Parasite loads and their relationship to herd health in the Highlands bighorn sheep herd in southwestern Montana

by Kerrie L Hoar

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:
A two year study was conducted to determine the parasite load of a bighorn sheep herd located in the Highlands and East Pioneer Mountain ranges in southwest Montana. This study involved the post-mortem examination of 52 bighorn sheep followed by microscopic examination of recovered parasites for identification purposes. Forty-five fecal samples from these sheep, as well as 35 samples from a group of bighorn sheep captured for transplanting, were tested via the Baermann and the modified Lane fecal flotation techniques.

Sixteen species of parasite belonging to eight genera were collected and identified during the course of this study-including ten nematode, one cestode, and five protozoan species. A modified Lane fecal flotation test, performed on 27 fecal samples, revealed the presence of five species of Eimerian Protozoa-E. ahsata, E. faurei, E. intricata, E. ovina, and E. ovinoidalis. The Baermann technique revealed an average LPG (the number of Protostrongylus spp. Larvae Per Gram of fecal material) level for this herd to be 10.7 ±27.6 in 1992 (n = 26) and 116.6 ±153.3 in 1993 (n = 19). The group of 35 bighorns that were captured for transplanting in December, 1992, had an average LPG level of 21.8 ±74.1. Statistical analysis showed no significant difference between any of these LPG levels.

One species of cestode, Wyominia tetoni, was recovered from the livers of nine bighorn sheep. The lungs from 50 sheep were examined and were found to harbor a mean Protostrongylus spp. infection level of 3.4 ±4.6 in 1992 and 3.1 ±5.1 in 1993. In addition, Ostertagia trifurcata, 0. ostertagi, Nematodirus abnormalis, N. davtiani, Chabertia ovina, and Trichuris spp. were recovered in low numbers both years-with all infections being less than twenty adult worms per animal. Marshallagia marshalli, recovered from 38 of the 44 abomasa examined, was the only species that occurred in significant levels in this herd with averages of 235.5200 and 114.4737 in 1992 and 1993, respectively. However, high standard deviations make these averages unreliable as a health index.

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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.

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Date December 6, 1995

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TABLE OF CONTENTS

	Page
LIST OF TABLES	vii
LIST OF FIGURES	viii
ABSTRACT	ix
Introduction	1
Study Objectives	5
Study Area	6
Methods	8
Post-Mortem Examination Procedure	11
Liver	11
Lungs	13
Gastrointestinal Tract	14
Baermann Technique	15
Modified Lane Fecal Flotation Technique	16
Statistical Tests	17
Results	19
Post Mortem Examination	19
Liver	20
Lungs	21
Gastrointestinal Tract	22
Baermann Technique	26
Modified Lane Fecal Flotation Technique	29
Herd Health vs. Parasite Level	30
Discussion	35
Post-Mortem Examination	35

Liver 35
Lungs 36
Gastrointestinal Tract 37
Baermann Technique 39
Modified Lane Fecal Flotation Technique 40
Herd Health vs. Parasite Level 41
Statistical Tests 41
Disease Transmission 42
Conclusions and Recommendations 45

THE REFERENCE MATTER 48

APPENDICES 55

Post Mortem Examination Results 56
Letters 66

LIST OF TABLES

Table		Page
1	Precipitation data collected at the NOAA weather station at Divide, Montana, for 1992 and 1993.	12
2	Summary of results obtained from post-mortem examination of 31 bighorn sheep received during the 1992 hunting season.	32
3	Summary of results obtained from post mortem examination of 21 bighorn sheep received during the 1993 hunting season.	33
4	Summary of results obtained from Baermann testing performed on fecal samples taken from a group of 35 bighorn sheep on December 23, 1992.	34
5	Summary of results of Baermann tests performed on fecal samples taken from 3 groups of bighorn sheep during the course of this study.	34
6	Results of a qualitative Modified-Lane Fecal Flotation test performed on fecal samples taken from hunter-killed bighorn sheep during the 1992 hunting season.	57
7	Results of a qualitative Modified-Lane Fecal Flotation test performed on fecal samples taken from hunter-killed bighorn sheep during the 1993 hunting season. All bighorn ages are given in years.	58
8	Results of a Baermann test performed on fecal samples taken from hunter-killed bighorn sheep during the 1992 hunting season.	59
9	Results of a Baermann test performed on fecal samples taken from a group of 35 bighorn sheep that were captured on December 23, 1992, and were transplanted to the Sleeping Giant region near Helena, Montana.	60
10	Results of a Baermann test performed on fecal samples taken from hunter-killed bighorn sheep during the 1993 hunting season.	61
11	Results of a post-mortem test performed on lungs and livers taken from hunter-killed bighorn sheep during the 1992 hunting season.	62
12	Results of a post mortem test performed on intestinal tracts taken from hunter-killed bighorn sheep during the 1992 hunting season.	63
13	Results of a post-mortem test performed on lungs and livers taken from hunter-killed bighorn sheep during the 1993 hunting season.	64
14	Results of a post-mortem test performed on intestinal tracts taken from hunter-killed bighorn sheep during the 1993 hunting season.	65

LIST OF FIGURES

Figure		Page
1	Probable distribution of bighorn sheep in the United States before (left) and after (right) the arrival of the white man (Buechner, 1960; Forrester, 1971)	4
2	Map of HD340 taken from (MDFWP, 1995b)	7

ABSTRACT

A two year study was conducted to determine the parasite load of a bighorn sheep herd located in the Highlands and East Pioneer Mountain ranges in southwest Montana. This study involved the post-mortem examination of 52 bighorn sheep followed by microscopic examination of recovered parasites for identification purposes. Forty-five fecal samples from these sheep, as well as 35 samples from a group of bighorn sheep captured for transplanting, were tested via the Baermann and the modified Lane fecal flotation techniques.

Sixteen species of parasite belonging to eight genera were collected and identified during the course of this study–including ten nematode, one cestode, and five protozoan species. A modified Lane fecal flotation test, performed on 27 fecal samples, revealed the presence of five species of *Eimerian* Protozoa–*E. ahsata*, *E. faurei*, *E. intricata*, *E. ovina*, and *E. ovinoidalis*. The Baermann technique revealed an average LPG (the number of *Protostrongylus* spp. **L**arvae **P**er **G**ram of fecal material) level for this herd to be 10.7 ±27.6 in 1992 (n = 26) and 116.6 ±153.3 in 1993 (n = 19). The group of 35 bighorns that were captured for transplanting in December, 1992, had an average LPG level of 21.8 ±74.1. Statistical analysis showed no significant difference between any of these LPG levels.

One species of cestode, *Wyominia tetoni*, was recovered from the livers of nine bighorn sheep. The lungs from 50 sheep were examined and were found to harbor a mean *Protostrongylus* spp. infection level of 3.4 ±4.6 in 1992 and 3.1 ±5.1 in 1993. In addition, *Ostertagia trifurcata*, *O. ostertagi*, *Nematodirus abnormalis*, *N. davtiani*, *Chabertia ovina*, and *Trichuris* spp. were recovered in low numbers both years–with all infections being less than twenty adult worms per animal. *Marshallagia marshalli*, recovered from 38 of the 44 abomasa examined, was the only species that occurred in significant levels in this herd with averages of 235.5200 and 114.4737 in 1992 and 1993, respectively. However, high standard deviations make these averages unreliable as a health index.

Introduction

At the beginning of the 19th century, there were between 1,500,000 and 2,000,000 bighorn sheep (*Ovis canadensis*) in North America (Buechner, 1960). By the end of the 19th century, bighorn sheep populations had been driven to the brink of extinction due to market hunting, degradation of and competition for habitat by domestic species, human intrusion into terrain favored by bighorns and epizootics. Today, there are less than 100,000 bighorn sheep left in North America, (see Fig. 1) (Buechner, 1960; Jessup, 1981). In the mid 1900's, strict hunting regulations were imposed on bighorn sheep hunters in an attempt to prevent the extinction of this species. Restrictions were also put on the grazing of domestic livestock to prevent habitat degradation and competition for resources between bighorn sheep and domestic livestock. Once these measures were in place, attempts were made to reintroduce bighorn sheep into areas where they had disappeared. The first bighorn sheep transplant in Montana took place in 1942 when 11 sheep from the Sun River herd were relocated to the Gates of the Mountains near Helena. Today, approximately one half of Montana's huntable populations of Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) have been established or augmented by transplants (Janson, 1974).

The original Highlands bighorn sheep herd, located in the Highlands and East Pioneer mountain ranges of southwestern Montana, disappeared in the early 1900's (Couey and Schallenberger, 1971). In the late 1960's, an effort was made to re-establish this herd through two transplants of bighorn sheep from the Sun River herd. The first transplant, consisting of 27 sheep, took place in 1967. This was

followed by a second transplant of 31 sheep in 1969 (Couey and Schallenberger, 1971; Janson, 1974). Both groups of bighorn sheep were released into the Camp Creek drainage of the Highlands mountain range. The success of these two transplants has been phenomenal, growing from a group of 58 animals occupying a single drainage to today's herd which exceeds 350 animals [1] and extends over two mountain ranges. The majority of the Highlands' mature rams exceed 180 points (by Boone and Crockett scoring methods) by the age of $6\frac{1}{2}$ (Frisina, 1992; Frisina and Atcheson, 1993).

In the past several years, the Highlands herd has developed a reputation among bighorn sheep hunters for its large rams. Since 1989, hunters have taken at least 19 trophy-size rams (196+ points) from this herd (Frisina, 1992). Each year Montana offers one bighorn sheep permit to the highest bidder to raise money needed to fund bighorn sheep management projects and research. The 1992 recipient purchased the tag for $88,000 and killed a $195\frac{4}{8}$ (green score) ram from the Highlands bighorn sheep herd. In addition, recipients of previous auctioned tags have hunted and successfully taken trophy rams from this herd (King, 1992). On April 21, 1992, the carcass of a $7\frac{1}{2}$ year old ram was found in this area which ranks as the largest bighorn ram in the United States and the fifth largest in the world with a score of $203\frac{5}{8}$ points. (This ram might have scored 208-210 points if it had not laid in the field for several months prior to scoring.) (Frisina, 1992; Frisina and Atcheson, 1993; Weigand, 1994).

Even though this re-established herd has been in existence for over 25 years, limited information is available about its current size, parasite load and general health status. Further, no information is available on whether the parasite load is stable or increasing in this population. Information on parasites could be very important, as Thorne, et al. (1982) believes that large populations of bighorn sheep are more likely

[1] In 1994, a major epizootic killed more than half of this herd. To date, it is not known exactly how many sheep survived.

to contract disease and/or parasites than small populations. It has also been documented that many bighorn die-offs occur in populations that appear to be thriving or have reached or exceeded the carrying capacity of their range. In addition, this herd may eventually be affected by the possibility of the BLM issuing a permit to allow four sections of public land, in and around bighorn winter range and rutting grounds in the western portion of the traditional range utilized by the herd, to be grazed by domestic sheep. This would allow for the possible transmission of disease and parasites from these domestic sheep to the bighorns. This action has been delayed until an ongoing study of the Highlands bighorn sheep herd's feeding and migration habits has been completed.

During this study, hunter-killed bighorn sheep were examined in order to determine the numbers and types of parasites present in the Highlands bighorn sheep herd. The greatest advantage in using hunter-killed sheep in a study of this type is that a large sample can be obtained in a short time from widely distributed areas without arousing public animosity (Hunter and Pillmore, 1954). These data were also used to determine if there is a relationship between population size and parasite loads. The latter is based on a procedure for using parasite levels to estimate range utilization, herd densities, and other health-related factors in free-ranging ungulate populations. This concept has been used to correlate parasite intensity with the health status of white-tailed deer herds in the southwestern United States (Demarais et al., 1983; Eve and Kellogg, 1977), and is used routinely to infer a relationship between the size of deer herds and available forage (Doster, 1985). The concept of using parasite levels, especially lungworm (*Protostrongylus spp.*) and stomach worm (*Marshallagia*) burdens in bighorn sheep as indicators of the balance between herd size and available range has been proposed (Worley and Seesee, 1988 and 1992).

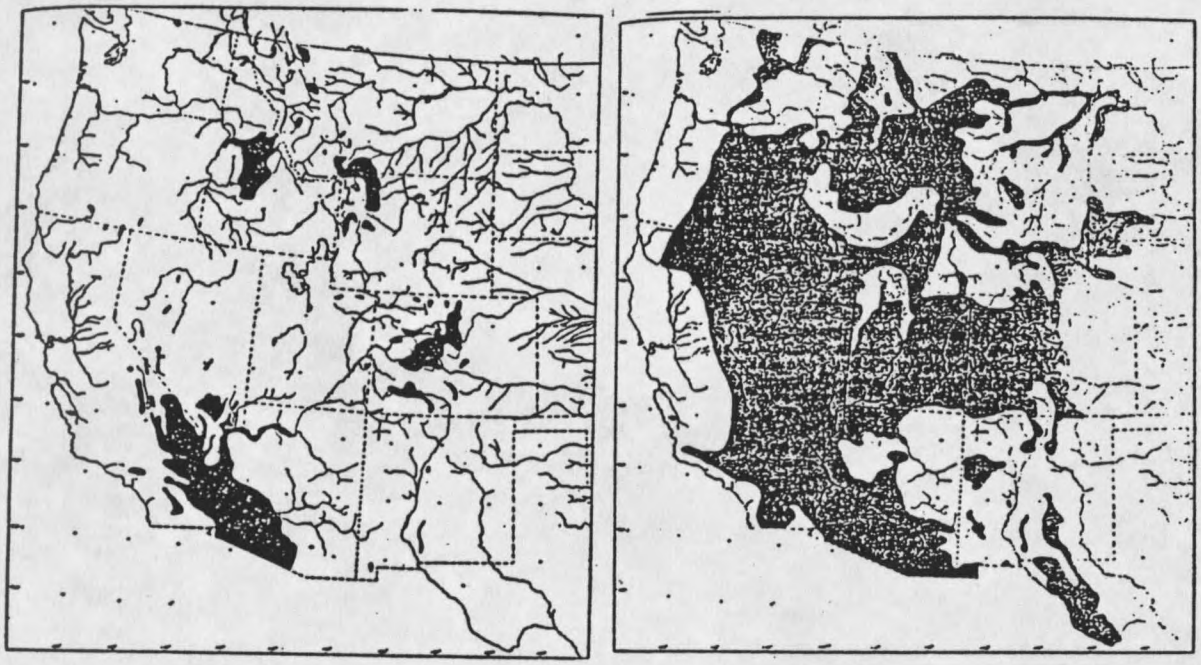

Figure 1: Probable distribution of bighorn sheep in the United States before (left) and after (right) the arrival of the white man (Buechner, 1960; Forrester, 1971).

Study Objectives

The objectives of this study were to:

1. Identify lung and gastrointestinal parasites present in the Highlands bighorn sheep herd.

2. Estimate the average level of lung and gastrointestinal parasite infections present in the Highlands bighorn sheep herd–both within individual sheep and within the herd.

3. Identify potential trends in the level of parasite infection in the Highlands bighorn sheep herd.

4. Determine if there is a correlation between sex, age, and/or level of precipitation and level of parasite infection in the Highlands bighorn sheep herd.

5. Identify potential relationships between bighorn sheep population size and level of parasite infection in the Highlands bighorn sheep herd.

Study Area

The Highlands bighorn sheep herd is located primarily within Montana Hunting District 340 (HD 340), (see Fig. 2) The boundaries of the study area were determined by the 1992-93 legal descriptions of HD 340 for bighorn sheep hunting issued by the Montana Department of Fish, Wildlife and Parks (MDFWP). The MDFWP bulletin states that HD 340 encompasses "those portions of Beaverhead, Madison, Jefferson and Silver Bow Counties lying within the following-described boundary: Beginning at Dillon, then northerly along Route 41 to Route 2, then northwesterly along said route to Interstate 90, then westerly along said interstate to Interstate 15, then southerly along Interstate 15 to Route 43, then westerly along said route to the Quartz Hill Road, then southerly along said road to Vipond Park and the Canyon Creek-Trapper Creek Road, then southerly and easterly along said road to Interstate 15 at Melrose, then southerly along said interstate to Dillon, the point of beginning" (MDFWP, 1992).

HD 340 encompasses 235km^2 with elevation ranging from 1593m along the Big Hole River to 3108m on Table Mountain in the Highlands range (Weigand, 1994). Land ownership is a combination of private, Bureau of Land Management (BLM), Forest Service, and state-owned parcels.

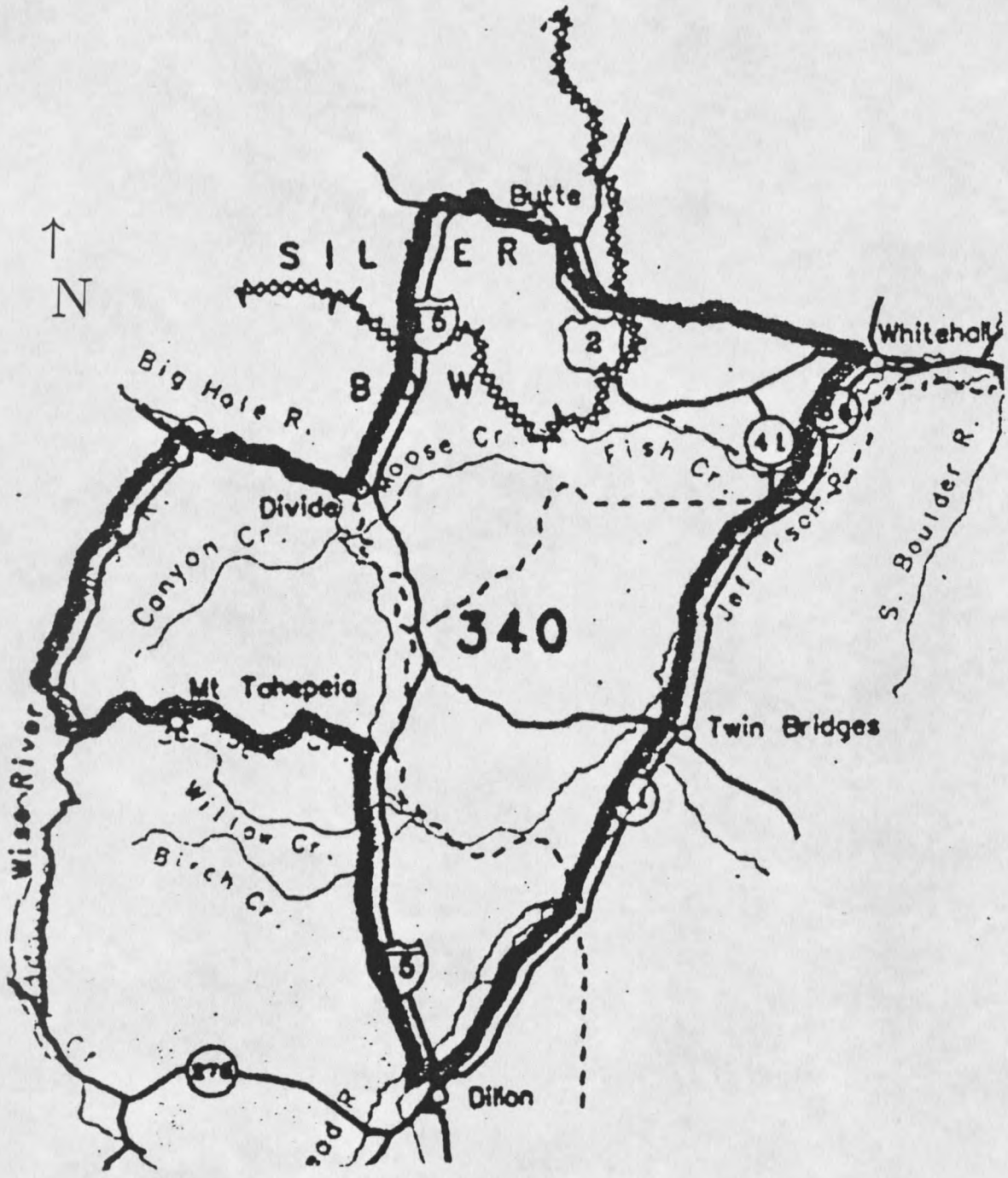

Figure 2: Map of HD340 taken from (MDFWP, 1995b).

Methods

Convenience sampling of hunter-killed sheep in HD 340 was accomplished in the following manner. Lists of HD 340 bighorn sheep permit holders for the 1992 and the 1993 hunting seasons were obtained from the Montana Department of Fish, Wildlife and Parks (MDFWP). The MDFWP Regional Supervisor for Region 3 wrote a letter requesting the cooperation of each permit holder in the collection of an incisor, lungs, liver, and gastrointestinal tract of each hunter-killed sheep for use in this study and that of another Montana State University graduate student, (see Appendix B). A copy of this letter, along with a collecting kit, was sent to each person on both the 1992 and the 1993 permit holder lists approximately one to two weeks prior to the beginning of the bighorn sheep hunting season (which begins on September 1 and ends on November 29). Each collecting kit consisted of one coin envelope for the storage of the incisor, one (1992) or two (1993) large garbage bags for the storage of requested organs, one pair of disposable gloves, and one tag for identification of hunter and other pertinent information such as date and location of kill and sex and age of sheep. The specimens that were obtained in 1992 were usually returned in an additional garbage bag (probably to prevent leakage); therefore, the 1993 collecting kits contained an additional garbage bag. Manila identification tags were used in 1992, but, upon receipt of specimens, it was discovered that moisture had obscured much of the information on many of the tags. For this reason, the tags used in 1993 were Tyvek and any known information (e.g., hunter name) was written on the tag with a permanent marker prior to the inclusion of the tag in the collecting kit. This measure allowed for the collection of sex and age information on a greater number of

sheep in 1993.

Each sheep carcass that was received underwent an extensive post-mortem examination. The fecal samples obtained during post-mortem examinations were analyzed in the lab with the use of the Baermann and the modified Lane fecal flotation procedures. Rumen contents were removed and frozen for examination by Weigand (1994) for inclusion in his food habits study on the Highlands bighorn sheep herd. Liver specimens were collected and frozen and are now being tested in a DNA analysis study. Any parasites recovered from these hunter-killed sheep were then counted and, when possible, their genus, species, and sex determined. These data were then used to determine the intensity and types of parasite infections present in the Highlands bighorn sheep herd.

The age of each bighorn was determined in one of three ways. First, some of the sheep were aged in the field through mandibular tooth replacement and wear and through annular horn rings by an MSU graduate student or a MDFWP employee when the animal was collected from the hunter. Second, records of all hunter-killed bighorn rams are kept by the MDFWP. These records include the age of the ram, as well as other information such as date and location of kill. Since the name of the hunter was known for some of the ram specimens, the MDFWP records were consulted to verify the age of those specimens. Finally, the original letter sent to each hunter requested the collection of an incisor. The incisors that were received were sent to Matson's Laboratory in Milltown, Montana, for age determination utilizing the cementum age analysis technique. A copy of the report made by Gary Matson for all teeth received by him is included in Appendix B. These age data were later analyzed in determining the presence or absence of an age/parasite load correlation for the Highlands bighorn sheep herd. Those animals for which no age data could

be determined were not included in this analysis. In one case in which the age was determined through both the annular horn ring and the tooth cementum age analysis techniques, the age differed by one year; therefore, the data from this sheep was not included in the age/parasite load correlation.

The sex of each bighorn was determined in one of five ways. First, Montana issues bighorn sheep permits for HD 340 in two categories–"either sex" and "adult ewe". Since the name of the hunter was known for some of the sheep specimens, the MDFWP permit lists were used to determine which type of tag was issued to that individual. Those hunters who received "adult ewe" tags are assumed to have taken a female. Second, the sex of some of the bighorn sheep was determined in the field by an MSU graduate student or a MDFWP employee when the animal was collected from the hunter. Third, some of the hunters indicated the sex of their bighorn on the identification tag included in their collecting kit. Fourth, the MDFWP records of hunter-killed bighorn rams was again consulted for those specimens for which the name of the hunter was known. Finally, many of the specimens contained reproductive organs that allowed the sex of that bighorn to be determined. These sex data were later analyzed in determining the presence of a sex/parasite load correlation for the Highlands bighorn sheep herd. Those animals for which no sex could be determined were designated Unknown, **U**, and were not included in this analysis.

Precipitation data were collected via modem using the U.S. Department of Agriculture, Soil Conservation Service's Centralized Forecast System (CFS), Operational Database located at the West National Technical Center in Portland, Oregon Some of the major data types contained in the CFS include snow course measurements, SNOTEL–telemetered sensor values, National Weather Service (NWS), and NOAA climate station data. These data types provide such data as snow water

equivalent (i.e., water content of snow pack), current and historical precipitation, and current and historical air temperatures. The SNOTEL computer polls remote telemetry sites, files site data (e.g., snow water equivalent, air temperature, precipitation, and soil temperature), and produces special reports of site conditions. In addition to the precipitation data that are collected at each SNOTEL site, the CFS also includes monthly precipitation data that are collected by the National Weather Service (NWS) and loaded into the Operational Database (ODB). All data in the CFS are formatted into a water year period which runs from October to the following September (SCS, 1988). Specific site information such as location, elevation, etc., as well as the data which were down-loaded from the CFS computer are included in Table 1.

Post-Mortem Examination Procedure

The post-mortem protocol followed was similar to that described by Thorne et al. (1982) and Worley and Seesee (1988). Each specimen that I received was first rinsed and then separated into its respective parts–lungs, liver, abomasum, small intestine and large intestine, and then each individual section was examined thoroughly for any abnormalities and/or parasites. The results of each individual post-mortem examination can be found in Appendix A. A summary of the overall herd results for each year are tabulated in the next section in Tables 2 and 3.

Liver

The liver was examined for the presence of tapeworms and/or liver flukes. First, the liver was examined externally for any nodules or lesions that may have been caused by a parasite. Any such deformities were removed and preserved in glycerin-

1992 and 1993 Divide Precipitation

Month	1992		1993	
	Ppt. (°F)	Dev. (°F)	Ppt. (°F)	Dev. (°F)
October	2.4	0.81	1.5	-0.09
November	2.4	0.49	2.2	0.29
December	1.1	-0.97	2.4	0.33
January	1.0	-1.29	2.4	0.11
February	2.3	0.33	2.2	0.23
March	1.0	-1.78	1.8	-0.98
April	1.8	-0.68	4.4	1.92
May	1.2	-1.93	2.4	-0.73
June	6.9	3.84	4.2	0.36
July	1.9	0.21	3.1	1.41
August	0.4	-1.2	3.0	1.4
September	1.7	-0.27	1.1	-0.87
TOTAL	24.1	-2.44	30.7	4.16

Table 1: Precipitation data collected at the NOAA weather station at Divide, Montana, for 1992 and 1993. This weather station is located near Divide, Montana, in Silver Bow county at an elevation of 1649 m. Abbreviation: Ppt. = Precipitation, Dev. = Deviation from average precipitation recorded for the Divide NOAA station.

alcohol (5% glycerin in 70% ethyl-alcohol) for further examination. A two-to-three inch square section was then removed from the posterior end of the right or left lobe of the liver. This liver section was frozen for use in a DNA analysis study which is currently being conducted by another MSU graduate student. The gallbladder and bile duct were then opened and examined for the presence of parasites. Finally, the liver tissue was sectioned with a post-mortem knife at 1 inch intervals and examined grossly for any parasites.

All cestodes were removed from the liver tissue (taking care to keep the worm intact with its scolex whenever possible), rinsed in tap water, and preserved in labeled

specimen vials filled with glycerin-alcohol. At a later date, these tapeworms were placed in a petri dish filled with tap water and examined under a dissecting microscope to determine genus or species utilizing Thorne et al. (1982).

Lungs

The lungs were examined for the presence of lungworms (*Protostrongylus* spp.). First, they were examined externally for any nodules or lesions which could have been caused by parasites. Any such abnormalities were excised and preserved in glycerin-alcohol for further examination. The lungs were then thoroughly washed over an 80-mesh screen and all of the major air passages opened with scissors. Again, the lungs were washed thoroughly to remove any lungworms that may have been present in the air passages. These washings were then examined in an illuminated tray with an attached magnifying glass. All suspicious material was removed to a water-filled petri dish for examination with a dissecting microscope to confirm their identity. All nematodes were then placed in labeled, glycerin-alcohol filled specimen vials for preservation until they could be examined in detail at a later date.

The washed lungs were then cut into one to two inch square sections, placed in a one-gallon container of tap water, and placed on a mechanical shaker for approximately 45 minutes to dislodge any nematodes which might be located in the lung parenchyma. The lung sections and washings were thoroughly rinsed over an 80-mesh screen. The lung tissue was discarded, and the washings were examined in an illuminated tray with a magnifying glass attachment. All suspicious material was removed to a water-filled petri dish for examination with a dissecting microscope to confirm their identity. All nematodes were then placed in glycerin-alcohol filled specimen vials for preservation until they could be identified specifically at a later

date.

Later, preserved nematodes were placed on a microscope slide with a drop of glycerin to preserve the mount, then covered with a coverslip. The specimens were then examined under a light microscope. Utilizing Honess and Winter (1956), Thorne et al. (1982), and Boev (1984), the genus, species, and sex of each nematode was determined whenever the sections of the parasite required for identification were intact.

Gastrointestinal Tract

The gastrointestinal tract was divided into abomasum, small intestine and large intestine. The contents were removed from the rumen and frozen. The rumen was then discarded. All fat and excess tissues were removed from each section and examined carefully to detect the presence of any larval tapeworm cysts. Cysts recovered were then crushed in a water-filled petri dish to release the larva and examined for identification using a dissecting microscope. Whenever possible, a fecal sample was taken from the rectum or large intestine and refrigerated in a plastic specimen cup to be analyzed at a later date using the Baermann and modified Lane fecal flotation techniques described below.

An enterotome was used to incise, wash, and scrape each section of the intestinal tract simultaneously over screens (Bizzell and Ciordia, 1962; Davis, 1944). The ingesta were thoroughly rinsed over these screens to rinse away much of the excess soluble debris. The screen sizes used were as follows: 60-mesh for the abomasal contents, 80-mesh for the small intestinal contents, and 24-mesh for the large intestinal contents. All washed ingesta were placed in jars and mixed with tap water. A small

amount of 10% formalin was then added to each labeled jar to preserve the ingesta for later study.

Later, the ingesta were again washed over the same screens used in the initial wash to remove any remaining soluble debris and then examined in an illuminated tray using a magnifying glass attachment. All suspicious material was then removed to water-filled petri dishes to confirm their identity. All nematodes were then placed in labeled specimen jars filled with glycerin-alcohol for identification at a later date. For identification, these worms were placed in a drop of glycerin on a microscope slide with a coverslip and examined with a light microscope. Utilizing Ransom (1911), Yorke and Maplestone (1962), Skrjabin et al. (1970), Levine (1980), and Thorne et al. (1982), each specimen was identified as to genus, or species, and sex whenever the required sections of the parasite were intact.

Baermann Technique

The Baermann fecal analysis procedure was first described in 1917 as a method to extract hookworm larvae from soil (Baermann, 1917). A modified Baermann technique was developed in 1922 and is now used widely to recover nematode larvae from feces (Beane and Hobbs, 1983; Dinaburg, 1942). In this study, the Baermann technique was used to determine the number and concentration of lungworm larvae (*Protostrongylus* spp.) present in a particular fecal sample.

When possible, a fecal sample was removed from the rectum of each sheep during the post-mortem examination. If the rectum was not collected, the fecal sample was obtained from the most distal portion of the large intestine. In a few instances, no fecal sample was available for examination (e.g., those bighorns for

which the large intestine was not collected).

The Baermann apparatus consisted of a plastic funnel into which a wire screen was placed. A piece of rubber tubing was slipped over the stem of the funnel and closed with a clamp. Fecal material collected during post mortem examination was weighed into 5g[2] samples, wrapped in two layers of cheesecloth, and placed on the screen. The funnel was then filled with enough warm tap water to immerse the test material. The funnels were left in place for approximately 24 hours to allow time for any larvae present in the sample to migrate out of the feces into the water. These larvae would sink to the bottom of the tubing and were withdrawn into a gridded Petri dish and examined with a dissecting microscope to determine the number of first-stage larvae present (Thorne et al., 1982). First-stage larvae (L_1) are the non-infective stage of the parasite that is passed from the gastrointestinal tract of its host. It is most easily identified by its characteristic tail inflection (Lange, 1974). The weight of the sample and the total larvae count were then used to determine the **L**arvae **P**er **G**ram of fecal material (LPG) for each individual fecal sample.

Modified Lane Fecal Flotation Technique

The modified Lane fecal flotation procedure was developed by Dewhirst and Hansen in 1961, and is used routinely to recover many nematode and trematode ova and protozoan oocysts (and some larvae) (Dewhirst and Hansen, 1961). In this study, the modified Lane fecal flotation technique was used to determine the presence

[2]In 1992, I began using 10g fecal samples. After a literature search was conducted, I replaced this practice with one which utilized 5g samples as used in Dinaburg (1942) and Beane and Hobbs (1983). The use of 5g samples is just as efficient, but less time-consuming during the counting of larvae.

In addition, fecal samples taken from bighorn sheep captured for the purpose of being transplanted to another range consisted of only 2-5 pellets; therefore, the samples weighed less than 5g.

of gastrointestinal nematode ova and coccidian oocysts in a particular fecal sample.

This apparatus consisted of a glass centrifuge tube into which fecal material was mixed with a small amount of a saturated salt solution. This mixture was stirred for 1 to 3 minutes to break up the fecal pellets. The mixture was then transferred through a small screen into a clean centrifuge tube. This tube was then filled with a saturated salt solution until a slight bulge or positive meniscus formed. A cover-glass was then placed on top of the tube, and the apparatus was left alone for approximately 10 minutes to allow enough time for ova to rise and adhere to the underside of the coverslip. The coverslip was then lifted carefully from the tube and placed on a clean microscope slide and examined under a light microscope to determine the type and number of ova and oocysts present (Thorne et al., 1982).

Statistical Tests

For the purpose of conducting statistical tests on the data that were collected, some basic assumptions were made. One, the samples were assumed to be random samples, so that each individual bighorn sheep in the Highlands herd had an equal chance of being included in the sample. Second, it was assumed that each sample was independent and representative of the whole herd.

Statistical tests for equality of two population means were based on techniques for both normal populations with equal variances and large samples from arbitrary populations (Neter et al., 1988 (p404)). The common t-test (based on the assumption that $\sigma_x^2 = \sigma_y^2 = \sigma^2$) was used for comparing normal populations with equal variances. The Z-test was used for comparing large samples from arbitrary populations. This test is based on the assumption that $\sigma_x^2 \neq \sigma_y^2$ and the assumption that the sample

sizes are large enough so that the estimated standard deviations of the sample means are roughly equivalent to the respective parameters (e.g., $\sigma_{\bar{X}} \approx \frac{s_x}{\sqrt{n}}$). An F-test for testing the equality of variance was used to determine which of these two tests, for the means, was appropriate. The method described by Neter et al. (1988 (p412)) was used for analyzing the difference between two population proportions.

A statistical test for determining the difference between two population means using matched samples was employed for analyzing precipitation data. This method simplifies to the analysis of a single population mean using a common t-test (Neter et al., 1988 (p407)).

The statistical test employed for determining the relationship between a dependent variable (e.g., LPG) and one or more independent variables (e.g., bighorn age) was based on a one-way analysis of variance (ANOVA) model, or F-test (Neter et al., 1988 (p719)). This test is based on the assumption that the probability distributions of $\bar{Y}$ corresponding to the different treatments (e.g., age groups) are each normal with the same variance. Therefore, differences in treatment effects (e.g., LPG levels of age groups) are associated with differences between the treatment (e.g., age group) means, μ_j.

Results

Post Mortem Examination

In 1992, 42 bighorn sheep hunting permits were issued for Hunting District 340 (12 'either sex' and 30 'adult ewe' tags). During the 1992 hunting season, 37 bighorn sheep were actually taken in HD 340. Of these 37 sheep, all or part of the requested organs from 28 animals were received for post mortem study. In addition, three bighorn sheep were found dead in the field (field mortalities) by MDFWP employees, and samples were collected from them and examined for inclusion in this study. In 1993, 16 'either sex' and 30 'adult ewe' tage were issued for HD 340. Of these, all or part of the requested organs from 21 animals were received for post mortem study.

Incisors from 17 bighorn sheep were received during this study (7 in 1992 and 10 in 1993). These teeth were sent to Matson's for age analysis, the results of which are included in Appendix B.

Parasites recovered from the Highlands bighorn sheep herd include *Wyominia tetoni*, *Protostrongylus rushi*, *Protostrongylus stilesi*, *Marshallagia marshalli*, *Ostertagia ostertagi*, *Ostertagia trifurcata*, *Nematodirus abnormalis*, *Nematodirus davtiani*, *Chabertia ovina*, *Trichuris* spp., and cysts of *Taenia hydatigena*. Only *M. marshalli* occurred in significant levels in the 52 sheep examined in this study. The average annual infection levels of all other parasites were less than 20 worms per sheep.

Liver

Examination of the gall bladder and bile ducts of 51 sheep (30 in 1992 and 21 in 1993) led to the recovery of only one parasite type–that of *Wyominia tetoni*. *Wyominia tetoni* was first described by J. W. Scott in 1941 and has only one known host–*Ovis canadensis* (Scott, 1941; Thorne et al., 1982). This parasite was recovered in very low numbers (1-2 per sheep) from 9 of the 51 livers examined. In addition, a common t-test showed that there was no significant difference between the 1992 and the 1993 infection levels [3]. T-tests also showed that no correlation existed between tapeworm infection level and sex of sheep in either year [4]. The identification of a trend in cestode levels required that at least three sets of data be compared; therefore, no such analysis was attempted.

No evidence of liver flukes was seen during post mortem examination of any bighorn sheep specimens. Liver lesions were recovered from two sheep specimens in 1992 and were analyzed at the Department of Livestock Diagnostic Laboratory at Marsh Laboratory in Bozeman, Montana. Both lesions consisted of abundant fibrous connective tissue. One of the specimens also contained dark pigment, a reaction consistent with the presence of liver flukes.

[3] A 95% Confidence Interval (CI) was calculated using a t-test with $n_{92}=30$ and $n_{93}=21$ and $t_{29}=2.045$ and $t_{20}=2.086$. The resulting intervals ($-0.18 \leq \mu_{92} \leq 0.78$ and $-0.24 \leq \mu_{93} \leq 0.72$) overlapped, indicating that no significant difference existed between these two means.

[4] A 95% CI was calculated for 1992 using a t-test with $n_{ram}=9$ and $n_{ewe}=17$ and $t_8=2.306$ and $t_{16}=2.120$. The resulting intervals ($-0.39 \leq \mu_{ram} \leq 0.61$ and $-0.40 \leq \mu_{ewe} \leq 0.99$) overlapped, indicating that no significant difference existed between these two means. A 95% CI was also calculated for 1993 using a t-test with $n_{ram}=10$ and $n_{ewe}=11$ and $t_9=2.262$ and $t_{10}=2.228$. The resulting intervals ($-0.58 \leq \mu_{ram} \leq 1.38$ and $-0.31 \leq \mu_{ewe} \leq 0.49$) overlapped.

Lungs

The lungs from 30 sheep were received in 1992. Of these, 19 contained parasites in either the air passages or lung tissue. In 1993, the lungs from 17 sheep were received, twelve of which contained parasites. All of the recovered lungworms were later identified as either *Protostrongylus rushi*, which lives in the air passages, or *Protostrongylus stilesi*, which lives in the lung tissue. A third species, *P. frosti*, has been observed in bighorn sheep in Wyoming, but was not observed in the bighorn sheep of the Highlands herd (Honess and Winter, 1956). The average infection rate per sheep was 3.4 $\pm$4.6 (n = 30) in 1992 and 3.1 $\pm$5.1 (n = 17) in 1993.

Infection levels of both rams and ewes were compared to determine if a correlation existed between sex and lungworm infection level as indicated by Festa-Bianchet (1988 and 1991). A common t-test showed that no such correlation existed in this herd [5]. Statistical tests also revealed that lungworm infection levels in 1993 had not changed significantly from those in 1992 [6]. The identification of a trend in *Protostrongylus* spp. levels required that at least three sets of data be compared; therefore, no such analysis was attempted.

Three bighorns were diagnosed with verminous pneumonia by a veterinary pathologist in the Department of Livestock Diagnostic Laboratory at Marsh Laboratory in Bozeman, Montana. The diagnosis was made after a thorough examination of lung nodule sections recovered during post mortem examination. These nodules

[5] A 95% CI was calculated for 1992 using a t-test with n_{ram}=9 and n_{ewe}=17 and t_8=2.306 and t_{16}=2.120. The resulting intervals ($-3.12 \leq \mu_{ram} \leq 12.67$ and $2.12 \leq \mu_{ewe} \leq 8.03$) overlapped, indicating that no significant difference existed between these two means. A 95% CI was also calculated for 1993 using a t-test with n_{ram}=8 and n_{ewe}=9 and t_7=2.365 and t_8=2.306. The resulting intervals ($-6.52 \leq \mu_{ram} \leq 12.52$ and $-4.01 \leq \mu_{ewe} \leq 10.24$) overlapped.

[6] A 95% CI was calculated using a t-test with n_{92}=30 and n_{93}=17 and t_{29}=2.045 and t_{16}=2.120. The resulting intervals ($0.04 \leq \mu_{92} \leq 6.77$ and $-2.07 \leq \mu_{93} \leq 8.19$) overlapped, indicating that no significant difference existed between these two means.

contained "innumerable nematode parasites and large numbers of ova" of the genus *Protostrongylus*. In addition, the pulmonary parenchyma immediately surrounding the nodules contained areas of necrosis and evidence of inflammatory cell response both of which are consistent with verminous pneumonia. This same phenomenon was seen in lung nodules removed from one bighorn sheep in 1993, and this animal was also diagnosed with verminous pneumonia.

Gastrointestinal Tract

The examination of portions of the gastrointestinal tract in 1992 and 1993 revealed the presence of 8 species of parasites. Statistical testing was conducted to determine if the average infection level of each of these parasites differed significantly during the two years of the study. In all cases no statistically significant difference existed between parasite levels between years. These data were to be further compared to the environmental conditions (e.g., precipitation amounts) during those two years to determine if parasite levels could be correlated to precipitation levels as suggested by Forrester and Littell (1976). Since parasite levels did not differ significantly between years, no comparison testing was conducted with the environmental data. In addition to 'between year' comparisons, all parasite data were also tested to determine if a correlation existed between host sex and infection level. In all cases, no such correlation could be detected. The identification of a trend in gastrointestinal nematode levels required that at least three sets of data be compared; therefore, no such analysis was attempted.

Abomasum. The abomasa from 25 sheep were received in 1992. Of these, 19 contained parasites. *Marshallagia marshalli* were recovered from 18 of these abomasa,

while specimens of *Ostertagia trifurcata* and *Ostertagia ostertagi* were found in only five. In 1993, all 19 abomasal specimens that were examined contained parasites. One abomasum contained the scolex of the bighorn sheep tapeworm *Wyominia tetoni*. The liver, where such cestodes are normally found, was not available for examination. However, it was assumed that this tapeworm specimen had been displaced from the liver to the abomasum of this sheep. In addition to *W. tetoni*, nematodes of the species *M. marshalli*, *O. trifurcata*, and *O. ostertagi* were also found in 1993.

Marshallagia marshalli was the most common parasite found in bighorns during this study, with an average infection level per sheep of 235.5 $\pm$287.7 (n = 25) in 1992 and 114.5 $\pm$99.4 (n = 19) in 1993. In addition, *Marshallagia* was the only parasite found in significant numbers during this study. At first glance the 1992 average appears to be much higher than the 1993 average, but testing showed that the difference was only marginally significant [7]. In addition, statistical testing was conducted to determine if *M. marshalli* infection levels were sex related. Testing was conducted for both 1992 and 1993 and resulted in no significant difference between *Marshallagia* burdens in rams versus ewes in either year [8]. Statistical tests indicated that infection levels of *Ostertagia* spp. did not differ significantly between the two years of this study [9]. In addition, t-tests showed that infection levels were not correlated with host sex [10].

[7] A Z-test was conducted with n_{92}=25 and n_{93}=19, using an α risk of 0.05 (Z(0.05)=1.96). The resulting statistic, Z_{obs}=1.96, indicated that the difference between years was marginally significant.

[8] A 95% CI was calculated for 1992 using a t-test with n_{ram}=6 and n_{ewe}=16 and t_5=2.571 and t_{15}=2.131. The resulting intervals ($-36.07 \leq \mu_{ram} \leq 694.73$ and $-118.24 \leq \mu_{ewe} \leq 577.49$) overlapped, indicating no that significant difference existed between these two means. A 95% CI was also calculated for 1993 using a t-test with n_{ram}=9 and n_{ewe}=10 and t_8=2.306 and t_9=2.262. The resulting intervals ($-61.60 \leq \mu_{ram} \leq 309.8196$ and $-3.41 \leq \mu_{ewe} \leq 215.01$) overlapped.

[9] A Z-test was conducted with n_{92}=25 and n_{93}=19, using an α risk of 0.05 (Z(0.05)=1.96). The test statistic, Z_{obs}=1.63, indicated that no significant difference existed between these two means.

[10] A 95% CI was calculated for 1992 using a t-test with n_{ram}=6 and n_{ewe}=16 and t_5=2.571 and t_{15}=2.131. The resulting intervals ($-15.77 \leq \mu_{ram} \leq 27.77$ and $-5.11 \leq \mu_{ewe} \leq 13.11$) overlapped, indicating that no significant difference existed between these two means. A 95% CI was also calculated for 1993 using a t-test with n_{ram}=9 and n_{ewe}=10 and t_8=2.306 and t_9=2.262. The