



Evaluation of lungworm, nutrition and predation as factors limiting the recovery of the Stillwater bighorn sheep herd
by Lee Carroll Jones

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Biological Sciences
Montana State University
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Abstract:

The Stillwater bighorn sheep herd is currently at a low of approximately 30 animals. From November 1991 to August 1993, Protostrongylus spp. lungworm levels, nutrition, and predation were evaluated on the winter range in relation to the recovery of the Stillwater bighorn herd. A total of 259 fecal pellet groups was collected and analyzed for lungworm larvae. For purposes of comparison, a total of 53 pellets was also collected and analyzed for lungworm from the Cinnabar bighorn herd. Although during both years in the Stillwater herd, the prevalence of lungworm larvae did increase in the spring to 60% or more, the average level of infection, no more than 4 larvae per gram for any month sampled, suggests that lungworm does not play a significant role in limiting the herd. Fenbendazole medicated salt, as administered in the last four years, appears to have been effective in controlling protostrongylid lungworm numbers. In comparison, the Cinnabar herd exhibited characters of an unmedicated herd, with 57 % prevalence, and an average larvae per gram output of 29, ranging up to a maximum of 225 larvae per gram. Stillwater fecal pellets were also analyzed for nitrogen content, an index of nutrition. Results suggest that nutrition is not a limiting factor to the Stillwater herd. Additionally, a study of known mortalities in the Stillwater herd suggests that mountain lion (*Felis concolor*) predation does play a role in limiting sheep overwinter survival. However, bacterial pneumonia resulted in approximately 50% of the known mortalities in the winter of 1992-1993. Additionally, since November of 1991, approximately 56% of the mortalities have been from unknown causes. Although protostrongylid lungworm and nutrition do not appear to be limiting factors in the recovery of the Stillwater herd, additional monitoring of these factors, as well as the study of predation, should continue. Future studies should be conducted to identify mortality factors for the Stillwater bighorn sheep herd on the summer range.

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STILLWATER BIGHORN SHEEP HERD**

by

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Lee Carroll Jones

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

May 21, 1994
Date

David E. Worley
Chairperson, Graduate Committee

Approved for the Major Department

May 23, 1994
Date

Harold R. Peterson
Head, Major Department

Approved for the College of Graduate Studies

6/6/94
Date

R. Brown
Graduate Dean

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ABSTRACT

The Stillwater bighorn sheep herd is currently at a low of approximately 30 animals. From November 1991 to August 1993, Protostrongylus spp. lungworm levels, nutrition, and predation were evaluated on the winter range in relation to the recovery of the Stillwater bighorn herd. A total of 259 fecal pellet groups was collected and analyzed for lungworm larvae. For purposes of comparison, a total of 53 pellets was also collected and analyzed for lungworm from the Cinnabar bighorn herd. Although during both years in the Stillwater herd, the prevalence of lungworm larvae did increase in the spring to 60% or more, the average level of infection, no more than 4 larvae per gram for any month sampled, suggests that lungworm does not play a significant role in limiting the herd. Fenbendazole medicated salt, as administered in the last four years, appears to have been effective in controlling protostrongylid lungworm numbers. In comparison, the Cinnabar herd exhibited characters of an unmedicated herd, with 57 % prevalence, and an average larvae per gram output of 29, ranging up to a maximum of 225 larvae per gram. Stillwater fecal pellets were also analyzed for nitrogen content, an index of nutrition. Results suggest that nutrition is not a limiting factor to the Stillwater herd. Additionally, a study of known mortalities in the Stillwater herd suggests that mountain lion (Felis concolor) predation does play a role in limiting sheep overwinter survival. However, bacterial pneumonia resulted in approximately 50% of the known mortalities in the winter of 1992-1993. Additionally, since November of 1991, approximately 56% of the mortalities have been from unknown causes. Although protostrongylid lungworm and nutrition do not appear to be limiting factors in the recovery of the Stillwater herd, additional monitoring of these factors, as well as the study of predation, should continue. Future studies should be conducted to identify mortality factors for the Stillwater bighorn sheep herd on the summer range.

INTRODUCTION

The number of bighorn sheep (Ovis canadensis) in North America has declined drastically since the turn of the century (Buechner 1960). These declines have often been attributed to the lungworm-pneumonia disease complex (Honest and Frost 1942, Buechner 1960, Forrester 1971, Forrester and Senger 1964). Buechner (1960) suggested that bighorn sheep may have brought lungworm with them from Asia.

The lungworm-pneumonia complex is indeed very "complex", and is often associated with other physiological stress factors, such as bacterial and viral infection, poor nutrition, inclement weather, multiple parasitism, overcrowding, predation, and human caused disturbances (Forrester 1971, Hudson and Stelfox 1976, Hibler et al. 1982, Foreyt and Jessup 1982, Onderka and Wishart 1984, Belden et al. 1990, Foreyt 1990). Some authors have suggested that due to the complex nature of pneumonia and to the incomplete understanding of most predisposing factors or stresses, the lungworm-pneumonia complex be renamed "stress-related pneumonia" (Spraker et al. 1984, Festa-Bianchet 1988). Therefore, until the many factors involved in the pneumonia complex are better understood, it is necessary to examine any possible predisposing or stress factors that may be present in bighorn sheep populations.

Festa-Bianchet and Samson (1984) concluded that parasitism may be both a cause and result of stress. In many cases of pneumonia, Protostrongylus spp. lungworm is clearly a predisposing or stress factor for bighorns (Couey 1950, Buechner 1960, Worley et al. 1976, Wishart et al. 1980, Silflow and Foreyt 1988), especially in the case of summer lamb mortality due to transplacentally derived lungworm infections (Hibler et al. 1976, Schmidt et al. 1979, Festa-Bianchet and Samson 1984, Samson et al. 1987, Festa-Bianchet 1988). Most bighorn sheep are infected with lungworm (Forrester and Senger 1964, Foreyt and Johnson 1980, Festa-Bianchet and Samson 1984, Yde et al. 1988, Fougere-Tower and Onderka 1988, Festa-Bianchet 1988). Herds in areas with heavily used ranges may be more susceptible to infection due to a denser population of the terrestrial snails that serve as the intermediate host (Schmidt et al. 1979). Both prevalence (the proportion of hosts infected) and the intensity of infection (indicated by the first-stage larval output per gram of feces (LPG)) are used to measure the parasite pressure on bighorns (Forrester and Senger 1964, Gregory and Blackburn 1991). Lungworm levels may be affected by other factors, such as herd density and nutrition of the animal (Schwantje 1986, Festa-Bianchet 1988).

Nutrition of the animal is thought by many to be important in relation to pneumonia-induced mortality (Honest and Frost 1942, Samson et al. 1987, Forrester and Senger 1964, Foreyt and Jessup 1982). Although current information suggests that poor nutrition is not necessarily a causal factor in relation to pneumonia (Jessup 1981, Foreyt and Jessup 1982, Bailey 1986, Ryder et al. 1992), the pneumonia complex is multi-factorial, and poor nutritive condition may likely be one predisposing or stress factor (Samson et al. 1987, Dunbar 1992). Additionally, nutrition has been shown to influence other important

population parameters of bighorn sheep (Honest and Frost 1942, Hebert et al. 1984). Fecal nitrogen has been shown to be an effective measure of bighorn nutrition and population condition (Hebert et al. 1984, Irwin et al. 1993).

Predation may constitute an additional source of mortality. Williams (1992) found that of 56 mountain lion kills and 27 lion scats examined in the Sun River area, bighorn sheep accounted for 18% of total kills and occurred in 20% of the scats. Bighorns accounted for 14% of total prey biomass. In some cases, predation may disproportionately affect one segment of a herd. Although some authors have suggested that rams, during or just after the rut may be more susceptible to predation than other classes (Geist 1971, Harrison and Hebert 1988), Sun River bighorn ewes accounted for 9% of the total prey biomass for mountain lions, more than all the deer sex and age classes combined (Williams 1992). Geist (1971) stated that lamb mortality due to predation was unlikely in the precipitous terrain in which lambing occurs. Although Hass (1989) reported significant lamb mortality due to predation, steep rocky terrain was not available to sheep in their study.

The Stillwater bighorn sheep herd, one of the last 12 native herds in Montana (Thorne et al. 1985), was reported to have peaked in the late 1940s and early 1950s at more than 100 animals (Buechner 1960, Pallister 1974), although this peak in the early 1950s may have been overestimated (Stewart 1975). Numbers declined in the 1960s, and then again increased in the 1970s to about 60 sheep (Pallister 1974, Stewart 1975, Stewart 1980, Farmer 1986). During this period of increase in the 1970s, lamb to ewe ratios ranged from approximately 40-60 : 100 (Pallister 1974, Stewart 1975). Summer and winter mortality of lambs was relatively low (Stewart 1980). However, the Stillwater herd has again been in

decline since the early 1980s, with lamb recruitment averaging 20% from 1982-1988, and no recruitment at all in 1987 (Farmer 1992). When compared to the estimate of 50% recruitment needed for population growth, it is obvious that the Stillwater herd may have a problem maintaining a viable population (Lawson and Johnson 1982). Stillwater lambs born in the summer are not arriving on the winter range (Farmer 1990). From 1989 to 1992, known summer lamb mortality was at least 50% (Farmer 1993). Of 10 lambs observed on the summer range in 1989, only 4 arrived on the winter range (Farmer 1990). The high levels of lungworm in relation to pneumonia were thought to be a primary cause for mortality of bighorn ewes and lambs (Final Environmental Impact Statement: Stillwater Mine Expansion... 1992). The Stillwater bighorn sheep herd currently consists of an estimated 30 sheep. In early winter of 1992-1993, there were an estimated 6-8 rams, 18-20 ewes, and 3 lambs.

The Stillwater herd has been tested periodically for lungworm. In 1964, this herd had one of the highest average levels of infection of herds surveyed in Montana at 900 larvae per gram of feces and 100% prevalence (Forrester and Senger 1964). Stewart (1975) recorded Protostrongylus spp. larval output levels at an average of 5.5 LPG in 91% of Stillwater sheep samples. However, in the mid to late 1980s, average levels again increased to approximately 100 LPG in more than 80% of samples tested. One lamb was known to have died in 1988 from lungworm-pneumonia (Farmer 1986, 1988, 1990).

Efforts were made in 1989 to treat the main segment of the Stillwater herd with fenbendazole medicated alfalfa pellets. Although some researchers have reported medicated pellets as palatable to sheep (Huschle and Worley 1986, Foreyt et al. 1990), the Stillwater

herd apparently did not find them so (Worley unpublished). An older captive ewe that was accustomed to pelletized feed was even released into the Stillwater herd, hoping she might induce other sheep to eat the medicated feed (Farmer 1990). Later, salt blocks and loose medicated salt were placed on the winter range, with no response (Worley and Seesee, unpublished). However, with the use of apple pulp as an attractant, sheep were finally observed consuming 0.5% fenbendazole medicated salt on the main winter range early in the summer of 1990. Salt was also placed on the summer range (Farmer 1991). Initial findings the following winter suggested that fenbendazole consumption had been adequate, with average lungworm LPG values below 1. Additionally, a young ewe that died from a fall in spring of 1991 had very low lungworm levels (Farmer 1991). Medicated salt has not been successfully placed on any Stillwater secondary winter ranges. The Stillwater sheep have had continued access to medicated salt on the main winter range every year since 1990.

In comparison, the Cinnabar herd has an estimated 60 - 100 animals, and may have declined in the last several years (L. Irby, personal communication). The Cinnabar herd also has been examined for lungworm. Arnett et al. (1993) reported that from 1984 - 1987, 100 % of Cinnabar sheep were infected with a mean (\pm SE) of 234 (\pm 51), 112 (\pm 17), and 194 (\pm 34) larvae per gram for lambs, ewes, and rams, respectively. The Cinnabar herd has not been medicated for lungworm in the past (D. Worley, personal communication).

Beginning in 1986, periodic measurements of Stillwater sheep fecal nitrogen values were made to assess the nutritional status of the herd. Although sampling was not done regularly and sample sizes were at times small, nitrogen values were generally within the range described by Hebert et al. (1984). However, a response to spring "green-up" was not

seen in some years (Farmer 1992). Irwin et al. (1993) suggested that fecal nitrogen values below 1.3% may be indicative of nutritional deficiencies. For winter range samples from 1986 - 1991 (n=18), 17% of Stillwater fecal samples contained less than 1.3% fecal nitrogen (Farmer 1992).

The Stillwater area has resident populations of bobcats, coyotes, black bears, mountain lions, and eagles. Prior to the late 1980's, predation was not considered a problem for the Stillwater bighorn herd. However, populations of coyotes and mountain lions appeared to be increasing. Although this increase was not documented quantitatively, reports of both species in the area increased. The first documented predation loss was in 1990; a mountain lion killed the captive ewe introduced earlier that year (Farmer 1991). Predation may affect bighorns directly through mortality or indirectly by harassment or displacement of animals (Farmer 1986).

The objectives of this study were to examine three factors possibly limiting the recovery of the Stillwater herd: 1) measure the prevalence, intensity and seasonal patterns of Protostrongylus spp. infection and determine the effectiveness of a free-choice fenbendazole medicated salt program in a free-ranging bighorn herd; 2) measure fecal nitrogen as an index to herd nutrition and condition; and 3) attempt to learn the cause of mortalities in order to estimate the extent of predation on the herd.

STUDY AREA

The study area is located at the Stillwater Mining Co. facility near Nye, Stillwater County, Montana, approximately 80 miles (130 km) southwest of Billings in the Beartooth Mountains. The Stillwater Mine is the only significant source of platinum and palladium in North America (Nelson 1990, Stillwater Mining Company pamphlet). Development began in 1986, and the mine activity areas are situated on both sides of the Stillwater River, one of 10 blue-ribbon trout streams in Montana (Stillwater Mining Company pamphlet). The Stillwater herd's primary winter range is approximately a 3 square mile (5 square km) parcel contained within the permit area for the Stillwater Mine. The elevation of the primary winter range is approximately 5,000 feet (1600 m) above sea level, with most bedding areas located about 600 feet (200 m) higher on a rocky outcrop referred to as the "reef". The secondary winter range on the West Fork of the Stillwater is used by a few individuals that generally join the main herd segment in spring.

The Cinnabar bighorn herd is located approximately 8 miles (13 km) north of Gardiner, Park County, Montana, on the west side of the Yellowstone River in the Gallatin Mountains. The elevation of the winter range is approximately 5000 feet (1600 m) above sea level.

METHODS

Attempts were made to collect feces at 2-3 week intervals on the Stillwater primary winter range where the main habituated segment of the herd is located. Additionally, collections were made on the West Fork winter range during the winter of 1991-1992. All known bedding and activity areas were searched, and all fresh pellets known to be from sheep were collected. Due to the small size of the herd and to the large number of fecal samples collected, it is unlikely that sampling methods affected results. Also, samples from individually recognizable sheep were obtained from direct observation. Although larvae of Protostrongylus spp. are known to remain viable in samples that are several months old (Buechner 1960, Hibler et al. 1982), no samples were collected that were estimated to be more than a few weeks old. In the field, pellets were classified according to age and sex of the donor when possible. Samples from animals of known sex were obtained from direct observation, or in the case of a few females, from the position of the pellets directly adjacent to lamb pellets in a bed. The samples were placed inside reclosable plastic bags and were placed in a refrigerator as soon as possible. A few of the samples collected by Stillwater Mine personnel were not refrigerated for several weeks. However, due to the viability of Protostrongylus spp. larvae (Hibler et al. 1982), the lack of refrigeration was unlikely to have a significant effect on the results.

Collections from the Cinnabar range were conducted from February to April, 1993. Fecal pellets were collected from the lower portion of the winter range where sheep graze. However, since many other ungulates frequently use the Cinnabar bighorn winter range, all bighorn samples were taken only when defecation was observed.

The Baermann technique (Forrester 1971, Hibler et al. 1982) was used to extract larvae from seven gram samples of feces. Samples were left in small funnels (10 cm diameter) for 24 hours; however, plastic funnels were used since suitable glass ones were not available. Funnel material may have reduced the number of larvae recovered up to 34%, but since funnel material was consistent throughout the study, no effects on comparisons or trends should occur (Beane and Hobbs 1983). Approximately 10 ml of fluid containing first-stage Protostrongylus spp. larvae were withdrawn into petri dishes for examination. Larvae were counted under a 25 x dissecting microscope; results were expressed as first-stage larvae per gram of feces (LPG). These data were also used to calculate prevalence of Protostrongylus spp. Fecal analysis data is not reliable in assessing parasitism in individual animals due to large variation in larval shedding rates; however, it is useful in consideration of levels of parasitism in the herd as a whole (Forrester and Senger 1964).

Nitrogen analysis on Stillwater samples was performed by the Montana State University Chemistry Station Laboratory on monthly composite samples. Thirty pellets, 2 each from 15 pellet groups, were randomly subsampled from the pellet groups collected for lungworm analysis. The Kjeldahl method was used, and total fecal nitrogen values are given on a percent dry weight basis.

Mortality data for the Stillwater herd were collected when a collared sheep died, during fecal pellet collection ground surveys, and by Stillwater Mine personnel. Ground surveys included all areas of known sheep activity on the winter range. Surveys of the winter range were also conducted three times during the summer, as several sheep visit the winter range during the summer, presumably to obtain salt. Fresh carcasses were examined by the Montana Department of Fish, Wildlife and Parks Research Laboratory in Bozeman, the Parasitology Laboratory at Montana State University Veterinary Molecular Biology Department, and by the state of Montana Department of Livestock Diagnostic Laboratory.

Statistical analysis was done on MSUSTAT, version 5.10, developed by Richard E. Lund, Montana State University, Bozeman, MT 59717-0002. Fecal collections from the main winter range and from the West Fork winter range were treated separately, even though West Fork sheep often join the main segment on the main range in late winter or early spring. Loglinear fit for p-way tables (LOGLIN) analysis was performed to test for a relationship between age and prevalence of Protostrongylus spp. in the Stillwater herd. Chi-square analysis was used to evaluate all other lungworm data. Statistical significance was determined at $p < 0.05$.

RESULTS

Lungworm

Sheep of all age and sex classes were observed using the medicated salt. The number of sheep observed using salt at a time and the frequency of observations of salt use suggest that most, if not all, Stillwater sheep were using salt. No sheep were observed coughing.

The prevalence of Protostrongylus spp. in the main segment of the Stillwater herd generally increased over the sampling period for each year. As shown in Figure 1, the trends were similar between years, except for the value for December 1992. Each of the three samples from August 1993 were negative. The increases in prevalence in both years became statistically significant in late winter and early spring ($p < 0.05$) (Table 1). The December 1992 sample also was significantly different from the following January and February samples but was not different from April 1993. No difference was found between the prevalence of lungworm in males and females for either winter. Results of the LOGLIN analysis suggested that there was no difference between the prevalence of lungworm in sheep less than 2 years old and adults. All 26 samples from the West Fork segment of the herd were negative.

The Cinnabar herd did not have access to fenbendazole at any time. Figure 2 shows the difference between the prevalence of Protostrongylus in each herd, both sampled in February and April, 1993 ($p = 0.004$). It is clear that the statistical significance was derived

Figure 1. Prevalence of *Protostrongylus* spp. in Stillwater bighorn sheep on the main winter range: November 1991 to August 1993.

**Sample sizes represent total number of samples examined.

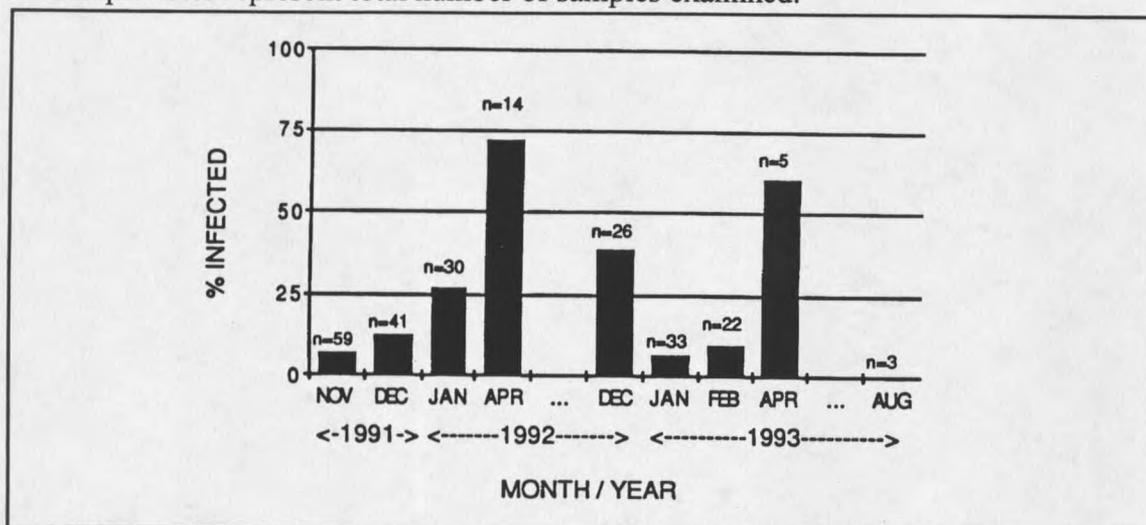


Table 1. Differences between months sampled for prevalence of *Protostrongylus* spp. in the Stillwater bighorn sheep on the main winter range: November 1991 to August 1993.

Month	P-value
Nov 91 to Dec 91	0.352
Dec 91 to Jan 92	0.119
Nov & Dec 91 to Jan 92	0.012*
Jan 92 to Apr 92	0.005*
Nov & Dec 91 to Apr 92	0.000*
Apr 92 to Dec 92	0.046*
Dec 92 to Jan 93	0.002*
Jan 93 to Feb 93	0.672
Feb 93 to Apr 93	0.008*
Dec 92 to Apr 93	0.371
Apr 93 to Aug 93	0.090

* indicates statistical significance at $p < 0.05$

from the prevalence differences in February, as they were similar in April. No difference was found for prevalence among sex or age classes.

The average LPG values of the positive samples for the Stillwater herd were all below 4 LPG (Figure 3). The maximum LPG values recorded were approximately 11 and 13 LPG in December 1992, from a 2-year-old ram and a 6-year-old ram, respectively. No difference was found in the LPG distributions between age or sex categories.

Comparison to the Cinnabar herd showed that there was a significant difference between LPG distributions of a medicated and an unmedicated herd (collapsed to a 2 category distribution, $p = 0.018$), as shown in Figure 4.

There were several individually recognizable sheep in the Stillwater herd. Although samples were collected from 15 - 16 recognizable sheep, only 7 sheep had more than one sample collected. As lambs matured, they were no longer identifiable by association with their mothers. Rams often disappeared later in the winter, and sometimes were replaced by other rams. Table 2 summarizes the results of known individual fecal collections.

From November 1991 - August 1993, all known ewe samples were below 2 LPG. Of 10 samples from 4 known adult ewes, 3 samples (each from a different ewe) were positive for lungworm larvae. No ewe ever tested positive for Protostrongylus spp. larvae more than once.

In 6 known lamb samples from 5 different lambs, only 2 were positive, each from different lambs. Although the mother of only 1 of the 2 positive lambs was known, the known mother had tested positive the winter before. All known lamb samples were below 1 LPG. Of 3 known yearling samples from 3 yearlings, 2 were positive. Of 3 samples from

2 2-year-olds, only one was positive (from a 2-year old ram), at approximately 11 LPG, the second highest LPG recorded during this study.

Of 6 samples from 2 - 3 known rams, 4 were positive, from 1 or 2 individuals. It is not known if the 5-year-old in 1991-1992 was the same sheep as the 6-year-old seen in 1992-1993. There may have been two sheep in that age class that were indistinguishable. The maximum LPG recorded for this herd during this study came from this 6-year-old ram.

One interesting parasitological observation of several dorsal-spined larvae was made in a Stillwater December 1992 fecal sample. Since adult worms were not available for verification, it was difficult to determine the species. Measurements of larvae, however, suggested that they may have been Muellerius spp. Although few researchers have reported this lungworm in bighorns (DeMartini and Davies 1976, Layne and McCabe 1986), this genus has been commonly reported in domestic sheep and goats in North America and Europe (Levine 1980, Anderson 1992).

Figure 2. Prevalence of Protostrongylus spp. in the Stillwater and Cinnabar bighorn sheep herds, 1993.

* Fecal pellets were not available from the Stillwater herd

