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Abstract:

Three herds of Rocky Mountain bighorn sheep (Ovis canadensis canadensis) in southeastern British Columbia were evaluated for health status during 1983 and 1984. Each herd was of similar size but varied in disease occurrence and herd dynamics, both historically and over the period Samples taken from six sheep in each herd were examined for nutritional condition, microbiological, virological and serological status, general and pulmonary parasite loads, blood chemistry and trace mineral levels, as well as gross and histological pathology lesions. A low elevation wintering herd at high density on poor quality range was demonstrated to have high levels of lungworm infection, low total serum protein, fecal nitrogen and liver selenium levels. Higher total serum protein, fecal nitrogen, liver selenium levels and lower lungworm levels were present in bighorns from a lower density high elevation wintering Adrenal glands were larger and clinical and subclinical diseases were common in adults and lambs from a low elevation wintering herd 2 years after an all-age dieoff. Parameters were discussed for relevance in describing the herd status and usefulness to the wildlife manager. The first herd was treated with trace minerals and an anthelmintic after the 1983 collection. Lungworm larvae output was less in four sheep examined in the following year, but no change was seen in trace mineral levels.

The Rocky Mountain bighorn sheep populations of North America have markedly decreased in number and distribution since the human settlement of their native habitat (Buechner 1960). The factors responsible for the decline are suggested to include heavy hunting pressures, the introduction of domestic animal diseases and, especially in this century, the widespread reduction of available and suitable ranges (Buechner 1960, Stelfox 1971).

More recently, bighorn sheep have suffered epizootic dieoffs in captive and wild situations in Canada and the U.S.A.. The sheep have nearly always succumbed to pneumonia caused by opportunistic bacteria with varying degrees of lungworm involvement (Forrester 1971). Reports of sheep dieoffs have outlined a large number of environmental and animal related factors which have been present before dieoffs and are believed to predispose sheep to disease. For example, Spraker et al. (1984) described a series of conditions preceeding a dieoff at Waterton Canyon in Colorado. They included increased levels of vehicular traffic, noise, human harassment, high animal density and a reduction in forage quality and quantity. Reports of other dieoffs have included factors such as low trace mineral levels, inclement weather, habitat deterioration and loss, the presence of domestic sheep, concurrent infectious diseases and high lungworm or other parasite levels (Buechner 1960, Lange et al. 1980, Foreyt and Jessup 1982, Thorne et al. 1982, Schwantje 1983, Onderka and Wishart 1984).

Such factors are believed to function through direct and indirect mechanisms, their presence and effect varying with each situation. Chronic exposure to some of these conditions may act cumulatively to impair protective immune functions. The collective influence is believed to create a state of chronic stress (Spraker et al. 1984). The physiological responses of individual animals to chronic stress include adrenal gland

enlargement and increased levels of adrenal corticosteroids (Feldman 1983). Long term maintenance of high levels of corticosteroids in other species is associated with an inhibition of immune responses and increased susceptibility to bacterial infections, particularly of the respiratory tract (Hunningshake and Fauci 1977, Kelley 1980). Studies in man and domestic animals have shown that concurrent viral respiratory infections (Yates 1982), malnutrition (Scrimshaw et al. 1968) and low tissue levels of selenium and copper (Chandra 1983) are associated with, and can directly cause depressed immune responses.

The proper function of immune systems is closely related to the overall nutrition of an animal (Chandra and Newberne 1977). Many of the factors discussed as influencing bighorn sheep herds play important roles in their nutritional condition. Forage quality and quantity have obvious direct influences. Other factors, such as intra and interspecific competition, harassment and high gastrointestinal parasite burdens may indirectly reduce the amount and type of feed consumed and utilized. Indices of nutritional status have been examined in controlled (Kirkpatrick et al. 1975, Seal et al. 1978, Bahnak et al. 1979, Warren et al. 1982) and free-ranging studies of white-tailed deer, pronghorn and wild sheep (Franzmann 1972, White and Cook 1974, Seal and Hoskinson 1978, Hebert et al. 1984. Spraker et al. 1984). The levels of total serum protein (TP), blood urea nitrogen (BUN), fecal nitrogen (FN) and body condition scores varied with season, habitat and physical condition in white-tailed deer and bighorns (Franzmann 1972, Seal et al. 1978, Hebert et al. 1984). parameters were the most frequently used in these studies and were easily available from live animals.

Depressed blood proteins have long been accepted as occurring with protein-energy malnutrition in man (Sauberlich 1983), and studies have demonstrated reductions in TP with starvation in wildlife species (Hebert 1973, Bahnak et al. 1979, Warren et al. 1982). Blood urea nitrogen levels were proportional to protein consumption in cattle (Biddle and Evans 1973), domestic sheep (Preston et al. 1965), white-tailed deer (Seal et al. 1972, Kirkpatrick et al. 1975) and pronghorn antelope (Seal and Hoskinson 1978). Franzmann (1972) felt that the nutritional status of bighorn sheep was best estimated by BUN and suggested that values below 15 mg/dl indicated low protein intakes.

The comparison of fecal nitrogen content with feed nitrogen content has been investigated to determine the protein status of wildlife Species examined include free-ranging elk (Gates and Hudson populations. 1979), East African ungulates (Arman et al. 1975) and wild and captive bighorns (Hebert 1973, Hebert et al. 1984). In most cases, studies have followed seasonal trends in FN and related these to environmental changes (Seip and Bunnell 1985), feed quality and animal weight (Mould and Robbins Hebert et al. (1984) showed changes in animal condition between seasons and years in captive, supplementary fed, free-ranging and non-migratory free-ranging California bighorn sheep. He advised the use of FN determination over time to assess the effect of changes in population density and grazing on sheep ranges and herds. Bahnak et al. (1979) suggested that periodic monitoring of all nutritional indices was more valuable than conclusions drawn from samples taken at a single point in time.

The definition of trace mineral deficiencies requires the association of clinical signs of deficiency with low mineral concentrations in animal tissues, diet and soil. Deficiency syndromes are often subclinical and may

be difficult to identify due to interactions and imbalances between individual minerals and the protein-energy content of the diet. Studies related to two of the more commonly recognized deficiencies, selenium and copper, are limited in free-ranging wild species; however, both minerals have been extensively reviewed for domestic cattle and sheep (Hidiroglou 1979, Underwood 1977, Van Vleet 1980).

Subtle or marked syndromes of muscle degeneration, ill thrift, neonatal weakness and infertility are responsive to the addition of selenium and vitamin E (Se/E) to the diets of ruminants (MacDonald et al. 1976, Maas 1983). In addition, suppressed cell mediated immune functions are restored (Sheffy 1979). An acute stress induced muscle degeneration (capture myopathy) has been well recognized in wild ungulates and is associated with Se/E deficiency (Hebert and Cowan 1971). The other livestock syndromes are not well defined in wild species.

Copper deficiency in domestic sheep is also often subclinical but can reduce growth rate, reproductive performance and haircoat pigmentation (Ward 1978, Hidiroglou 1979). Studies in Alaskan moose and Idaho mule deer suggested reductions in fertility and abnormal hoof keratinization (Flynn et al. 1977, Dunbar and Foreyt 1985). Low copper levels are also associated with decreased immune responses and an increased susceptibility to parasitism (Chandra 1983). A possibility of interaction between copper and selenium has been proposed because of a lack of response to supplementation until both elements were provided to domestic livestock (Blood et al. 1983).

Investigations into all-age dieoffs of bighorn sheep in the East Kootenay (E.K.) region of British Columbia in the 1960s and 1980s determined that these dieoffs were preceded by high population densities, heavy interspecific competition for forage, contact with domestic sheep, severe winter weather, high lungworm levels and low copper and selenium levels in some sheep (Stelfox 1971, Davidson 1982, Schwantje 1983). It was concluded that the herds in which dieoffs occurred were predisposed to pneumonia by chronic stress conditions caused and complicated by high animal density, poor nutrition, parasitism and trace mineral deficiencies. The presence of concurrent or subclinical diseases was unknown (Schwantje 1983).

Sheep herds wintering at high elevations were not affected by either E.K. dieoff. This suggested that the absence of some of the previously mentioned factors allowed the high elevation sheep herds to be maintained in better overall health than those which suffer from dieoffs, and that they have thus avoided health disease epizootics.

A definitive study was required to evaluate the presence of these factors in E.K. herds with and without the history of dieoffs. The following project surveyed three herds of similar size which differed in herd dynamics and disease occurrence. Samples were compared and evaluated to describe the presence and influence of predisposing factors and to describe the overall health of each herd.

# STUDY HERDS

The three herds selected for this project are designated by their winter ranges; Columbia Lake (CL), Wigwam (WW) and Ewin Ridge (ER) (Figure 1).

CL and WW sheep graze summer pastures at elevations up to and over 2170 metres. In the fall they migrate to low elevation, predominantly

seral ranges along the eastern boundary of the Rocky Mountain Trench (Bandy 1968). Arrival on winter range is followed by the rut in November to December. Both of these herds have been affected by all age dieoffs previously.

Vehicular access to both winter ranges is possible until December 1. The roads tend to be heavily travelled for recreational and hunting use. The CL range is adjacent to a small community and shares a portion of the range with a growing residential subdivision. A lumber mill and small community are located across a river from a portion of the WW range, but most of the range is out of visual and acoustic contact from these developments.

The CL range consists of alluvial fans, parkland areas and rocky bluffs on the eastern shore of Columbia Lake. The quality of this range has been considered poor since at least 1970 (Demarchi 1970, Davidson pers. commun.). Exclosures erected in 1983 showed obvious differences in forage growth by 1984. Forest succession is very prominent around most meadows. Livestock have been absent from the range for over 20 years, however, mule deer and particularly elk are abundant.

The CL herd suffered a dieoff in 1966, reaching a low of 28 from approximately 100 animals (Bandy 1968, Demarchi 1970). At present the herd numbers approximately 150 animals. Annual lamb:ewe ratios were considered to be gradually decreasing (Davidson pers. commun.) and were 40-50% in 1983.

The WW range is made up of fire formed grasslands, some natural parkland and thickly forested areas between the Elk and Wigwam Rivers. The range condition appears to improve following reductions in the wildlife stocking rate, as was seen after a 1965 sheep dieoff and subsequent increases in elk harvests (Demarchi 1970). It is presently considered to be improving after a 1981 sheep dieoff. Livestock are not permitted on the range but mule deer and elk are abundant.

An all-age dieoff in 1965 removed 40-50% of the WW herd. By 1981 the 150 animals remaining had increased to at least 424, the highest density ever reported for the range. At this time elk and mule deer numbers were considered high, similar to levels prior to the 1965 dieoff.

Another all-age dieoff began in December 1981 and reduced the population to less than 150 bighorns. Animals in poor condition and/or coughing are still occasionally reported at this time. The herd now numbers approximately 100-150 sheep with lamb:ewe ratios of less than 5% in 1983.

ER sheep winter on virtually inaccessible, windswept subalpine and alpine ridges up to 2700 metres in elevation (Schuerholz 1984). The high elevation allows an approximately one month earlier onset of the rut and winter conditions. This herd has never been reported to suffer large scale mortality although, because of the location, we can only rely on evidence that aircraft classified counts have remained stable at 100-150 sheep since 1971 (Warkentin, Schuerholz pers. commun., 1984). Lamb:ewe ratios have averaged 55% since 1971 and were 78% in 1984. An open pit coal mine is present on ram summer range but the winter range common to both sexes is undisturbed. Extensive range studies demonstrate excellent quality and a lack of competition from elk or mule deer. The amount of available range is reduced with heavy snowfalls or the late arrival of spring. With these conditions intraspecific competition decreases the quantity of forage for overwintering sheep (Schuerholz 1983).

#### MATERIALS AND METHODS

In 1983 and 1984, the CL and WW herds were observed and counted from the ground (foot and automobile). ER population data were supplied by Crowsnest Resources and Transamerica Environmental Science Consultants (TAESCO) as well as minimal direct observation during early winter. Fixed wing or helicopter classified counts were performed routinely on all herds in January or February by B.C. Wildlife Branch personnel.

From late October to early December 1983 observations of major CL and WW groups were made, usually from a vehicle. The animals were observed for 0.5 to 1 hour before an individual was collected for sampling. Disturbance was considered minimal before collection. ER animals were observed from helicopter and collected after hazing towards a ground based hunter during

late October and early November 1984.

Six sheep were collected from each herd. Selection criteria were designed to select for those animals which were of less than the average body condition or demonstrated symptoms of disease so that they appeared different from other sheep in the group. Criteria included body condition grading and clinical symptoms of disease or signs stated by wildlife biologists or experienced observers as indicative of poor condition or subclinical disease. The signs ranged from isolation behaviour to pale or rough haircoats, loss of muscle mass, contagious ecthyma lesions and mild to obvious respiratory symptoms. Animals were also selected from three age classes; lambs of the year, yearlings and adult ewes. The ER collections used only these criteria. Four additional sheep from CL were examined in 1984. Three were shot and a fourth poached carcass was examined.

Sheep were shot in the caudal skull or anterior cervical region. Blood was collected immediately, chilled and harvested for serum on the following day. Each animal was weighed and a necropsy was performed. Carcasses were evaluated subjectively for body condition on the basis of body fat deposits and muscling for a total body score of 100. Major organ systems were examined and representative portions were preserved in 10% neutral buffered formalin. The lungs were removed intact and photographed. Lesions were drawn and 2 cm slices of each lobe were preserved. Portions of anterior or grossly affected lobes, as well as a retropharyngeal lymph node and main stem bronchial swabs, were obtained for bacterial and viral culture. These tissues were frozen and chilled respectively and transported to the Alberta Agriculture Laboratory, Edmonton, Alberta for culture.

Kidney, liver and sera were frozen and assayed for trace minerals at the British Columbia Veterinary Pathology Laboratory, Abbotsford, B.C.. Serum was frozen and analyzed later for antibodies to infectious bovine rhinotracheitis (IBR), parainfluenza 3 virus (PI3), bovine virus diarrhea (BVD) and bovine respiratory syncytial virus (RSV) and levels of blood urea nitrogen, glucose and total serum protein at the Western College of Veterinary Medicine, Saskatoon, Saskatchewan. Cortisol levels in serum and urine were assayed by the T.R. Spraker laboratory at the College of Veterinary Medicine, Fort Collins, Colorado. Serological evaluations for bovine lymphosarcoma virus (WW4), bluetongue (BT), epizootic-hemorrhagic disease (EHD), maedi/visna viruses and Johne's disease (Mycobacterium pseudotuberculosis) were performed by the Canadian Animal Disease Research Institute, Nepean, Ontario.

Samples of feces were frozen and analysed for nitrogen content by the Soil, Feed and Tissue Testing Laboratory, Kelowna, B.C. Gastrointestinal

parasite ova counts were done with fecal flotations by A. Gajadhar, W.C.V.M.. Fecal lungworm larvae counts were performed on air dried feces by the W.M. Samuel laboratory, University of Alberta, Edmonton, Alberta, using a Baermann technique (Samuel and Gray 1982).

Microscopic tissues were mounted in paraffin, sectioned at 5 um thickness and stained with hematoxylin-eosin. Special stains (Masson's trichrome, PTAH, PAS, Grocott, Brown and Brenn) were used as required. Three microscopic sections were taken from each lung slice as well as any additional lesions. Histopathological findings were noted in all organs. Pulmonary changes were categorized on the basis of microscopic changes. The left kidney and adrenal from each animal were preserved, weighed and photographed. The thoracic thymus was collected where identifiable, photographed and sectioned.

#### RESULTS

#### CLINICAL EVALUATION

#### Columbia Lake

The sheep in this herd were usually seen in groups of 40 to 65 animals. They could be approached to within 50 feet and remained in the immediate area during all procedures. On clinical inspection individual sheep appeared in good to excellent body condition. The pelage was occasionally long and/or pale or yellow. Three sheep (CL 1,4,5) were selected for these haircoat changes. One ewe was selected because of a mild dry cough (CL 6). This was the only respiratory sign noted during all observations. Some sheep had oronasal depigmented areas, suggestive of healed contagious ecthyma lesions. Body condition scores in collected sheep averaged 86 of a possible 100.

#### Wigwam

This herd was wary and was scattered as pairs or small groups over the range. Approach was possible by vehicle but sheep dispersed rapidly with disturbance. Field inspection revealed mostly adults of moderate to good body condition. Some groups were uniformly thin. Sheep selected had long pale haircoats and were thinner than average. The two lambs collected (WWI, 3) were very small, thin and lethargic. Both had nasal discharges and obvious oral contagious ecthyma lesions. Oral contagious ecthyma was also severe in WW4, mild in WW5 with residual nasal scars in WW6. Diarrhea with perineal staining was present in WW3 and 5. All but WW5 had Otobius megnini and Dermacentor albipictus ticks in light to heavy numbers. No signs of coughing were observed. Body condition scores averaged 57 out of 100.

# Ewin Ridge

Clinical evaluation of these sheep was not possible but the carcasses were examined after collection. The only noticeable abnormality was that the yearling (ER 4) had a pale but otherwise normal haircoat and was of small size. The other sheep were in good body condition with an average body condition score of 81.

#### 2. GROSS NECROPSY

#### Columbia Lake

Five of the six sheep were classified as having moderate to severe verminous pneumonia. Animal CL 1 was classified with mild lesions. One third to one half of the caudal lung lobes were diffusely swollen and the overlying pleura was pale and thickened. Individual nodules from 0.2 - 2 cm protruded from the lung over the dorsal surface of the diaphragmatic lobe and occasionally other lung lobes. Smaller nodules were more transparent with yellow-grey mottling. On cut section the nodules were firm. Airways were surrounded by prominent white glistening cuffs. The lung changes were consistent with Protostrongylus stilesi infection. P. rushi adults were present in the airways of all six sheep. A small focus in the cranial right lung of CL 6 was atelectatic with multiple tiny white foci. The kidney:adrenal weight ratios ranged from 24.6 to 37.3 with a mean of 30.1 (Table 1).

Wyominia tetoni tapeworms were found in four sheep. Heavy infections were accompanied by bile duct and gall bladder dilation and fibrosis. Cl 4 had two 1-2 cm green lamellated abscesses adjacent to these structures. Multiple 0.2 cm nodules on the abomasal rugae and over cecal and colonic serosae and mucosae were common and were considered to indicate gastrointestinal parasitism due to various Ostertagia and Marshallagia spp..

# Wigwam

Mild lesions of verminous pneumonia were present in five sheep with WW5 classified as moderately affected. Opaque white individual nodules rather than diffuse swelling of caudal lobes were more common in these animals. Sheep WW6 was the only animal in which P. rushi adults were identified.

Chronic bronchopneumonia and fibrous pleuritis were noted in four sheep. These ranged from a small area of cranial lobe consolidation in WW5 to bilateral ventral adhesion and focal consolidation in all lobes of WW4. Consolidation was extensive in both lambs with sharp demarcation of affected anteroventral and ventral middle and caudal lobes. The firm, plum-coloured tissue had a cobblestone texture with scattered white foci and a mucoid airway exudate. Extensive fibrous pleuritis and pericarditis was also present in the lambs.

Gastrointestinal parasitism lesions were present in all sheep and were more severe in those with chronic pulmonary lesions. WW4 and 5 had generalized enlargement of lymphoid organs and multifocal pale renal cortical streaking. Contagious ecthyma was very severe throughout the oral cavity and rumen of WW4 and mild in WW5. The lambs had lesions associated with thymic atrophy, serous atrophy of fat, large numbers of gastrointestinal parasites and oral contagious ecthyma. WW 3 was more severely affected with fibrinous peritonitis, serous polyarthritis, decubitus ulcers and contagious ecthyma of the coronet bands. The kidney:adrenal ratio ranged from 9.4 to 29.2, with a mean of 21.8. The lowest ratio, or largest adrenal size was in WW3.

Table 1. Kidney and adrenal gland weight ratios in bighorn sheep collected from the East Kootenays in 1983-84.

| Sheep<br>number | Age<br>(Yr) | Sex              | Body Weight (Kg) | K:A <sup>a</sup> |
|-----------------|-------------|------------------|------------------|------------------|
| CL 1            | 2.5         | м                | 77.3             | 29.4             |
| CL 2            | 0.5         | M                | 40.9             | 37.3             |
| CL 3            | 0.5         | M                | 32.7             | 33.0             |
| CL 4            | 4.5         | F                | 72.7             | 24.6             |
| CL 5            | 1.5         | M<br>F           | 64.5             | 26.5             |
| CL 6            | 3.5         | F                | 71.4             | 30.9             |
|                 |             |                  | X = 30.1         |                  |
| WW 1            | 0.5         | м                | 29.1             | 23.5             |
| WW 2            | 3.5         | P                | 71.8             | 23.8             |
| WW 3            | 0.5         |                  | 14.5             | 9.4              |
| WW 4            | 6.5         | F<br>F<br>F<br>M | 64.5             | 29.2             |
| WW 5            | 3.5         | F                | 65.5             | 19.3             |
| WW 6            | 1.5         | м                | 53.2             | 25.5             |
|                 |             |                  | X = 21.8         |                  |
| ER 1            | 0.5         | F                | 23.6             | 34.2             |
| ER 2            | 4.5         | F<br>F<br>M<br>F | 67.3             | 26.3             |
| ER 3            | 3.5         | F                | 68.2             | 26.1             |
| ER 4            | 1.5         | н                | 41.8             | 27.8             |
| ER 5            | 7.5         | F                | 81.8             | 26.7             |
| ER 6            | 9.5         | F                | 72.7             | 26.7             |
|                 |             |                  | X = 28.0         |                  |

aK:A = kidney (g):adrenal (g)

# Ewin Ridge

Three sheep were classified to have mild verminous pneumonia and the remainder had mild-moderate or moderate lesions. Nodules were mostly small and transparent. The most caudal area of the diaphragmatic lobes was frequently contracted with pleural fibosis. Two ewes (ER 5,6) had small contracted areas of middle lobes with occasional fibrous pleural adhesions. P. rushi adults were present in ER 2 and 3.

The lamb (ER 1) lacked the right ovary with partial absence of the right oviduct. No depigmented areas or active contagious ecthyma lesions were present. Gastrointestinal parasitism lesions were restricted to the abomasum in ER 1,2 and 4 and were moderate in number and severity. Kidney:adrenal ratios varied from 26.1 to 34.2 with a mean of 28.0.

#### HISTOPATHOLOGY

The most consistent findings in all sheep were lesions due to liver and gastrointestinal tapeworm and nematode parasitism. The major liver changes were fibrosis, mononuclear cell infiltration and hyperplasia of bile ducts in portal regions, as well as small, cellular foci scattered throughout liver lobules. These foci were often associated with portal areas and were primarily mononuclear cells with occasional eosinophils and individual degenerative hepatocytes.

Abomasal inflammation consisted of nodular and diffuse mononuclear cell infiltrates. This lesion was less common in CL sheep. intestinal changes were diffuse mononuclear and eosinophilic infiltrations of the lamina propria. The more severely affected sections also demonstrated mucosal hyperplasia. Occasional areas of superficial necrosis were associated with intracellular stages of Eimeria spp.. intestinal lesions were less severe with less hyperplasia. Eosinophilic chronic inflammation, suggestive of granulomas and focal migration, were present in the intestines, liver, lymph nodes and abomasum in sheep from all herds. All cardiac and skeletal muscles contained cysts of Sarcocystis spp., except in CL sheep.

Kidney changes primarily involved glomeruli or were interstitial with secondary glomerular and tubular effects. Glomerular basement membranes were irregularly thickened and the mesangium was hypercellular. Severely affected glomeruli had adhesions and were shrunken. None of these lesions were considered to be extensive or significant enough to cause clinical signs. The most severe kidney lesions were in WW sheep and were associated with primary diseases in other organs.

Lymphoid tissue was generally hyperplastic in most animals. Systemic illness was accompanied by lymphoid atrophy in WW3. The epithelium overlying the pharyngeal lymphoid tissue in most sheep was altered. Changes ranged from hyperplasia to necrosis. Invasive protozoa were found in the pharynx submucosa or retropharyngeal lymph nodes of ER 4, 5 and 6. There did not appear to be any relationship of the nasopharynx changes to other respiratory pathology. Trachea mucosal pathology was mostly mild hyperplasia, mononuclear cells and/or necrosis. predominantly seen in WW sheep. The generalized lymphadenopathies of WW4 and 5 were the result of a multisystemic lymphoid tumor (lymphosarcoma) and multiorgan infection of an intracellular parasite, Encephalitozoon, respectively.

Pulmonary changes were similar in all sheep, differing primarily in the amount of lung affected by lungworm activity, types of tissue response to inflammation and degree of bacterial bronchopneumonia involvement. The predominant changes involved airways, vessels, interstitial tissue, alveolar spaces and locally diffuse inflammatory reactions to lungworm reproduction. In summary, CL and ER lung histopathology was similar. WW sheep had markedly fewer lungworm stages and accompanying inflammation, as well as less chronic changes associated with the parasite (i.e. fibromuscular hyperplasia). A mild focal bronchopneumonia was present in CL 6, resolving bronchopneumonias were present in ER 5 and 6 and mild to severe chronic bronchopneumonias were present in WW 1,3,4 and 5.

#### 4. LABORATORY ANALYSES

## Microbiology

No respiratory pathogens were cultured from CL sheep. E. coli was found in the liver abscess from CL 4.

Pasteurella haemolytica biotype T was present in the bronchial swabs and right cranial lung of WW 1 and the retropharyngeal lymph node of WW 4. A non T biotype of P. haemolytica was cultured from the bronchial swabs, mediastinal lymph node and left lung of WW 4. Staphylococcus aureus was present in joint swabs from WW 3 and the retropharyngeal lymph nodes of WW 3 and WW 5. All other lung cultures from WW sheep were negative or produced insignificant contaminants.

P. haemolytica biotype T was present in the lungs and retropharyngeal lymph node of ER 5. No further significant pathogens were recovered from ER sheep.

No viruses were cultured from any sheep tissues. ER 2,5,6 and WW 2 and 6 had titres to PI3 virus. ER 6 also had a positive titre to RSV virus. All sera were negative to Johne's disease, maedi/visna, EHD and BT virus. A titre to bovine lymphosarcoma virus was not present in WW4.

# Parasitology

Fecal flotations indicated mild to moderate levels of usually mixed intestinal parasitism in all sheep. Ova were recovered in larger numbers from lambs, particularly those with systemic illness (WWI and 3).

Protostrongylus spp. lungworm larvae levels were higher in feces from CL sheep than from WW or ER sheep, with average larvae per gram feces of 799 (CL), 162 (WW) and 166 (ER). Lambs and yearling bighorns from CL had the highest larval outputs while the lambs from WW and the lamb and yearling from ER had very low fecal larvae. The WW yearling had moderate numbers of larvae (Table 2).

# Trace Minerals

Liver and kidney selenium levels in all CL sheep were consistent with levels considered to be deficient in domestic sheep. Marginal levels were present in three sheep. Tissue copper levels were considered marginal in two CL, three WW and four ER bighorns (Table 2).

Table 2. Fecal larvae output, liver copper and selenium levels in bighorn sheep collected from the East Kootenays in 1983-84.

| Sheep   | LPGA      | Liver levels (ppm) |             |  |  |
|---------|-----------|--------------------|-------------|--|--|
|         |           | Copper             | Selenium    |  |  |
| CL 1    | 172       | 77                 | 0.10        |  |  |
| CL 2    | 665       | 14                 | 0.10        |  |  |
| CL 3    | 1971      | 67                 | 0.06        |  |  |
| CL 4    | 543       | 43                 | 0.07        |  |  |
| CL 5    | 959       | 20                 | 0.11        |  |  |
| CL 6    | 484       | 73                 | 0.06        |  |  |
| WW 1    | 9         | 91                 | 0.16        |  |  |
| WW 2    | 58        | 16                 | 0.32        |  |  |
| WW 3    | 3.5       | 17                 | 0.29        |  |  |
| WW 4    | 167       | 6.6                | 0.25        |  |  |
| WW 5    | 321       | 37                 | 0.19        |  |  |
| WW 6    | 414       | 63                 | 0.61        |  |  |
| ER 1    | 13        | 6.5                | 0.30        |  |  |
| ER 2    | 347       | 8.3                | 0.27        |  |  |
| ER 3    | 142       | 32                 | 0.32        |  |  |
| ER 4    | 51        | 24                 | 0.30        |  |  |
| ER 5    | 109       | 8.5                | 0.29        |  |  |
| ER 6    | 336       | 5.7                | 0.29        |  |  |
| ormalsb | deficient | 0.5 - 4.0          | 0.005 - 0.1 |  |  |
|         | marginal  | 5.0 - 20           | 0.15 - 0.25 |  |  |

a LPG - larvae per gram feces liver levels of domestic sheep (R. Puls 1981)

# Chemistry

Blood urea was within normal domestic sheep levels in two CL and two ER animals. Levels were less than normal in all other sheep except for increased levels in WW 1, 3 and 4. Serum glucose values were mildly to moderately increased in five CL, four WW and four ER sheep. Two ER sheep had extremely high glucose levels. Total serum protein levels were reduced in four CL, four WW and two ER sheep. Average values were the highest in ER animals and lowest in CL sheep. Average fecal nitrogen levels were similar in CL and WW sheep and considerably higher in ER sheep (Table 3).

Serum and urine cortisol values varied greatly within herds without a consistent pattern. Kidney:adrenal ratios were similar in CL and ER bighorns but lower on average in WW sheep indicating larger adrenal glands in the WW sheep (Table 1).

#### DISCUSSION

The CL herd appeared to be in good health on the basis of field observation. Animals were calm and in large groups. Clinical signs were restricted to slight variations in the degree of body muscling, fat deposits and coat colour, and a single mildly coughing ewe. Body condition scores were high. In spite of declining lamb ratios and heavy lungworm infections, individual lambs were large and showed no evidence of secondary bacterial bronchopneumonia at 5-6 months.

The WW sheep were wary and were found scattered over the range in small groups. Observers have found that these sheep grazed in large groups, such as seen on the CL range, when at higher population density prior to the 1981-82 dieoff (Davidson pers. commun.). A larger proportion of WW sheep were in obvious poor health. Clinical signs included pale coats, tick infestations, lower body condition scores and active contagious ecthyma in all ages. Chronic respiratory disease was still present in both lambs and adults two years after the acute dieoff. Lamb mortality was very significant by 6 months of age.

The ER bighorns primarily utilize winter ranges where dispersal is limited by the surrounding snow pack (Schuerholz 1984). Animals are therefore generally grouped on snow free slopes. The use of a helicopter prevented visualization of groupings and clinical signs at the time of collection. Body condition scores of the ER herd were high. The lamb and yearling examined were small but in good condition. Mild chronic respiratory disease in adult ewes appeared well localized without debilitating systemic effects. The lesions associated with P. stilesi infections were extensive but contained and were surrounded by increased amounts of fibrous tissue indicating old, healing lung damage.

Gross pathological findings indicated the most severe lungworm infections occurred in CL sheep, followed by ER and WW. Microscopic pulmonary changes and fecal larvae numbers supported the gross estimations of lungworm pneumonia severity and chronicity. Microscopic hepatic and intestinal parasitic lesions were present to similar degrees in all sheep. Gross and fecal examination frequently missed lesions and parasite species and showed little correlation with severity of histologic lesions.

Tissue changes with bacterial pneumonias were the most severe and extensive in WW lambs. The chronic pneumonias of adult sheep on all three ranges varied in extent. There was localization to single lobes in CL and

Table 3. Serum, fecal and urine chemistry values in bighorn sheep collected from the East Kootenays in 1983-84.

|      | eep<br>mber | BUN<br>(mg/dl) | TP<br>(g/L) | FN<br>(%) | Glucose<br>(mg/dl) | Cortisol<br>serum | (ng/ml)<br>urine |
|------|-------------|----------------|-------------|-----------|--------------------|-------------------|------------------|
| CL   | 1           | 2.8            | 50          | 0.9       | 77.4               | 3.6               | 18.2             |
| CL   |             | 10.9           | 57          | 1.0       | 77.4               | 7.2               | 63.5             |
| CL   | 10.00       | 11.5           | 46          | 1.0       | 59.4               | < 1               | 66.5             |
| CL   |             | 3.9            | 61          | 1.0       | 70.2               | < 1               | NE               |
| CL   |             | 2.8            | 42          | 1.1       | 64.8               | 9.5               | 60.3             |
| CL   |             | 1.1            | 64          | 1.1       | 52.2               | 12.8              | 12.8             |
| X    |             | (5.5)          | (53.3)      | (1.0)     | (66.9)             |                   |                  |
| WW   | 1           | 24.9           | 57          | 1.8       | 55.8               | 15.6              | 77.5             |
| WW   | 2           | 7.6            | 60          | 0.8       | 72                 | 3.1               | NE               |
| WW   |             | 19.3           | 61          | 0.5       | 52.2               | 23.8              | 178.8            |
| MM   |             | 28.6           | 46          | 1.4       | 81                 | 0.3               | 39.9             |
| WW   |             | 3.4            | 58          | 1.1       | 59.4               | 1.0               | NE               |
| WW   | 6           | 3.4            | 58          | 0.9       | 108                | 1.2               | 41.1             |
| х    |             | (14.5)         | (56.7)      | (1.1)     | (71.4)             |                   |                  |
| ER   | 1           | 9.8            | 78          | 2.0       | 59.4               | 5.9               | NE               |
| ER   | 2           | 3.6            | 66          | 1.8       | 108                | 22.4              | NE               |
| ER   | 3           | 2.0            | 63          | 1.7       | 626                | 2.6               | NE               |
| ER   | 4           | 3.1            | 61          | 1.4       | 93.6               | 27.7              | 31.2             |
| ER   | 5           | 5.6            | 56          | 1.8       | 46.8               | 16.9              | NE               |
| ER   | 6           | 10.6           | 57          | 1.8       | 327                | 32.1              | NE               |
| x    |             | (5.8)          | (63.5)      | (1.8)     | (210)              |                   |                  |
| orma | lsa         | 8 - 20 6       | 0 - 79      |           | 34 - 56            |                   |                  |

NE - not examined domestic sheep values

ER ewes. These local reactions suggest an association with focal injury and inflammation as would result from aspiration of Protostrongylus larvae.

Fragments of larvae surrounded by focal granulomas are occasionally present, usually in anterior lobes, in many sheep (Spraker 1979). This was seen in this study, however, the foci were usually without changes related to secondary bacterial infection. The focal bacterial pneumonia in CL 6 did contain larval remnants.

In contrast, chronic pneumonia in WW sheep, when present, affected more than one lobe, extended into the pleural cavity and appeared older. Microscopic changes in chronic pneumonias were similar in all herds and only varied with the age of the lesion.

P. haemolytica type T, a bacterium strongly associated with the recent B.C. and Alberta dieoffs (Onderka and Wishart 1984) was present in a WW lamb with active pneumonia and was cultured from lymphoid and lung tissues of WW4 and ER5, ewes with chronic pneumonias. The presence of the organism in association with other pathogenic opportunistic bacteria in WW 4 may be incidental as her immune system was undoubtedly compromised by the lymphoid tumor. The presence in the focal, mild, resolving lesion in an otherwise healthy ER ewe supports the hypothesis that P. haemolytica type T is an opportunistic pathogen (Onderka and Wishart 1984), and that pneumonias can occur and resolve in individual bighorn sheep without disease epidemics.

The high incidence of chronic diseases, secondary infections and continued presence of lesions from pathogenic pulmonary bacteria in adult and juvenile WW sheep in 1983, strongly suggests residual effects of stressors associated with the acute dieoff in 1982. Spraker et al. (1984) described the mortality of bighorn lambs in captive and free-ranging herds during years following all-age dieoffs. Lambs less than 6 months of age died with bronchopneumonia caused by opportunistic bacteria and showed marked atrophy of the thymus gland. The authors suggested that stressors similar to those which precipitated the all-age dieoff may continue to affect pregnant ewes. Continued high levels of glucocorticoids secreted by the highly stimulated adrenal gland can inhibit the development of the thymus and immune system of the fetus. In addition, the steroids may restrict the production of colostral antibodies. Without a fully functional immune system and the passive immunity provided by the colostrum, the neonatal lamb would be extremely vulnerable to normal microflora. Adult sheep harbouring chronic respiratory infections could easily transmit large doses of pathogens to the susceptible lambs. The pattern of lamb disease and mortality in the WW herd appears to fit this pathogenesis.

Titres to the respiratory viruses PI3, IBR, RSV and other systemic disease viruses which may cause respiratory or debilitating disease, such as BT, EHD and BVD, have been present in all North American wild sheep species examined (Howe et al. 1966, Parks and England 1974, Zarnke 1983, Clark et al. 1985, Dunbar et al. 1985). Isolations of PI3 (Parks et al. 1972, Jessup 1985), RSV (Spraker et al. 1986) and BT (Robinson et al. 1967, Kistner et al. 1975) have been made from bighorns with clinical disease. Maedi/visna is a viral disease of domestic sheep which causes a slowly progressive interstitial pneumonia and has not yet been identified in bighorn sheep. Serological results in this study suggested little exposure of the E.K. bighorns to these viruses or to Johne's disease.

Marginal copper levels were present in all three herds, but not in all samples. Marginal selenium levels in some WW sheep and levels consistent with deficiency in all CL sheep suggest the presence of deficiency related disorders in these two herds. Similar levels of copper and selenium were present in tissues from some WW bighorns which died in the E.K. dieoff in 1981 (Davidson 1982). Since that time WW mineral licks have been supplemented with a livestock trace mineral mix. It is possible that those sheep collected from the WW range had artificially high trace mineral levels and that deficiencies may have played a more important role in the herd prior to supplementation.

It is recognized that the soils of the E.K. area are generally deficient in selenium and that domestic animal syndromes are diagnosed in animals grazing on pasture in this area (Puls per. commun.). Hebert and Cowan (1971) examined mountain goats, forages and mineral licks from the E.K. area and suggested that myodystrophies seen in that species were predisposed by selenium deficiency. Eastman et al. (1971) determined that copper levels in forage plants common on low elevation winter ranges in the E.K. were especially low.

The consistently higher levels of selenium present in ER bighorns supports the findings of Hebert (1973) that alpine forages contain higher levels of trace minerals and suggests that these sheep do not suffer from selenium deficiency.

Serum chemistry profiles for healthy captive and wild bighorn sheep (Davies 1976, Franzmann 1971a, Franzmann and Thorne 1970, Woolf and Kradel 1970), stone sheep (Franzmann 1971b), Dall sheep (Foreyt et al. 1983) and Nelson desert sheep (McDonald et al. 1981), as well as for sick captive and wild bighorn sheep (Woolf and Kradel 1973, Spraker et al. 1984) have been examined. In this study healthy and sick sheep were shot, usually without prior disturbance. Samples were expected to reflect sctual physiologic values in GL and WW sheep, while the use of a helicopter was expected to increase parameters affected by acute stress, such as cortisol and glucose (Franzmann and Thorne 1970), but not total serum proteins and blood urea nitrogen. The study chemistry values were compared to the above reported levels for wild sheep species as well as those for domestic sheep.

East Kootenay bighorn glucose values were similar to literature reports as well as to domestic sheep levels. Mild to extreme increases were present with excitement, the highest in helicopter herded ER sheep. Glucose levels vary with excitement and habitat, however, without further forage evaluation no habitat quality assumptions may be made from this data (Franzmann 1972). Blood and urine cortisol values varied too greatly for accurate interpretation. In general, disturbed, clinically ill and chased sheep serum cortisols were high, although these findings were not consistent and there was no relationship with the size of individual Urine cortisol levels should not have been affected by adrenal glands. scute stresses, however, they were not corrected for urine concentration when analyzed. There was then a trend towards higher cortisol values in the order WW > CL > ER, however, the diagnosis of stress levels by this method requires more rigorous sampling technique and further research before proper assessments can be made.

The TP of the study animals was within normal domestic and bighorn sheep limits in ER sheep, decreased in GL juveniles and low normal in GL adults. The sick WW lambs also had normal TP in spite of their poor body condition. TP in these animals may have been artificially elevated due to dehydration and/or high circulating immunoglobulin levels in response to systemic infections. Other WW sheep had low normal TP except for low levels in the ewe with lymphosarcoma.

The levels of BUN were low in the majority of the study sheep. Mild increases were present in sick WW sheep, likely due to body protein catabolism. Levels similar to moderately low CL, WW and ER values were reported in healthy and sick bighorns (Franzmann and Thorne 1970, Spraker et al. 1984) and were suggested to reflect the low protein content of forages. Many of the apparently healthy sheep in good condition from CL and ER ranges had BUN values far below those reported.

The project bighorn FN values were comparable to Hebert et al. (1984) values for sheep from October to December. The average ER values were highest and corresponded with levels in migratory sheep seen in that study. WW and CL values were equal and matched the lowest levels that were found during winter months in Hebert et al. (1984) evaluations. Variations in FN of sick WW animals were ilkely due to nitrogen losses from systemic illness.

The low TP, BUN and FN values of CL sheep suggest very inadequate winter range protein content. This substantiates estimate of poor quality winter range quality for this herd. Similar findings in w sheep should be further investigated due to the variations seen in chronically ill animals. In contrast, low BUN values of ER sheep were present with normal TP and higher FN content than either of the other herds. Hebert (1972) compared high and low altitude forages in the E.K. and found those from high altitude pastures to be of higher nutrient quality. Stelfox (1976) and Schuerholz (1983) assessed the mountain grasslands of national park and E.K. bighorn habitats and found them to be of high productivity. They were also easily available due to the preference of sheep for south facing, snow free slopes. Schuerholz (1983) also noted a lack of interspecific competition for forage at this altitude. The low BUN values and marginal tissue copper levels do not completely support the prsence of higher quality of feed on the ER winter range, howver, sample numbers were low without actual feed analysis of these animals. Larger numbers of coordinated samples of forages, soils and animals over time would be required to fully understand these results.

#### Columbia Lake Treatment

The B.C. Wildlife Branch was advised to attempt treatment of CL sheep for the high levels of lungworm infection and mineral deficiencies. A reduction of animal density was considered to be a necessary part of this remedial action.

Sheep on Columbia Lake winter range were conditioned to eat fermented apple mash during the spring of 1984. Fenbendazole anthelmintic was added to treat each sheep three times at weekly intervals. Mineral licks had been supplemented with livestock trace mineral mix since the fall collection. A group of 25 sheep of mixed ages and sexes were live captured after treatment and moved to a suitable unoccupied winter range at some distance from CL.

An additional three CL sheep were collected and a posched animal examined in the fall of 1984. Similar procedures were performed as described for project sheep. Fecal samples were taken from transplanted sheep and the CL range for lungworm analysis.

Three of the sheep collected in 1984 had deficient and one had marginal selenium levels. One of the four had marginal copper values. Langworm larvae were very low in an adult ewe and a yearling, moderate in one lamb and high in a second (Figure 2). Fecal larvae levels in range collected or trapped sheep were zero following spring anthelmintic treatment but returned to near previous levels in some animals by the fall.

It is believed that the committment to intensive management of this herd has allowed a reduction in parasite load of CL bighorns. Therapy and research must continue, however, in the face of constant reinfection from the range and lack of significant changes in selenium and copper levels. Range improvement and reduction of sheep numbers through transplantation must be considered extremely important and integral remedial elements for the successful treatment of this herd.

#### CONCLUSION

The low sample size of this study prevents any conclusive statements. however, the results do suggest that there are differences between the low and high elevation wintering bighorn herds of the East Kootenay region of British Columbia. The differences were primarily related to the factors which were suggested to predispose E.K. bighorns to an all-age dieoff in 1981, high animal density, poor nutrition, parasitism and trace mineral deficiencies. High lungworm levels, reduced nutritional indices and lower selenium levels occurred in the CL herd at high animal density. wintering at high elevation on the ER range, at lower density, appeared to have access to better quality nutrition and maintained lower levels of lungworm infection. The effects of a previous pneumonia outbreak appeared to have a longterm influence on the WW herd and resulted in residual chronic disease in adult bighorns and a marked reduction in the viability of young lambs for at least 3 years post-dieoff, in spite of lower levels of lungworm infection and reduced animal density on winter range.

Although there may be a background level of some or many of the previously discussed factors in all sheep herds, at a certain population density, additional or increased levels of preexisting stressors appear to overwhelm the functional immune system and precipitate the occurrence of epidemic disease. Bighorn sheep management and research must continue to emphasize the identification of density and stressor levels where outbreaks occur. The status of a population appears to be best determined by the use of a group of indices. Hanks (1981) advises the characterization of population condition by the assessment of physiological parameters together with reproductive capacity, habitat condition and trend. Comparison of population nutritional condition and parasite levels between herds over time may then indicate and predict when stress conditions and sheep density move into the critical range before dieoffs occur and allow remedial actions to maintain and increase wild sheep populations.

# RECOMMENDATIONS

The method of sampling used in this study may not be practical or indicated for many management or monitoring schemes. The results, however, underline the importance of invasive and noninvasive monitoring techniques. Of these, we believe the collection of fecal samples from individual sheep over successive seasons and years remains the method of choice, economically and practically. Feces may be examined for lungworm larvae per gram, (Uhazy et al. 1973) to determine parameters such as mean LPG, LPG annual trends and the proportion of heavily infected (> 1400) sheep per herd (Holmes and Samuel 1974). Fecal nitrogen levels have proved useful but require further studies in field situations, again, over time. Although blood and urine cortisol levels were not useful in this study, further studies with these and a potential method for fecal cortisol

metabolites (Spraker pers commun.) may allow the determination of sheep baseline cortisol secretion and allow the evaluation of levels indicative of overall herd stress. The submission of freshly dead animals or hunter kills for necropsy and liver tissue for trace mineral analysis is also advised whenever possible.

These sampling procedures must be considered only as corollaries to the observation and study of stable and fluctuating sheep herds, their dynamics and range conditions for trends over time.

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# Questions

- Jim Bailey, Colorado -- You said your blood and urine cortisol levels were highly variable.
- Schwantje -- Yes, they were generally high in animals that I could predict would be high, like those hazed by helicopter. Some of the levels from those sheep were low. In the other herds other animals I expected to be high were not.
- Bailey -- I guess that answers my question. I was going to ask you if you attempted to correlate cortisol levels with any other factors, in particular, the thymus and adrenal glands.
- Schwantje -- The blood and urine cortisol levels for those animals with small thymuses and large adrenals were higher than other sheep in the same herd but were similar to sheep with normally sized glands in other herds.