl's Prussian blue for hemosiderin (hemosiderophages). BAL fluid from SS1 was examined for L1 at 25× magnification, whereas BAL fluid from SS2 and SS3 was centrifuged at 1,200 rpm at 4 C for 12 min. Sediment was resuspended in 2% formalin, centrifuged again, and resuspended in a drop of methylene blue and examined for protostrongylid eggs and L1 at 40–100× (modified Knott's test).

For SS1, thoracic radiographs were obtained at each BAL procedure. Images of transverse sections (7 mm, and also 1 mm at

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126 and 173 dpi) through the entire lung field were obtained using a High Speed Computed Tomography/i® helical scanner (GE Medical Systems, Milwaukee, Wisconsin, USA) at the Royal University Hospital, Saskatoon, Saskatchewan (SK), Canada, at 37 days before infection, at 70 and 126 dpi, and on excised, inflated lungs at 173 dpi (Cadore et al., 1994; Kutz et al., 1999; Kutz et al., 2004).

A routine necropsy was performed on each sheep, involving gross and histologic examination of all major organs, including the brain and meninges. The spinal cord and canal were examined under a dissecting scope, and for SS3 only, sections of spinal cord were taken for histology. Adult nematodes were recovered from characteristic hemorrhages in 5-mm sections of the entire skeletal musculature (as per Kutz et al., 2001) and identified as *P. odocoilei* as described in Jenkins et al. (2005b). Larvae were recovered from rectal feces and quantified using a modified beaker Baermann technique (Forrester and Lankester, 1997; Jenkins et al., 2005a). Samples from lungs of all three sheep were cultured for bacteria, and for SS2 and SS3; samples were examined using immunohistochemistry for respiratory syncytial virus (RSV), parainfluenza III (PI3), and infectious bovine rhinotracheitis (IBR) (Haines and Chelack, 1991).

"Healthy" Dall's sheep from Mackenzie Mountains

In 2000, 2001, and 2002, we sent kits and instructions to the eight outfitters who guide nonresident hunters in the Mackenzie Mountains, NT, who collected 2×2-cm samples from the dorsum of the left cranial and caudal (diaphragmatic) lung lobes, feces, blood (pooled in the chest cavity), and throat swabs (Cary-Blair or Amies transport media, BBL CultureSwab®, Becton Dickinson, Sparks, Maryland, USA). We also received submissions of lungs (entire) and feces from Dall's sheep taken by resident hunters. Samples were frozen within 48 hr of collection and shipped by air to the Western College of Veterinary Medicine (WCVM), Saskatoon, SK.

In November 2001, three ewes and one young ram (FC23–26) were opportunistically selected from the Katherine Creek area in the Mackenzie Mountains (65°01′N, 127°35′W) and shot. Samples were collected as described above but processed immediately, including direct inoculation of swabs on to agar plates for culture, and separation of sera from whole blood. Washes of the airways were examined for adult lungworms (Oakley, 1980), and adult *P. odocoilei* were recovered from the left half of each carcass and archived in the United States National Parasite Collection (USNPC accession nos. 98890–98893). The brains, meninges, spinal cords, and spinal canals were examined grossly, and sections of the brains were fixed for histology. We also obtained data and slides of lung tissue from six adult ewes from the Mackenzie Mountains collected in October 1998 (FC1–3) and April 1999 (FC5–7) (Kutz et al., 2001). These "straggler" ewes, slower than others in the herd, were selected to maximize chances of parasite recovery.

Sick and dead Dall's sheep from Mackenzie Mountains

Reports of one to three coughing, dyspneic, and lethargic rams with nasal discharge were received in each of September 1999, July 2001, and September 2002. In two of these instances, we inspected lung samples from affected rams (SK13 and SK18) by using histologic examination and culture. Twelve carcasses of Dall's sheep found in the Mackenzie Mountains between 1999 and 2002 (SC15, FC21, FC27, and all with identifier MO [for mortality]) were kept cool and shipped within a few days to WCVM for routine necropsy. We determined cause of death and pathogens present (by using bacterial culture, immunohistochemistry, and parasitologic techniques), and we estimated body condition by using the marrow-fat technique (Mech and Delgiudice, 1985).

Processing of samples

When the entire lungs were available, the parenchyma was examined grossly for lesions, and airways were examined for adult lungworms. Tissue samples for histologic examination were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin by Prairie Diagnostic Services (PDS), Saskatoon, SK, Canada. Histologic sections from cranial and caudal lung were examined for protostrongylid eggs, larvae, and adult nematodes at 100× magnification. Adult nematodes in lung parenchyma and *Protostrongylus* spp. larvae in feces were assumed to be *P. stilesi*, the only lung-dwelling nematode identified in Dall's sheep in the Mackenzie Mountains, NT (Kutz et al., 2001). Basophilic, loosely morulated eggs of *P. odocoilei* were clearly distinguishable from eosinophilic, dense, freshly deposited eggs of *P. stilesi* (Fig. 1), although larvae could not be differentiated unless the tails were visible (Kutz et al., 2001). Protostrongylid eggs and larvae in 10 arbitrarily chosen 100× fields in the cranial lung section were counted to provide an estimate of parasite density. The

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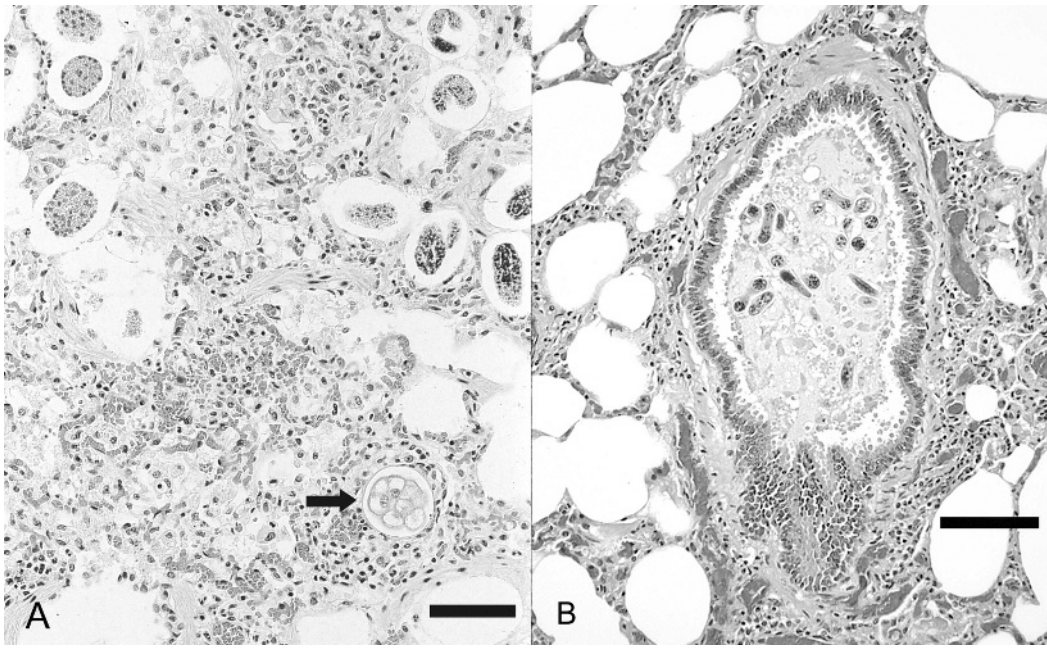


FIGURE 1. Protostrongylid parasites in histological sections of the lungs of wild Dall's sheep found dead in the Mackenzie Mountains, Northwest Territories, Canada. (A) Dense, eosinophilic eggs and developing larvae of *P. stilesi* and loosely morulated, basophilic egg of *P. odocoilei* (arrow) in lung parenchyma of SC15 (see Table 1). Bar=50 μm. (B) Larvae trapped in debris in bronchiole of sheep with bacterial bronchopneumonia (MO8; see Table 3). Bar=100 μm.

dimensions (length and width) of the largest granuloma surrounding eggs of *P. odocoilei* observed in each section of cranial lung were measured using a calibrated slide micrometer (Pybus and Samuel, 1984). When examining histologic sections of lung, the observer did not know the results of Baermann analyses for protostrongylid larvae in matching fecal samples. For all Dall's sheep, the prevalence of infection with each protostrongylid was determined separately for lung and fecal samples, and the two methods were combined to obtain the overall prevalence. For Dall's sheep without bacterial pneumonia, density and dimensions of granulomas in the cranial lung were compared with intensity of L1 of *P. odocoilei* in feces by using Pearson's correlation coefficient (two-tailed, significant at the 0.01 level) (Statistical Package for the Social Sciences 12.0, SPSS Inc., Chicago, Illinois, USA). Histologic sections were archived in the department of Veterinary Pathology at the Western College of Veterinary Medicine (accessible through the PDS database).

Bacteria isolated from throat swabs and lung samples were identified to genus, and when possible, to species level, by using standard techniques by PDS and the Bacteriology Research Laboratory, WCVM. Sera from four sheep collected in November 2001 were tested for antibodies to *Mannheimia haemolytica* (serotypes A1 and A2) and *Pasteurella trehalosi* (T10) in direct agglutination assays as well as a leukotoxin neutralizing assay, by the Department of Pathobiology, Ontario Veterinary College, Guelph, Ontario, Canada. Sera from these four animals and chest fluid from seven sheep killed by hunters in 2001 were tested by PDS for antibodies to respiratory viruses (RSV, PI3, and IBR), bovine viral diarrhea (BVD), maedi-visna virus, and *Mycobacterium paratuberculosis*. Histologic sections from the lungs of seven sheep with bacterial pneumonia were stained using immunohistochemistry for antigens of *M. haemolytica* and respiratory viruses (IBR, PI3, and BRSV) as well as *Mycoplasma* spp. ($n=5$), *Mycobacterium* spp. ($n=2$), *Haemophilus somnus* ($n=2$), and BVD ($n=2$).

RESULTS

Experimentally infected Stone's sheep

There were no atypical findings on bronchoscopy or on thoracic radiography

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TABLE 1. *Parelaphostrongylus odocoilei* in lung, feces, and muscles of experimentally infected Stone's sheep (SS1–3) and naturally infected, "healthy" Dall's sheep collected in spring (SC) and fall (FC) from the Mackenzie Mountains, Northwest Territories (NT), 1998–2002.

ID	Collection	Age (yr)	Sex	Density in lung[a]	Granuloma size in lung[b]	Fecal DSLPG[c]	Muscle, No. worms	Percent worms recovered				Nematode M:F ratio
								Axial	Trunk	Hindquarters	Forelimbs	
SS1	173 dpi	2	Male	27	1,400	3,508	75	37	13	33	16	0.8
SS2	87 dpi	16	Female	27	88	1,187	27	33	33	11	22	0.8
SS3	92 dpi	15	Female	60	399	4,499	14	14	21	64	0	1
SC15	April	0.9	Female	27	588	187						
SC5	April	2.5	Female	60	840	5,993	17	12	0	53	35	0.4
SC6	April	Adult	Female	60	880	1,983	30	17	0	40	43	1.3
SC7	April	7	Female	60	2,000	2,293	48	13	15	33	40	1.7
FC27	August	1.3	Female	2	121	0						
FC21	September	5	Female	8	196	484						
FC1	October	Adult	Female	9	140	896	6[d]	17	0	17	67	0.7
FC2	October	Adult	Female	6	168	198	3[d]	67	0	0	33	1
FC3	October	Adult	Female	8	150	408	0[d]	0	0	0	0	
FC23	November	5.5	Female	7	224	157	11[e]	18	18	36	27	1.3
FC24	November	1.5	Male	40	340	794	18[e]	22	17	39	22	2
FC25	November	11.5	Female	27	160	1,301	28[e]	0	29	21	50	1.7
FC26	November	4.5	Female	8	143	220	6[e]	17	0	50	33	3

[a] Number of protostrongylid eggs and larvae in 10 fields at 100× magnification in cranial lung.

[b] Length × width × 100 μm^2 of largest granuloma surrounding egg(s) of *P. odocoilei* in cranial lung.

[c] Dorsal-spined larvae of *P. odocoilei* per gram wet feces.

[d] Right hind leg not examined, first attempt at recovery.

[e] Only left side of carcass examined.

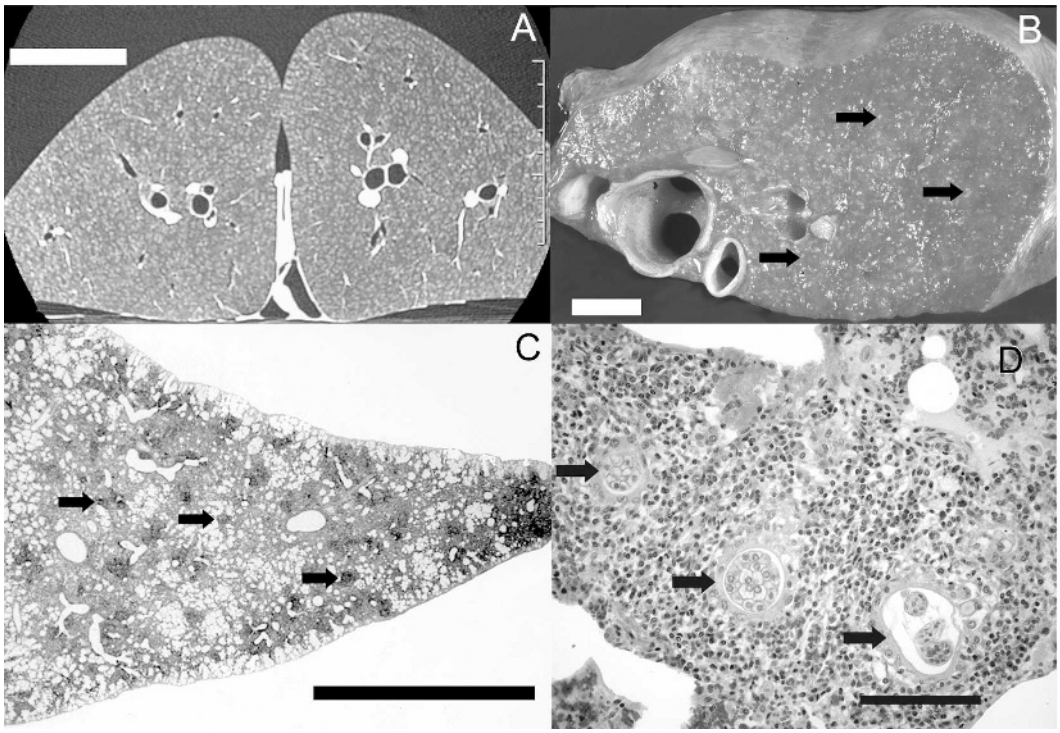


FIGURE 2. Granulomas associated with eggs and larvae of *P. odocoilei* in the lungs of an experimentally infected captive Stone's sheep (SS1; see Table 1). (A) Computed tomography scan of excised lungs (1-mm section). Solid white bar=5 cm. (B) Cut section at necropsy, arrows indicate granulomas. Bar=1 cm. (C) Histological section, subgross, arrows indicate granulomas. Bar=5 mm. (D) Histologic section, 400×, arrows indicate eggs and developing larva of *P. odocoilei* within a granuloma. Bar=100 μm.

of SS1. A diffuse reticulonodular infiltrate was first observed on CT of the lungs at 126 dpi, and SS1 died of respiratory failure following exertion at 173 dpi (101 days after patency). At necropsy, nodules observed on computed tomography (CT) images of excised lungs corresponded with grossly visible granulomas that, on histological examination, consisted of lymphocytes, plasma cells, and macrophages surrounding three to 13 eggs and larvae of *P. odocoilei* (Fig. 2). In addition to this generalized, granulomatous, interstitial pneumonia, there was diffuse pulmonary edema and hemorrhage (acute and chronic), with petechial and ecchymotic hemorrhages visible on the pleural surface of the lung.

Sheep SS2 was euthanized at 87 dpi (19 days after patency) after developing bacterial pneumonia and unilateral hindlimb "lameness," recumbency, and behavioral changes. Chronic, locally extensive, fibrinopurulent bronchopneumonia and pleuritis were confined to the ventral portions of the cranial lobes and to the right middle lung lobe, from which *Pasteurella* sp. and *Corynebacterium* sp. were isolated. There was little inflammatory response surrounding eggs of *P. odocoilei* in the lungs. On histologic examination of the cerebellum, there were discrete, multiple foci of eggs and developing larvae of *P. odocoilei* associated with encephalomalacia, and, at one site, hemorrhage (Fig. 3A, B). There was lymphoplasmacytic cuffing of blood vessels nearby and in the choroid plexus, and there were also plasma cells and hemosiderophages in the meninges of the medulla. Sheep SS3 died at 92 dpi (19 days after patency) of unknown causes, possibly

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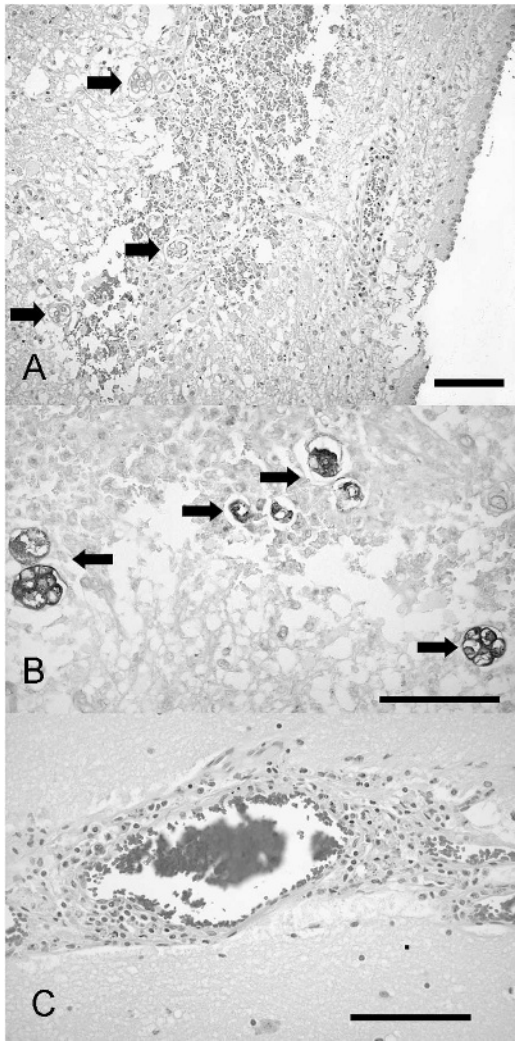


FIGURE 3. Neural lesions associated with *P. odocoilei* in experimentally infected Stone's sheep (SS2; see Table 1) and naturally infected Dall's sheep (SC15; see Table 1). Bars=100 μm. (A) Hemorrhage and encephalomalacia in cerebellum of SS2, with intralesional eggs of *P. odocoilei* (arrows). (B) Eggs of *P. odocoilei* (arrows) in section adjoining that shown in a, stained with Alcian blue periodic acid Schiff. (C) Eosinophils, lymphocytes, plasma cells, and hemosiderophages surround a blood vessel in the cerebral cortex of SC15.

as a complication of anesthesia, with high densities of eggs and larvae of *P. odocoilei* in the lungs (Table 1). There was no evidence of infection with respiratory viruses in SS2 or SS3.

Before experimental infection of the three Stone's sheep, the majority of cells recovered from BAL were macrophages (70–99%) and small mononuclear cells/lymphocytes (0–29%), with few neutrophils (generally <10%), eosinophils (generally ≤2.5%), and hemosiderophages (≤2 of 100 macrophages). In BAL fluid from SS1, increased numbers of hemosiderophages were observed starting at 56 dpi, larvae of *P. odocoilei* were detected at 77 dpi, and total protein increased starting at 105 dpi. In BAL fluid from SS2, 27 protostrongylid eggs (40 × 80 μm) were observed at 35 dpi, and one egg was present at 63 dpi. In BAL fluid from SS3, *Protostrongylus* spp. larvae were observed before, but not after, infection with *P. odocoilei*. One protostrongylid egg was recovered from BAL fluid at 63 dpi, and larvae of *P. odocoilei* and increased numbers of hemosiderophages (23 of 100 macrophages) were present in BAL fluid at 91 dpi.

In the muscles of SS1, SS2, and SS3, adult *P. odocoilei* were associated with inflammatory infiltrates and chronic hemorrhage in fascial planes. Tails of female nematodes were frequently observed inside blood vessels. In SS1, adult worms were evenly distributed throughout the body; in SS2, adults were predominantly in the axial and trunk muscles; and in SS3, adults were predominantly in the hindquarters (Table 1).

"Healthy" Dall's sheep from Mackenzie Mountains

Lung samples from 47 Dall's sheep with no evidence of bacterial pneumonia (Table 2) were examined from seven of the eight outfitting zones in the Mackenzie Mountains (primarily from the northern half). When the whole lungs were available (n=20), petechial and ecchymotic hemorrhages were observed throughout the pleural surface, and firm, pale, consolidated lesions typical of *P. stilesi* were grossly visible in the parenchyma of the dorsum and apices of the diaphragmatic lobes. Chronic fibrous adhesions between the lung and chest wall were reported or observed in a few sheep. No airway-

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TABLE 2. Prevalence (%) and intensity of *P. odocoilei* and *P. stilesi* in lungs and feces of Dall's sheep from the Mackenzie Mountains, NT, 1998–2002.

Collection	*n*	Sex	Age (yr)[a]	*P. odocoilei* (%)			*P. stilesi* (%)			Density in lung[a,b]	Granuloma size[a,c]	Fecal DSLPG[a,d]	Fecal PrLPG[a,d]
				Lungs	Fecal	Overall	Lungs	Fecal	Overall				
Killed by NH[e]	27	Male	10.1	93	96	100	93	63	96	8	114	109	155
July–August 2000–02			(7.5–12.5)							(0–50)	(49–330)	(2–748)	(5–1,075)
Killed by RH[f]	7	6 male/1 female	5.7	100	100	100	86	57	86	11	158	182	51
August–October 1999–2002			(3.5–9.5)							(2–40)	(110–286)	(44–387)	(22–123)
Collected in fall	9	1 male/8 female	4.9	100	89	100	89	67	89	14	182	557	194
August–November 1998–2002			(1.3–11.5)							(2–50)	(121–340)	(157–1,301)	(15–458)
Collected in spring	4	Female	3.5	100	100	100	100	100	100	52	1,077	2,614	1,044
April 1999–2000			(0.9–7)							(27–60)	(588–2,000)	(187–5,993)	(8–2,800)
Natural mortalities	9	3 male/6 female	6.8	56	11	56	89	67	100	8	NA[g]	1	66
June–August 1999–2002			(0.2–10)							(0–30)			(1–300)

[a] Mean (range).

[b] Number of protostrongylid eggs and larvae in 10 fields at 100× magnification in cranial lung.

[c] Length × width × 100 μm^2 of largest granuloma surrounding egg(s) of *P. odocoilei* in cranial lung.

[d] Larvae per gram of wet feces (LPG), DS = dorsal-spined larvae of *P. odocoilei*, Pr = larvae of *P. stilesi*.

[e] Nonresident hunter.

[f] Resident hunter.

[g] Not available due to pathology associated with bacterial pneumonia.

dwelling nematodes, such as *P. rushi* or *Dictyocaulus* spp., were observed.

On histologic examination, eggs of *P. odocoilei* were observed within alveolar septa and occasionally within capillaries, surrounded by granulomatous inflammation with the occasional eosinophil and multinucleated giant cell. Protostrongylid larvae were observed in the lumen and epithelium of alveoli and airways. Density and dimensions of granulomas in the cranial lung were positively and significantly correlated with intensity of larvae of *P. odocoilei* in feces (Pearson's coefficient 0.729 for density, 0.630 for granuloma size, $n=47$, $P<0.001$). Density, granuloma size, and larval counts were higher in sheep collected in spring than those collected in summer and fall (Table 2). In straggler sheep collected in spring (SC5–7) but not those collected in fall (FC1–3), granulomas coalesced and surrounded terminal and respiratory bronchioles, leading to impairment of entire alveolar units. One yearling (SC15) died of respiratory failure after exertion, with gross and histologic lesions similar to those observed in SS1.

Locally severe, lymphoplasmacytic, granulomatous inflammation and fibrosis were associated with adults of *P. stilesi* that were actively producing eggs. Adult *P. stilesi* without associated eggs or developing larvae elicited little inflammatory reaction. In sheep collected in summer and fall, the prevalence of *P. stilesi* was greater based on detection in the lungs (86–93%) than in feces (57–67%) (Table 2). Prevalence of *P. odocoilei* was relatively consistent year-round, regardless of method of detection (lung or feces), and it was 100% overall when both methods were combined.

The majority of adult *P. odocoilei* were in the appendicular musculature of sheep collected in spring (81% of adult nematodes) and fall (66%) (Table 1). No nematodes were observed in the central nervous system (CNS) on gross examination. On histologic examination, focal eosinophilic and lymphoplasmacytic meningitis, as well as hemosiderophages, surrounded a blood vessel deep in the cerebral cortex of one wild Dall's sheep (SC15) (Fig. 3C).

Arcanobacterium pyogenes was isolated from throat swabs plated immediately after collection from two healthy sheep. Other bacteria isolated were consistent with normal fauna of the upper respiratory and gastrointestinal tract (*Streptococcus* spp., usually alpha or nonhemolytic; *Escherichia coli*; *Staphylococcus* sp.; *Enterobacter* sp.; *Enterococcus* sp.; and *Lactobacillus* sp.). There were low titers (12–108) of antibody to a pestivirus related to BVD in three of 11 samples from "healthy" sheep; otherwise, there was no serologic evidence of exposure to bacterial or viral pathogens of domestic livestock.

Sick and dead Dall's sheep from Mackenzie Mountains

We examined samples from two sick sheep that were shot (SK), and nine carcasses of sheep found dead (MO) with evidence of bacterial bronchopneumonia, septicemia, or both (Table 3). Older ewes were frequently lactating and in poor body condition. Most submissions were from the northern Mackenzie Mountains in the NT, with one from the southern Mackenzie Mountains (MO17) and one from the Yukon Territory (MO18). In September 1999, carcasses of three adult sheep with no visible signs of predation or trauma were found in proximity to each other in the northern Mackenzie Mountains; necropsies were not performed.

In sheep with bacterial bronchopneumonia, the cranial and middle lung lobes, and occasionally the cranial portion of the diaphragmatic lobes, were consolidated and contained purulent foci ranging from 1 mm to several centimeters in diameter (Fig. 4). Fibrinous and fibrinopurulent pleuritis, as well as fibrous adhesions, also were observed. On histologic examination, normal lung architecture was obliterated by focal necrosis and abscesses. Protostrongylid eggs and larvae in various stages

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TABLE 3. Pathology and microbiology in sick (SK) and dead (MO) Dall's sheep from the Mackenzie Mountains, NT, 1999–2002.

ID	Age(yr)	Sex	Condition (marrow fat)	Mo	Diagnosis	Culture results	IHC	Parasite status[a]
SK13	11.5	Male	Thin	September 1999	Cough, dyspneic, depressed, purulent abscess	*A. pyogenes*[c]	NA[b]	Po
SK18	NA[b]	Male	NA[b]	July 2001	Cough, dyspneic, depressed	*Mannheimia* sp.[c]	NA[b]	Po and Ps
MO8	10	Female	Thin (45%)	June 1999	Chronic, fibrinopurulent bronchopneumonia	*A. pyogenes*[c,d]	Neg[e,f]	Po and Ps
MO9	8	Female	Thin (51%)	July 1999	Chronic, fibrinopurulent bronchopneumonia	*Mannheimia* sp.[c]	Neg[e]	Ps
					Dental disease, lumpy jaw	*A. pyogenes*[c,d]	Pos[f]	
MO16	6	Female	Thin (6%)	June 2000	Chronic, fibrinopurulent bronchopneumonia	*A. pyogenes*[c,d]	Neg[e,g]	Po and Ps
MO17	7	Female	Thin (14%)	July 2000	Chronic, fibrinopurulent bronchopneumonia	*A. pyogenes*[c,d]	Neg[e,g]	Po and Ps
MO19	7	Male	Good (88%)	August 2000	Acute, fibrinonecrotizing, bronchopneumonia	*A. pyogenes*[c,d]	Neg[e,g]	Po and Ps
MO20	9	Male	Good (89%)	August 2000	Acute, fibrinonecrotizing, bronchopneumonia	*A. pyogenes*[c,d]	Neg[e,g]	Po and Ps
MO22	0.2	Female	Good	July 2001	Acute, fibrinous pleuropneumonia	*P. multocida*[c,d]	NA[b]	Ps
MO18	9	Female	Thin (33%)	August 2000	Septicemia, dental and renal disease	*A. pyogenes*[c,d]	Neg[e,g]	Ps
MO28	0.25	Male	Moderate	August 2002	Suppurative meningoencephalitis	*E. coli* (brain)[d]	NA[b]	Ps

[a] *Parelaphostrongylus odocoilei* (Po) and *P. stilesi* (Ps).

[b] Not available.

[c] Lung.

[d] Septicemia.

[e] Immunohistochemistry (IHC) on lung for PI3, BRSV, IBR, and M. haemolytica.

[f] IHC for BVD, *Mycobacterium* spp., and *H. somnus*. MO 9 positive for *Mycobacterium* spp., probably *M. avium*.

[g] IHC for *M. bovis*.

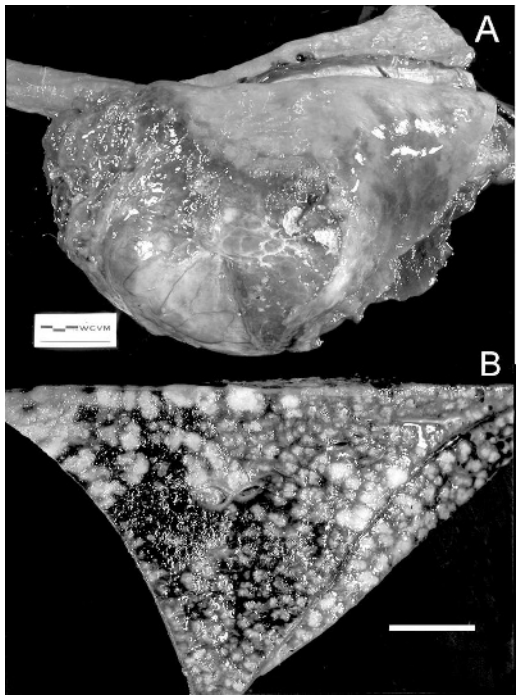


FIGURE 4. Chronic, fibrinopurulent bronchopneumonia in Dall's sheep from the Mackenzie Mountains, Northwest Territories, Canada. (A) Classic cranio-ventral distribution of bacterial bronchopneumonia, left lateral view of excised lungs of MO9 (see Table 3). Each black bar is equal to 1 cm. (B) Purulent material ("microabscesses") in cross section through cranial lung of MO16 (see Table 3). Bar=1 cm.

of decomposition occasionally could be seen on the periphery of "microabscesses," and larvae were observed in the midst of necrotic and bacterial debris in airways (Fig. 1B). Parenchymal lesions typical of *P. stilesi* were observed in the relatively unaffected caudal portions of the diaphragmatic lobes. In these sheep, prevalence of both *P. odocoilei* and *P. stilesi* was higher based on detection of parasite stages in the lungs (56% and 89%, respectively) compared with feces (11% and 67%, respectively) (Table 2).

DISCUSSION

Patterns of parasite localization and transmission

Contrary to current understanding of the migration routes for species of *Parelaphostrongylus* (Platt and Samuel, 1978; Anderson, 2000; Lankester, 2001) and consistent with the hypothesis proposed in Jenkins et al. (2005b), our findings suggest that *P. odocoilei* undergoes a neural migration in thinhorn sheep. Although eggs of *P. odocoilei* in the brain of an experimentally infected Stone's sheep (SS2) might have been of hematogenous origin, histologic lesions (hemorrhage, malacia, and inflammation) in the brains of experimentally and naturally infected sheep were similar to those reported for *P. tenuis* and *E. rangiferi*, which establish in or migrate through the CNS (Anderson, 1965; Lankester and Northcott, 1979; Handeland and Norberg, 1992; Lankester, 2001). Migration of developing *P. odocoilei* through the CNS during the prepatent and early patency periods might be followed by movement into the surrounding axial and trunk muscles and subsequent dissemination throughout the musculature of thinhorn sheep. This pattern is consistent with observations in cervids, in which adult *P. odocoilei* have been observed in connective tissue beneath the spine in black-tailed deer (Hobmaier and Hobmaier, 1934), and in the epidural space and in association with nervous tissue in muscles of mule deer killed in early patency (Pybus, 1983).

Three yearlings (FC24, FC27, and SC15), but no lambs, were naturally infected with *P. odocoilei* (Table 1), consistent with the hypothesis that infection occurs in late summer or fall of the first year (Samuel et al., 1985; Jenkins et al., 2006). If our hypothesis about the timing of the neural migration is correct, examination of lambs in fall should demonstrate the presence of developing fourth-stage larvae or adults in the CNS. Examination of the CNS of adult sheep in October–November did not reveal evidence of *P. odocoilei*; however, nematodes may only be present in the CNS of young of the year, as observed for *E. alces* (Handeland and Gibbons, 2001) and *E. cervi* (Handeland et al., 2000). Dall's sheep infected as

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lambs may be subsequently immune to infection, as suggested for *P. andersoni* and *P. tenuis* (Lankester, 2001), with a long life span of adult *P. odocoilei* accounting for a prevalence approaching 100% in many populations of wild Dall's sheep (Jenkins et al., 2005a).

Infections with *P. stilesi* were present in Dall's lambs as young as 2 mo old (Table 3), suggesting that transplacental transmission, similar to that reported for this lungworm in bighorn sheep (Hibler et al., 1974), also may occur in thinhorn sheep. The prepatent period for *P. stilesi* in an experimentally infected thinhorn sheep was 45 days (Skific and Jenkins, pers. obs.), and newborn lambs less than 2 wk of age are unlikely to consume infected gastropods. Transplacentally derived *P. stilesi* have been implicated in a syndrome known as "summer lamb mortality" in bighorn sheep (Bunch et al., 1999); this syndrome has never been reported in thinhorn sheep, suggesting that other causes should be considered for this syndrome in bighorn sheep.

Verminous pneumonia in thinhorn sheep

Verminous pneumonia (granulomatous inflammation associated with eggs and larvae of *P. stilesi* and *P. odocoilei*, and adults of *P. stilesi*) was present in all "healthy" Dall's sheep examined from the Mackenzie Mountains. Pathologic findings associated with adults and eggs of *P. stilesi* were focal and often limited to the caudal lung, whereas protostrongylid larvae and hematogenously derived eggs of *P. odocoilei* were distributed diffusely throughout the lung. Seasonal patterns in pathologic findings associated with larvae and eggs in the cranial lung corresponded with seasonal patterns in shedding of larvae in feces, with a trough in summer and a peak in spring (Jenkins et al., 2006). We used staging adapted from interstitial lung disease of humans (Crystal et al., 1981) to describe pathologic findings in lung associated with eggs of *P. odocoilei*. In stage 1, discrete granulomas caused focal distortions of alveolar septa, as in most sheep collected in summer and fall. In stage 2, coalescing granulomas caused diffuse distortion of the lung architecture and may have compromised vascular walls, terminal respiratory bronchioles, and associated alveolar units, as observed in "straggler" sheep collected in spring. In stage 3, increased blood pressure during exertion may have led to fatal pulmonary edema and hemorrhage in one experimentally (SS1) and one naturally infected sheep (SC15). Respiratory changes associated with *P. odocoilei* are likely a direct, if infrequent, cause of mortality in wild thinhorn sheep, similar to naturally infected black-tailed deer with "overwhelming parasitemia" (Brunetti, 1969) and "indurations" of the lungs (Hobmaier and Hobmaier, 1934).

In experimentally infected sheep, pathogenicity of *P. odocoilei* varied with stage of infection, magnitude and chronicity of the inflammatory reaction, and density of eggs and larvae. Respiratory damage may occur in the prepatent period, because protostrongylid eggs consistent with those of *P. odocoilei* (Hobmaier and Hobmaier, 1934), or possibly *Protostrongylus* spp., were present in lung washes of SS2 and SS3 before patency. As observed in experimentally infected mule deer (Pybus and Samuel, 1984), the size of granulomas associated with eggs of *P. odocoilei* increased with time since infection. Interestingly, one experimentally infected sheep with concomitant bacterial pneumonia (SS2) had little inflammatory response to eggs of *P. odocoilei*, suggesting that the immune response may have been overwhelmed in this animal, which also had eggs and larvae in the brain.

Bacterial pneumonia in thinhorn sheep

Chronic, fibrinopurulent bronchopneumonia in Dall's sheep from the Mackenzie Mountains was associated with *A. pyogenes* and species of *Mannheimia*, and frequently also with lactation, emaciation, and dental disease (for details on MO8

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and MO9, see Jenkins et al., 2000). The emaciated condition of these animals may be a predisposing factor, or a consequence of the disease process, as reported in free-ranging bighorn sheep and captive thinhorn sheep (Spraker and Hibler, 1982; Black et al., 1988). Other wild Dall's sheep in good body condition died acutely with fibrinonecrotizing bronchopneumonia, also associated with *A. pyogenes* and *Mannheimia* spp. Differences in virulence of bacterial strains or host susceptibility may account for the different rates of progression of pneumonia. Observations of groups of two to three sick and dead sheep, often rams, suggest direct transmission of a primary pathogen or a common predisposing factor. Increased scrutiny by hunters may be responsible for this gender bias; however, older rams experienced greater losses in one pneumonia epizootic in bighorn sheep (Onderka and Wishart, 1984).

Arcanobacterium pyogenes was isolated from the upper respiratory tract of healthy Dall's sheep and the lungs of pneumonic sheep in the Mackenzie Mountains, suggesting that this bacterium is an opportunistic pathogen in the lower respiratory tract. Elsewhere, *A. pyogenes* is commonly isolated from mandibular osteomyelitis (lumpy jaw) in Dall's sheep (Glaze et al., 1982; Bowyer and Leslie, 1992). Species of *Pasteurella* and *Mannheimia* also may act as opportunistic invaders; however, we did not recover any of these bacteria from pharyngeal swabs, even those collected from freshly killed sheep and cultured immediately. For hunter-killed sheep, poor recovery may have been due to the choice of transport media, freezing, and long periods between collection and culture (Ward et al., 1997). Collection and freezing of tonsils or tonsillar biopsies (versus swabs) (Wild and Miller, 1991; Foreyt and Lagerquist, 1994) are recommended for future field investigations of the bacterial fauna of the upper respiratory tract of Dall's sheep.

Bacterial species and lung pathology in sporadic cases of pneumonia in Dall's sheep were similar to those observed during widespread outbreaks of pneumonia in bighorn sheep (Spraker et al., 1984; Schwantje, 1986; Aune et al., 1998). However, the strains of *Mannheimia* present in Dall's sheep in the NT (based on serology and immunohistochemistry) differed from those described in bighorn and domestic sheep. As well, we found minimal evidence of exposure to respiratory viruses common in bighorn and domestic sheep, consistent with a history of isolation and philopatry of thinhorn sheep (Loehr et al., 2006). Most populations of thinhorn sheep have had few, if any, opportunities for contact with domestic sheep, although recent genetic characterization of *Pasteurella* and *Mannheimia* strains suggests that Dall's sheep in Alaska may have had indirect contact with strains endemic in domestic sheep (Kelley et al., 2006); however, this interpretation may be complicated by the choice of locus used for molecular characterization. Further investigation of the diversity and significance of these bacterial species in Dall's sheep is warranted, in light of their importance in bighorn sheep.

Management significance

Parelaphostrongylus odocoilei is a newly recognized pathogen in thinhorn sheep with a year-round prevalence approaching 100% in Dall's sheep in the Mackenzie Mountains. High densities of eggs and larvae of *P. odocoilei* caused diffuse interstitial lung disease and even respiratory failure in naturally and experimentally infected thinhorn sheep. Based on observations of interstitial lung disease in other species (Crystal et al., 1981; Collie et al., 1993), hypoxemia due to reduced lung volumes, compliance, and gas exchange may compromise respiratory function of Dall's sheep, especially at high altitudes and when escaping predation. Infection with *P. odocoilei* also has been linked to poor body condition and respiratory failure in mountain goats and mule deer

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(Pybus and Samuel, 1984; Pybus et al., 1984), and it caused weight loss and neurological disease in experimentally infected thinhorn sheep (Jenkins et al., 2005b). Therefore, *P. odocoilei* has the potential to cause subclinical disease and even overt mortality in wild thinhorn sheep, primarily among young of the year in their first winter. Exposure of thinhorn sheep to increasing numbers of infective larvae and range expansion of *P. odocoilei* in northern North America associated with climate warming may augment the impact of this parasite in the near future (Jenkins et al., 2006).

Verminous pneumonia, especially the diffuse pattern associated with *P. odocoilei*, may act as a predisposing factor in the development of bacterial pneumonia; however, lesions of verminous pneumonia were frequently obliterated by necrosis and inflammation associated with bacterial pneumonia, complicating interpretation of the role of protostrongylid parasites in the pathogenesis of pneumonia in thinhorn sheep. In bighorn sheep, the "role of any nematode in the pneumonia complex … is poorly understood" (Demartini and Davies, 1977). If *P. odocoilei* becomes established in bighorn sheep, it could become yet another player in the multifactorial pneumonia complex. Because of their caudal distribution, lesions associated with the lungworm *P. stilesi* in bighorn and thinhorn sheep may compromise the only remaining functional lung tissue in sheep with concomitant bacterial bronchopneumonia (generally confined to the cranial lung).

In bighorn sheep, counts of protostrongylid larvae in feces are frequently used to monitor population health, and even to predict pneumonia outbreaks. In our study population of Dall's sheep, relying on detection of larvae in feces would have underestimated the true prevalence of infection with *P. stilesi* in sheep collected in summer and fall. This may reflect seasonal decreases in parasite reproduction, or newly acquired infections with *P. stilesi*, which were not yet patent. In animals with concomitant verminous and bacterial pneumonia, shedding of both types of protostrongylid larvae in feces was reduced or eliminated, probably due to inflammatory destruction and mechanical trapping of larvae in the airways. Caution must be exercised when interpreting fecal larval counts as a measure of health in individuals and populations of wild sheep.

Finally, we observed a sporadic pattern of mortality due to pneumonia caused by pathogens endemic in Dall's sheep in the Mackenzie Mountains. This differs markedly from the wide-scale die-offs due to pneumonia in bighorn sheep, often associated with pathogens introduced from domestic livestock. Thinhorn sheep occupy most of their historic range at high densities and abundance (Heimer et al., 1992; Worley et al., 2004), and no large-scale, disease-related die-offs have been reported (Hoefs and Cowan, 1979; Dau, 1981; Bowyer et al., 2000). Thinhorn sheep have not been extensively translocated (Hatter and Blower, 1996; Heimer and Taylor, 1996; Veitch, 1996) and have had minimal opportunities for contact with domestic livestock, which harbor parasites, viruses, and strains of bacteria to which thinhorn sheep are naïve (Nielsen and Neiland, 1974; Foreyt et al., 1996; Zarnke, 2000; Garde et al., 2005). Thinhorn sheep are likely susceptible to the same pathogens and ecologic factors (such as habitat fragmentation and degradation) that have hampered conservation efforts for bighorn sheep. Therefore, efforts to avoid translocation, contact with domestic animals, and anthropogenic stressors are a sound basis for proactive management for thinhorn sheep in North America, and they may forestall the breakdown of protective ecological barriers.

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