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A preliminary investigation of the protostrongylin lungworm-bighorn sheep relationships in Montana

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A PRELIMINARY INVESTIGATION OF THE PROTOSTRONGYLIN LUNGWORM-BIGHORN SHEEP RELATIONSHIPS IN MONTANA

by

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INTRODUCTION

Native sheep of the genus Ovis apparently originated in Asia in pre-Pleistocene time and part of the thick-horned subgenus Pachyceros eventually reached North America. This probably occurred by a crossing of the Bering Sea land bridge during the late Pliocene or early Pleistocene or by the crossing of an ice bridge during a glacial period of the Pleistocene. The formation of the Cordilleran ice cap during the Pleistocene divided this North American population into a northern and southern stock. These stocks evolved separately into Ovis dalli in the northern part of Alaska and O. canadensis south of the ice cap in the Rocky Mountains, Cascades, Sierra Nevadas, and certain badlands and desert ranges of Western United States. After recession of the ice the two groups spread toward each other until at present their ranges are about 100 miles apart near the Peace River in British Columbia. These two species are closely related to O. nivicola of northeastern Siberia and all three species are probably descendants of the same ancestral sheep (Cowan, 1940).

Seton (1929) estimated that prior to 1800 there were about two million bighorn sheep in North America. Settlement of the west by white man was followed by overhunting, range competition, and subsequently a decrease in bighorn populations (Seton, 1929). Disease was believed to be involved in

the decline, at least in later times.

Specific observations have been reported on several recent declines or die-offs of bighorn sheep. During the winter of 1924-1925 the Sun River herd in Montana dropped from 250 to 64 (Rush, 1927). National Bison Range records show an estimated die-off of 66 percent of 100 bighorns in 1930 and 53 percent of 65 bighorns in 1940. Contor (1959) reported that the bighorns in Rocky Mountain National Park decreased from 1,000 in 1920 to 329 in 1939 and to 210 in 1959. Hunter and Pillmore (1954) reported 499 known sheep deaths during the winter of 1952-1953 in Colorado and estimated that two-thirds of a herd of 1,500 was lost. The exact causes of these declines are still not known, but many theories have been advanced to account for the decrease in bighorn populations. Among the suggested causes are scabies (Mills, 1937; Potts, 1938; Honess and Frost, 1942; Spencer, 1943; Packard, 1946; Smith, 1954; Jones et al, 1957), coccidiosis (Packard, 1946; Contor, 1959), mineral and protein deficiencies (Packard, 1946), range competition and depletion (Mills, 1937; Honess and Frost, 1942; Packard, 1946), and predation (Packard, 1946).

Bighorn losses attributed to what has been called hemorrhagic septicemia with associated consolidation of lung tissue have been reported from Colorado (Packard, 1946; Hunter and Pillmore, 1954), Canada (Cowan, 1944; Green, 1949), Montana (Rush, 1927; Marsh, 1938; Couey, 1950), Yellowstone National Park (Mills, 1927), Glacier National

Park (Potts, 1938), and Rocky Mountain National Park (Potts, 1937; Spencer, 1943).

The parenchymal lungworm Protostrongylus stilesi was found associated with symptoms of pneumonia in bighorn lungs and described by Dikmans (1931). Later Dikmans (1937; 1943) described a new bronchiolar lungworm P. rushi. Honess (1942) described another parenchymal lungworm P. frosti, which was subsequently re-described by Dikmans (1957). Marsh (1938) found P. stilesi in lungs of bighorns which had died of chronic pneumonia. Hunter and Pillmore (1954) correlated these lungworms with lung pathology and pneumonia and suggested that the early losses, attributed to hemorrhagic septicemia, may have actually been caused by a lungworm-pneumonia complex.

Protostrongylin lung nematodes of the family Metastrongylidae have also been reported from mule deer (Odocoileus hemionus), cottontail rabbits (Sylvilagus spp.), and snowshoe hares (Lepus americanus) (Pillmore, 1957) and from domestic sheep and goats (Boev and Wolf, 1940; Gerichter, 1948; Mapes and Baker, 1950). Apparently all protostrongylin lungworms have an indirect life cycle involving an intermediate host, while other closely related lungworms such as the dictyocaulids have direct cycles.

Hobmaier and Hobmaier (1930), working with P. rufescens of domestic sheep, were first to report the life cycle of a protostrongylin lungworm. Mapes and Baker

(1950) added additional information to the cycle of P. rufescens and substantiated the work of Hobmaier and Hobmaier (1930). Further confirmation and expansion of the life cycle of protostrongylin lungworms have been reported by Boev and Wolf (1940), working with Synthetocaulus (=Protostrongylus) hobmaieri in Russia, Shults and Boev (1940), working with S. hobmaieri and S. raillieti in Russia, Gerichter (1948), working with P. kochi in Jerusalem, and Hunter and Pillmore (1954) and Pillmore (1958b), working with P. stilesi and P. rushi in the United States.

Pillmore (1958b) presents the life cycle of the lungworms P. stilesi and P. rushi, both of which infect Rocky Mountain bighorn sheep. The dioecious adult lungworms mate in the lungs of the host and the females deposit ova which hatch into first stage larvae. These larvae migrate up the respiratory passages, are then swallowed by the host, and pass out through the intestinal tract in the feces. Under favorable environmental conditions, the larvae emerge from the droppings and must come in contact with a suitable terrestrial snail and penetrate its foot to continue the cycle. Inside the snail the larvae undergo two molts before the infective stage is reached. Apparently the snails containing the infective larvae must be ingested by a bighorn to complete the cycle. Once the infective larvae are in the sheep they supposedly migrate to the lungs, mature and reproduce

to begin the cycle once more.)

It appears from the previous reports that protostrongylosis might have an important influence on bighorn populations in North America, but additional information is needed in order to evaluate this situation properly. This information might allow game biologists to manage and control populations of bighorn sheep more efficiently. With these relationships in mind the present investigation was undertaken.

The major objectives of this study were to investigate the distribution of land mollusks which could serve as intermediate hosts of protostrongylin lungworms infecting bighorn sheep, to determine the frequency and degree of protostrongylin infections in bighorn sheep in Montana, and to obtain additional information about the life cycle and bionomics of the lungworms of bighorn sheep.

Results from physiological studies of the first stage larvae will be presented first in order to provide a background for the subsequent sections on lungworm infections in Montana bighorn sheep and intermediate host bionomics.

PHYSIOLOGY OF FIRST STAGE LARVAE OF *PROTOSTRONGYLUS STILESI* AND *P. RUSHI*

Review of Literature

Studies on the physiology of first stage larvae of *Protostrongylus stilesi*, *P. rushi*, and other protostrongylids are very limited. Most reports have been concerned with temperature extremes and desiccation.

Several workers have observed the survival of larvae that have emerged from fecal pellets. Hobmaier and Hobmaier (1930) reported that first stage larvae of *P. rufescens* will survive in water for a year. In Russia, larvae of *Synthetocaulus* (=*Protostrongylus*) *hobmaieri* from domestic sheep and goats, which had migrated from fecal material, were found to survive for 40 days beneath turf at 90-100 percent relative humidity and temperatures of 64-68°F. (18-20°C.) (Matekin *et al*, 1954). Pillmore (1956) found first stage larvae of *P. stilesi* to survive desiccation in glass dishes for 30-50 days.

Additional observations have been given on the survival of larvae in fecal material. Honess (1942) reported that first stage larvae of *P. rushi* and *P. frosti* survived in droppings stored in paper bags in an out-building for 15 months, but observed no living larvae after 22 months. Air temperatures as low as -27°F. (-32°C.) were recorded during this time. Couey (1950) found that almost all of the *P. stilesi* and *P. rushi* in dried fecal pellets were alive after

20 months storage in a building during which time temperatures as low as -40°F. (-40°C.) were recorded.) Protostrongylus rufescens larvae were found alive in "completely dried" fecal pellets of sheep and goats in Jerusalem (Gerichter, 1951). Matekin et al (1954), while working on the biology of protostrongylin larvae in Middle Asia, found live larvae of Synthetocaulus hobmaieri, S. kochi, and S. raillieti in sheep and goat droppings which had been deposited on subalpine pastures nine months previously. Live larvae of S. hobmaieri and S. raillieti were observed in droppings which had been stored for 19 months at 50-68°F. (10-20°C.) and a relative humidity of 35-50 percent. Larvae of S. hobmaieri were found to survive for an undetermined length of time in partially dried droppings subjected to temperatures 77-133°F. (25-56°C.) and larvae of S. kochi and S. raillieti remained alive in pellets under snow for 150 days (Matekin et al, 1954). Schanzel (1958) found that larvae of P. kochi from domestic sheep in Czeckoslovakia survived unharmed in dry, semi-dry and diluted feces when exposed to temperatures of 68-72°F. (20-22°C.) for 72 hours.(Protostrongylus stilesi larvae in frozen lung tissue lived for over six months at a temperature of 3°F. (-16°C.) (Pillmore, 1956).)

These data suggest that protostrongylin larvae are physiologically able to withstand the environmental conditions of temperature and desiccation which would be encountered on bighorn ranges throughout the year.

In the present study, several controlled experiments were conducted in the laboratory in order to obtain more accurate information concerning the effects of temperature and humidity on survival of first stage P. stilesi larvae.

Methods and Materials

First stage protostrongylin larvae used in these experiments were obtained from the lung tissue and droppings of hunter-killed bighorn sheep and from fresh sheep droppings collected on sheep ranges in Western Montana. Experiments to determine the effects of temperature and humidity on the first stage larvae were conducted utilizing larvae in water, in 0.9 percent sodium chloride solution (saline), and in fecal pellets. The larvae extracted from lung tissue were in different stages of development, but the larvae in pellets were probably less variable.

Desiccation Experiment 1 (D-1). This experiment was initiated to determine the effect of drying on larvae isolated from lung tissue. First stage protostrongylin larvae were separated from lung tissue by use of the Baermann technique (Appendix A). Over 100 active larvae in several drops of 0.9 percent saline were placed on cover slips for each replication. These cover slips were then suspended over the drying agents anhydrous calcium chloride and calcium sulfate or over water. Each sample was sealed in a two-ounce glass jar with a tight fitting plastic cover and incubated at $36^{\circ} \pm 2^{\circ}C$. Samples were removed from the incubator after

one, two, four, and eight days, moistened and examined under 15 diameters magnification. The numbers of living and dead larvae were counted and recorded. The inactive, turgid, half-moon shaped larvae were assumed to be dead or dying.

Desiccation Experiment 2 (D-2). The second experiment was designed to determine the effects of long term drying on larvae in fecal pellets. Experimental droppings were placed on cotton over the drying agent, anhydrous calcium sulfate, while control pellets were suspended by heavy thread over water. Each of the two replications in each group was sealed in an individual two-ounce glass jar as in D-1 and groups incubated at 27°C. for logarithmically increasing periods of time. Samples were removed after one day, two days, four days, eight days, two weeks, eight weeks, 16 weeks, 32 weeks, and one year. The Baermann technique was used to recover active larvae from the fecal material. Theoretically only the living larvae will migrate from the fecal droppings during this treatment and dead larvae remain in the fecal material.

Desiccation Experiment 3 (D-3). An additional series of experiments was organized to determine the effects of various temperatures on first stage larvae. Desiccation Experiment 3 was essentially the same as D-2, except that samples of the experimental and control groups were subjected to temperatures of -24°, -10°, 9°, 22°, 32°, 42°, and 52°C. for one, two, and four days. The pellets were baermannized after the designated intervals and the living larvae counted.

Desiccation Experiment 4 (D-4). When it was found that some larvae survived desiccation at 52°C., Desiccation Experiment 4 was set up to study the survival of larvae at higher temperatures. This experiment was similar to D-3, but with incubation temperatures of 52°, 62°, and 72°C. and with four replications in each treatment group.

Freezing Experiment. Since Experiment D-3 investigated the survival of larvae in pellets when frozen, it seemed desirable to investigate the effects of freezing on larvae suspended in solution. In these experiments larvae collected from fecal pellets using the Baermann technique were frozen in distilled water at -9°C. and -22°C. At periodic intervals the samples were thawed, the larvae examined, and the samples refrozen for later examination.

Phototropism Experiment. Experiments were conducted to determine if the first stage larvae of P. stilesi and P. rushi were phototropic. Several hundred active larvae, obtained from fresh droppings, were dispersed in distilled water in a Petri dish half of which was painted black to prevent light penetration. The larvae were retained in this dish in a darkened room at about 20°C. with a bright light shining through the bottom of the container. After eight hours the larvae on the dark and light sections were counted.

Experimental Results

Desiccation Experiment 1 (D-1). The results from D-1 indicate that first stage protostrongylin larvae in solution

survive better than desiccated larvae (Table I). Detailed results are presented in Appendix B. The differences between survival of larvae at one, two, four, and eight day exposures under similar treatment seems to be small and relatively insignificant, although a slight decrease in survival with time is evident. Assuming that time has no effect on survival, almost 78 percent of the larvae suspended in saline survived, whereas only about 55 percent of the dried larvae recovered after being moistened. In most cases the survival of dried larvae was at least 20 percent lower than that of comparable wet larvae.

Desiccation Experiment 2 (D-2). Results of long term desiccation of first stage larvae in pellets at 27°C. indicate that larvae will survive longer in dried than in wet fecal material, although initially a higher percentage of larvae were found in the wet pellets (Figure 1). Detailed results are presented in Appendix C. All larvae were dead in the wet pellets after four months of incubation. Some living larvae were noted in dried pellets after four months, but none were noted at eight months.

Desiccation Experiment 3 (D-3). The effect of low and high temperatures on survival of larvae in moist and dry droppings was examined in Experiment D-3.

Table I. Survival of protostrongylin lungworm larvae subjected to dry and humid conditions at $36^{o} \pm 2^{o}C$. (Experiment D-1)

Duration of Exposure (Days)	Percent Survival		
	Anhydrous $CaCl_2$	Anhydrous $CaSo_4$	Water
1	63.5	67.7	94.9
2	59.0	59.7	88.3
4	51.2	38.9	52.5
8	51.4	53.3	76.0
Average	56.3	54.9	77.9

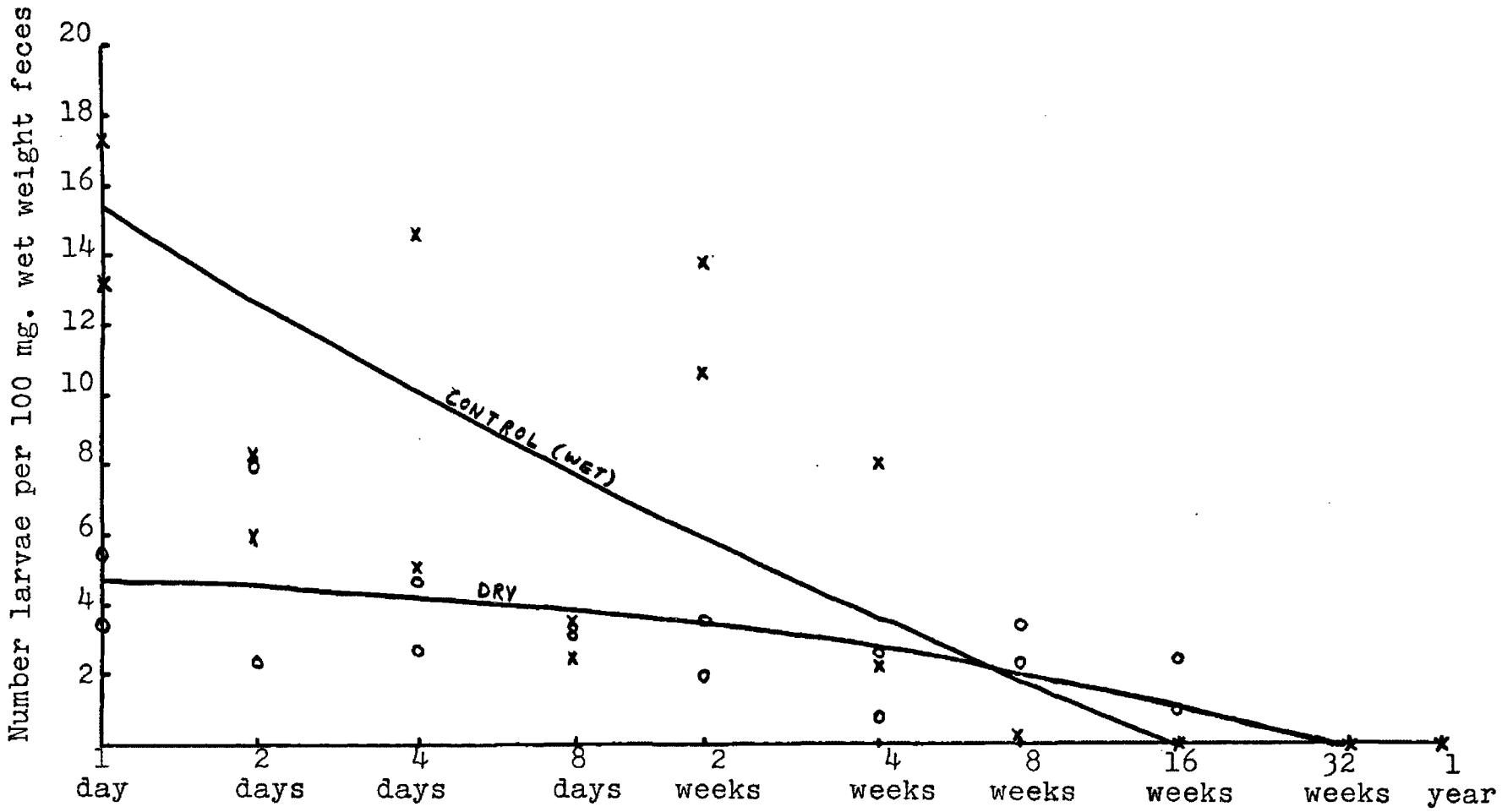

Figure 1. Survival of first stage protostrongylin lungworm larvae incubated in dry and wet feces at 27°C. for up to a year. (Experiment D-2)

x = wet

o = dry

Higher survival of larvae was noted in moist droppings at the lower temperatures, -24°, -10°, 9°, 22°, and 32°C., than in dried droppings. The reverse was true at temperatures of 42° and 52°C. (Figure 2). The highest survival of larvae was at 32°C. for the first two days and 22°C. after four days of incubation. This would suggest that the optimum temperature for survival of protostrongylin larvae in droppings during a four-day period is between 22° and 32°C. Extreme temperatures resulted in lower survival of larvae under both wet and dry conditions. However, because only two replications of each sample were tested in this experiment, the results are probably not statistically significant, but they do indicate a trend. For detailed results, the reader is referred to Appendix D.

Desiccation Experiment 4 (D-4). Incubation of droppings at high temperatures (D-4) resulted in a decrease in survival of larvae with time and increasing temperatures. For the first two days of treatment, the average decrease in survival of larvae in dried pellets was 51 percent, 74 percent, and 88 percent at 52°, 62°, and 72°C. respectively, whereas in wet pellets it was 74 percent, 88 percent, and 91 percent at 52°, 62°, and 72°C. respectively. More larvae appeared to survive in dried than in wet pellets at these temperatures (Figure 3). Surprisingly, 16 larvae even survived in three of the four dried droppings after incubation for four days at 72°C. Appendix E presents detailed results of this experiment.

Freezing Experiment. Preliminary investigations indicate that liberated larvae and larvae contained in fecal

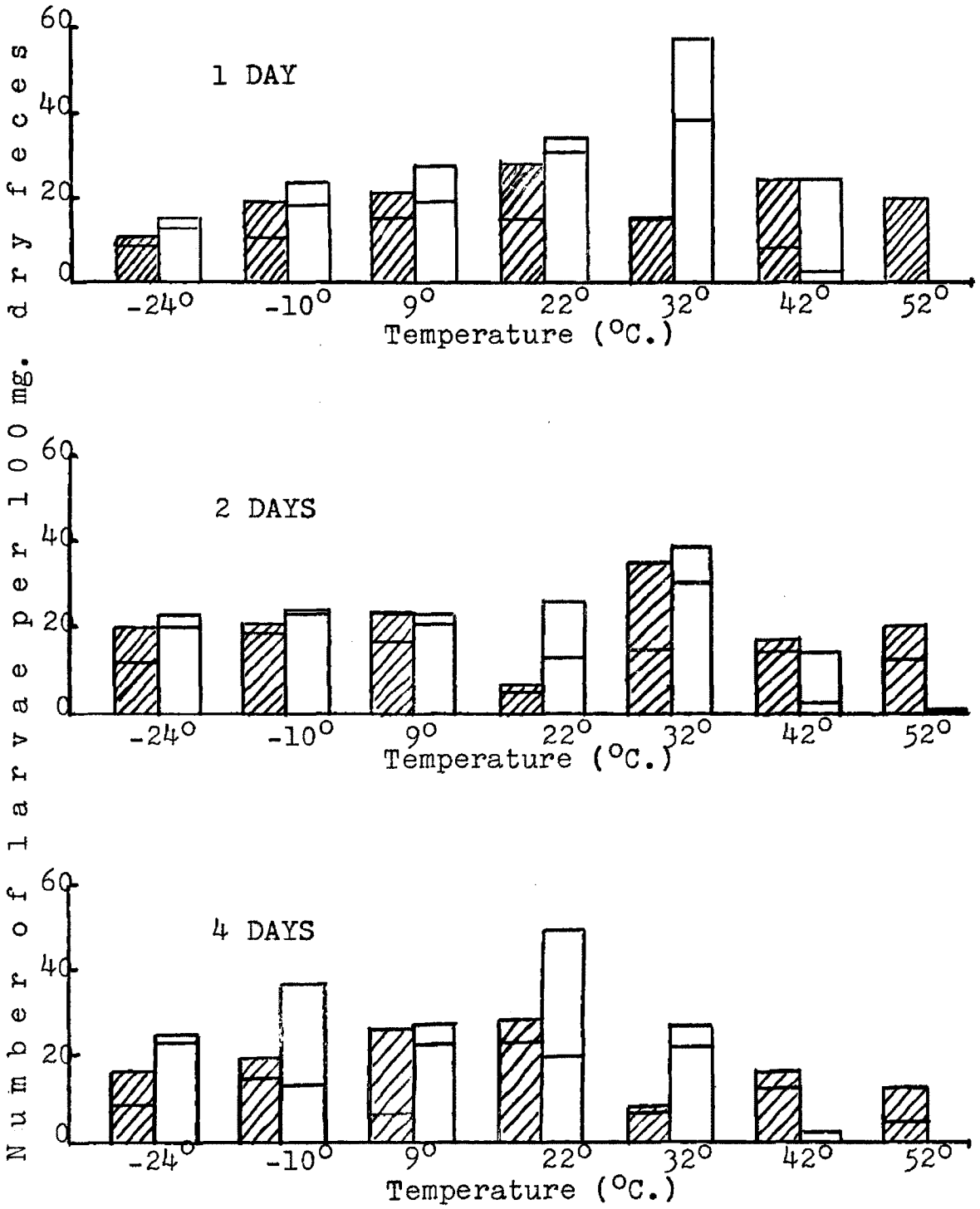

Figure 2. Survival of protostrongylin lungworm larvae in wet and dry feces incubated at -24°, -10°, 9°, 22°, 32°, 42°, and 52°C. for one, two, and four days. (Experiment D-3)

Lower block indicates survival for one replication and entire block indicates survival of the other replication

dry

wet

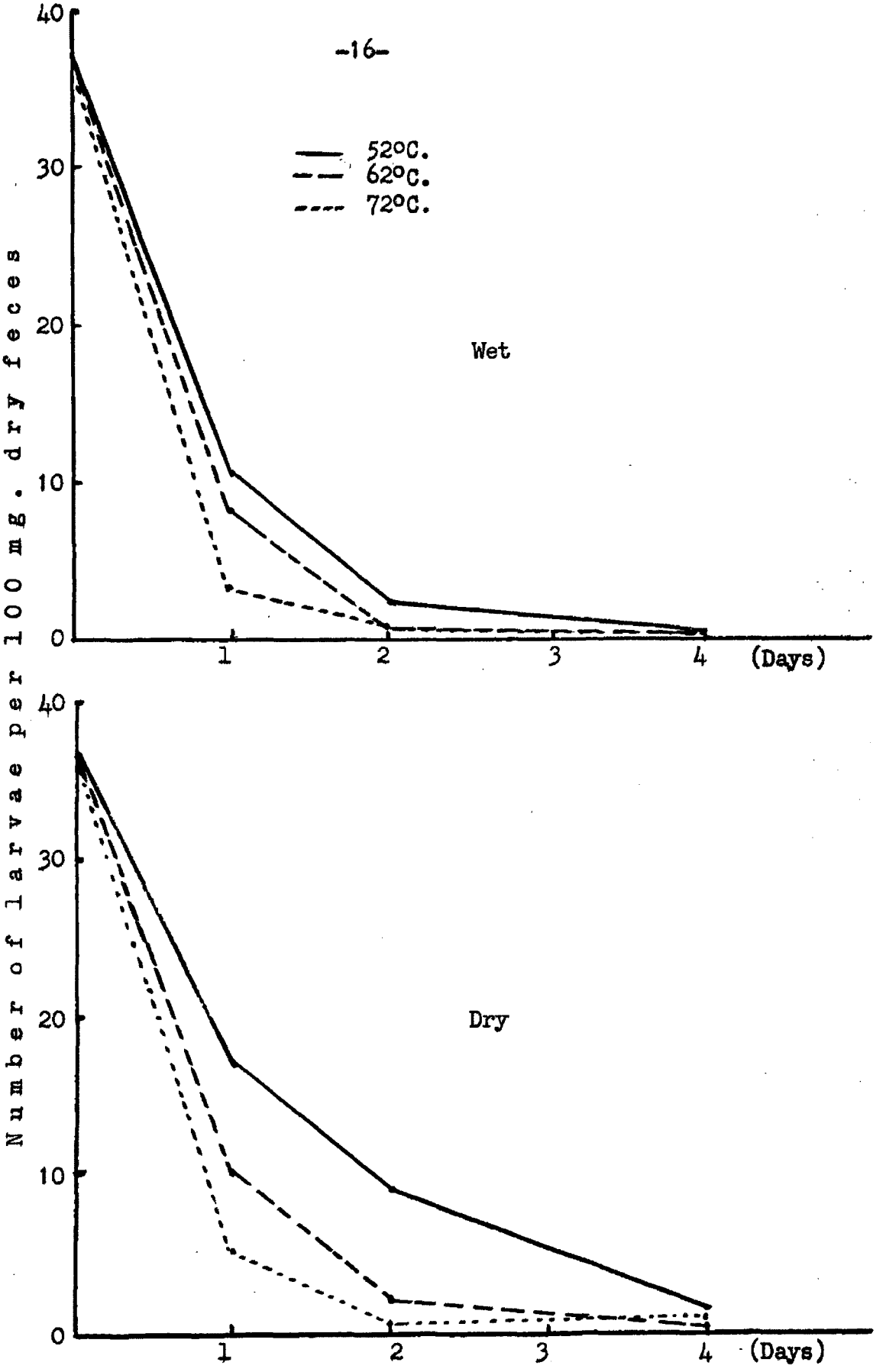

Figure 3. Survival of protostrongylin larvae in wet and dry feces incubated at 52°, 62°, and 72°C. for one, two and four days. (Experiment D-4)

material can survive during temperatures as low as -22°C. for five months. This was apparent in other miscellaneous observations as well as the freezing experiment. It was noticed that alternate thawing and freezing apparently had an adverse effect on survival of larvae suspended in water. Larvae in distilled water were thawed and refrozen at four intervals of one week and one interval of a month. Mortality of larvae treated in this manner was noted to increase. This particular effect was not studied in larvae frozen in fecal material.

Phototropism Experiment. First stage protostrongylin larvae were found to migrate to or remain on the light side of a Petri dish half of which had been painted black. Only ten larvae out of several hundred were observed on the dark side of the dish after eight hours. Similar observations indicating that first stage larvae are positively phototrophic have been reported by Pillmore (1955) who used light to concentrate larvae to one side of a container for collection purposes.

Discussion

The most apparent conclusion from the desiccation experiments is that first stage protostrongylin larvae in fecal material appear to be resistant to desiccation, but larvae that have migrated from feces survive better in a wet medium.

In general, there was an overall decrease in survival of larvae in dried and moist droppings with time and increasing temperature, although survival in dried droppings was

higher than in moist ones.

The higher survival of larvae in dried and low survival in wet droppings at higher temperatures is difficult to explain. Giese (1957) states that a "temperature which is most favorable for one function of a cell, a function such as respiration, may not at the same time be favorable for the cell's growth or longevity. Furthermore, a temperature which may appear to be favorable for a given function during brief exposure of a cell may prove harmful on longer exposure."

Possibly the larvae in moist pellets have a higher metabolic rate and therefore use up their available energy while the larvae in dried droppings go into some sort of "aestivation," accompanied by low metabolic rates and can survive for a longer period of time. The viability of larvae in these experiments was not determined after treatment and possibly the larvae that survived desiccation could have been physiologically affected in some manner and might not be able to complete the life cycle.

Analysis of weather data from the Bigfork and Polson Stations near Wildhorse Island, where a herd of lungworm-infected bighorns is found, indicates that the highest recorded temperature between 1931 and 1958 was 104°F. (40°C.) and the lowest was -30°F. (-34°C.) (Anon., 1952). These are extreme temperatures, however, and lasted only for a short time. The results of the desiccation experiments suggest that the survival of larvae in droppings during these temperature extremes would probably be less than survival during the more moderate

weather conditions normally occurring on Montana sheep ranges.

From analysis of the information presented in the literature and the results from this study, it seems unlikely that temperature and humidity can effectively influence the survival of first stage protostrongylin larvae in fecal material to a significant degree. However, some seasonal effects of weather will occur, but first stage larvae will undoubtedly be available in sufficient quantities to allow infection of the intermediate hosts on a range utilized by infected bighorns.

INTERMEDIATE HOST - LUNGWORM BIONOMICS

Review of Literature

The initial report on the life cycle of a protostrongylin lungworm listed the snails Helicella ericetorum, H. obvia, and H. bolli as suitable intermediate hosts for Protostrongylus rufescens, a lungworm of domestic sheep (Hobmaier and Hobmaier, 1930). In 1940, Boev reported the snails Succinea altaica, Pupilla muscorum, P. signata, Vallonia costata, and V. pulchella and Shults and Boev (1940) reported Deroceras (Agriolimax) agrestis, Parachondrula apthycha, Eva maientaki, Fruiticicola lantzi, F. rubens, and Cathaica semenowi as capable of serving as intermediate hosts of a protostrongylin lungworm of sheep and goats in southeast Kazakhstan, Russia. Boev and Wolf (1940) artificially infected snails, which they thought were Pupilla muscorum, with protostrongylin lungworm larvae. In 1947, further investigations in Russia confirmed Pupilla muscorum and P. signata and established Orcula doliolum as intermediate hosts (Davtian, 1947). Joyeaux and Gaud (1943) reported Helicella rugosiuscula and H. gigaxii to be naturally infected with larvae of Protostrongylus rufescens from sheep and goats in Morocco, and in 1946 (Joyeaux and Gaud, 1946) added Deroceras sp. Helicella barbesiana was added to the list in 1950 (Gerichter, 1950) and in 1951 Helicella vestalis and Monacha syriaca were found harboring infective larvae of P. rufescens from domestic

sheep and goats in Israel (Gerichter, 1951). Additional snail hosts were reported in Russia when Subzebrinus miser, S. fedtschenkoi, and Macrochlamys turanica were discovered containing larvae of P. hobmaieri (Matekin et al, 1954).

In Colorado Pupilla muscorum and Vallonia pulchella were reported as intermediate hosts for the bighorn sheep lungworms Protostrongylus stilesi and P. rushi (Pillmore, 1958b). These same two snails were also found to be intermediate hosts of protostrongylin lungworms in Russia (Davtian, 1947; Matekin et al, 1954. Pillmore (1958b) also lists Pupilla blandi, Gastrocopta armifera, G. pentodon, Pupoides albilabris, and Vertigo concennula of the family Pupillidae and Vallonia cyclophorella of the family Valloniidae as additional intermediate hosts. No previous investigations have been undertaken in Montana to determine which mollusks are serving as intermediate hosts of Protostrongylus stilesi and P. rushi.)

Methods of Collecting and Identifying Mollusks

Land mollusks were collected on ten bighorn sheep ranges in Western Montana during 1958 and 1959 (Figure 4). Shells or "bones" and living snails and slugs were collected by thoroughly searching under rocks, moss, and debris in appropriate habitats. Snail shells were usually lifted from the habitat by use of BB forceps and were then packed in cotton in one-dram glass vials. Live mollusks were stored in glass vials without cotton packing.

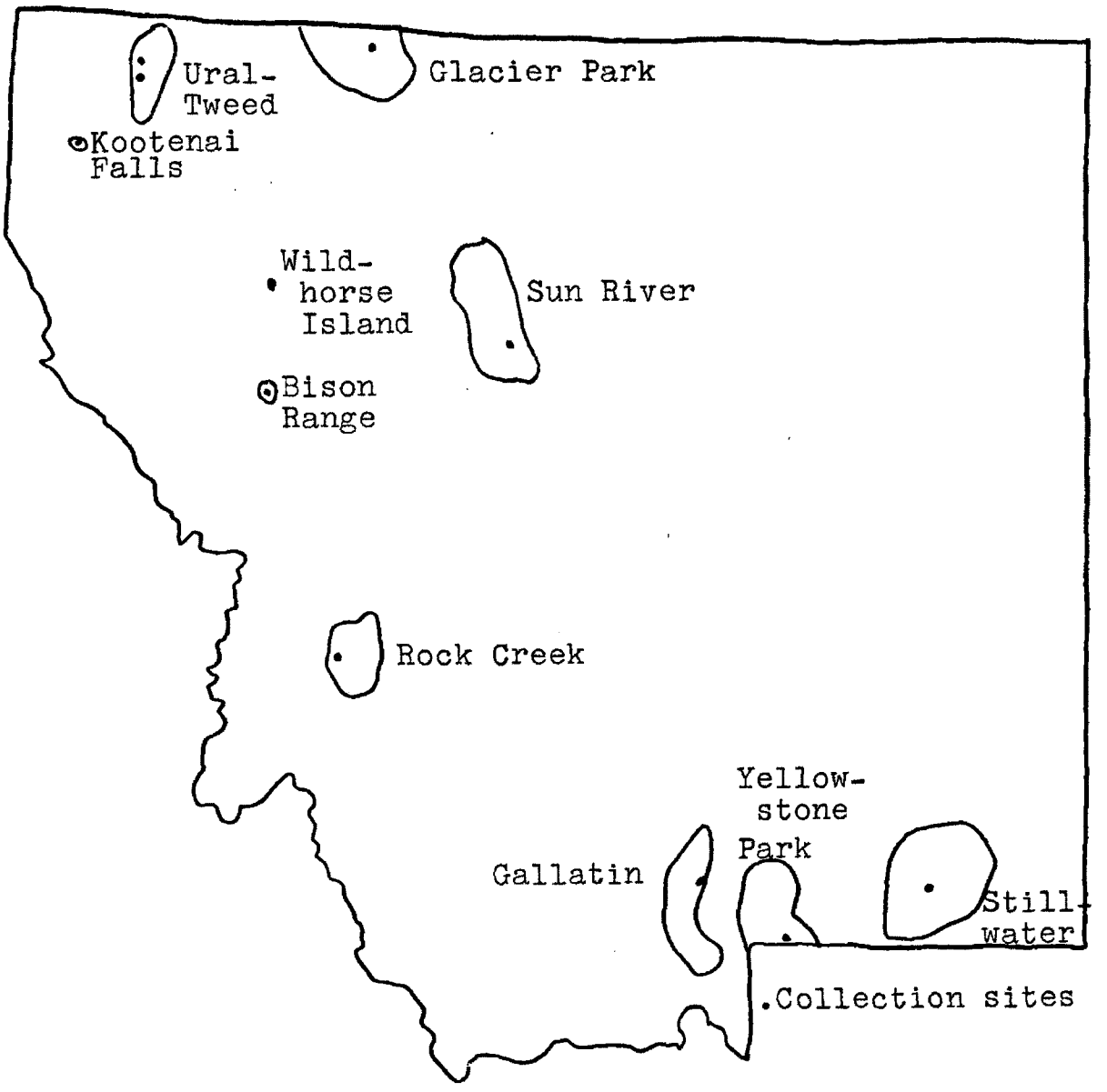

Figure 4. Collection sites of fecal droppings and mollusks on bighorn sheep ranges in Western Montana

Snail shells were later identified, packed in cotton, and permanently stored in glass vials. Mollusks were identified to genus by using keys provided by Dr. Royal B. Brunson of Montana State University and Pilsbry's (1939) "Land Mollusca of North America." The collection was sent to the Chicago Natural History Museum where species were determined by Drs. A. Solem and E. J. Roscoe. Living snails and slugs were also identified and were later examined for protostrongylin larval infections.

Wildhorse Island Mollusks

In 1954, Ogren reported finding snails of the genera Zonitoides, Retinella, Vitrina, and Euconulus and some slugs on Wildhorse Island. No other records concerning the land mollusks of the island were found.

An attempt was made in this study to obtain a sample of all land mollusks occurring on Wildhorse Island in order to determine which snails and slugs were present and might be serving as intermediate hosts for the protostrongylin lungworms infecting the bighorns. Eight genera of snails and two genera of slugs were found.

The most frequently encountered mollusks were Vitrina alaskana, Euconulus fulvus, and Zonitoides arboreus, while Zacoleus idahoensis, Oreohelix subridus, Vallonia cyclophorella, Deroceras laeve, and Retinella spp. were occasionally found; single specimens of Oxyloma decampi and Discus cronkhitei were also collected. These are summarized in Table II.

According to the literature, the species of Vallonia and Deroceras could serve as intermediate hosts, but all the other mollusks have been reported to be refractive to protostrongylin larval infections. Pillmore (1958b) proved Vallonia cyclophorella to be capable of serving as intermediate host of the bighorn sheep lungworms P. stilesi and P. rushi but found two species of Deroceras to be refractive (Pillmore, 1956). However, Shultz and Boev (1940) infected Agriolimax (=Deroceras) agrestis with larvae of Synthetocaulus (=Protostrongylus) hobmaieri and P. raillieti from domestic sheep and goats.

Mollusks from Other Bighorn Ranges

In addition to collections made on the Wildhorse bighorn range, specimens of snails and slugs were also collected on nine other ranges: The National Bison Range, Gallatin, Kootenai Falls, Many Glacier, Rock Creek, Stillwater, Sun River, Ural-Tweed, and Yellowstone. Table III shows the species of land mollusks found on each range. The snails in the families Pupillidae and Valloniidae are the most significant to this study because they have been reported by Pillmore (1958b) as intermediate hosts of protostrongylin lungworms of bighorn sheep in Colorado. Pupilla blandi was found on the Gallatin and Many Glacier ranges, P. hebes was found on the Stillwater and Yellowstone ranges; whereas, P. muscorum was found only on the Yellowstone Range. Vallonia cyclophorella was found on the Gallatin, Kootenai Falls, Sun River, and Ural-Tweed ranges

as well as on Wildhorse Island. Deroceras laeve was found on the Rock Creek, Ural-Tweed, and Wildhorse Island ranges, and possibly might serve as an intermediate host as was mentioned previously.

Table II. Land mollusks collected on Wildhorse Island from 1959 to 1960, their relative abundance, and habitats

Mollusk	Relative Abundance	Habitat
Arionidae		
Zacoleus idahoensis	Scarce	Under driftwood near shoreline
Camaenidae		
Oreohelix subrudis	Scarce	Under rocks in talus slides
O. strigosa	Scarce	Same habitat
Endodontidae		
Discus cronkhitei	Very scarce	Under rock
Limacidae		
Deroceras laeve*	Scarce	Under driftwood near shoreline
Succineidae		
Oxyloma decampi	Very scarce	Under driftwood near shoreline
Valloniidae		
Vallonia cyclophorella**	Scarce	Under rocks near sandy shore
Zonitidae		
Euconulus fulvus	Common	Under rocks and decaying logs in moss area
Retinella sp. (?)	Scarce	In rotten logs and under driftwood
Vitrina alaskana	Abundant	Under rotten logs, duff, and moss
Zonitoides arboreus	Common	In rotten logs and stumps

*Capable of serving as intermediate hosts of protostrongylin lungworm (Schultz and Boev, 1940)

**Capable of serving as intermediate hosts of Protostrongulus stilesi and P. rushi (Pillmore, 1958b)

(?) Identification tentative

Table III. Land mollusks collected on bighorn sheep ranges in Western Montana from 1958 to 1960

Family	Mollusks: Genus and Species	Bison Range	Gallatin	Kootenai Falls	Many Glacier	Rock Creek	Stillwater	Sun River	Ural-Tweed	Wildhorse	Yellowstone
Arionidae	Magnipelta mycophaga								?		
	Zacoleus idahoensis			?		?				?	
Camaenidae	Oreohelix strigosa	X		X					X	X	
	O. subrudis		X	X	X			X	X	X	
Cionellidae	Cionella lubrica		X								
Endodontidae	Anguispira kochi	X									
	Discus cronkhitei		X					?		X	
	D. shimeki		X					?	X		
Limacidae	Deroceras laeve					?			?	?	
Polygyridae	Allogona ptychophora								X		
	Triodopsis mullani			X							
Pupillidae	Pupilla blandi		X		X						
	P. hebes						X				X
	P. muscorum										X
Sagdidae	Microphysula ingersolli			X	X			X	X		

Table III (continued)

Mollusks Family Genus and Species	Bison Range	Gallatin	Kootenai Falls	Many Glacier	Rock Creek	Stillwater	Sun River	Ural-Tweed	Wildhorse	Yellowstone
Succineidae										
Oxyloma decampi									X	
Quickella rehderi					X					
Valloniidae										
Vallonia cyclophorella		X	X				X	X	X	
Zonitidae										
Euconulus fulvus		X	X	X				X	X	
Hawaiia minuscula						X				
Pristiloma wascoense			X	X						
Retinella sp.									?	
Vitrina alaskana	X	X		X					X	
Zonitoides arboreus		X		X				X	X	

?Tentative identification by the author

Examination of Land Mollusks for Natural Protostrongylin Infections

All of the living land mollusks collected in the course of the field work were examined for presence of lungworm infections. The technique of examination was similar to the methods used by Gerichter (1948) and Pillmore (1955). Mollusks were activated with a mist of water and allowed to crawl over the bottom of a Petri dish. The dish was then inverted and the feet of the mollusks were examined under a dissecting microscope using a magnification of 15 diameters. Infections are visible in the feet of mollusks and resemble dark half-moon objects. Suspicious objects were dissected from the foot of the slug or snail and mounted in polyvinyl alcohol on a slide. Mollusks found to be free from visible signs of larvae were then transferred to snail colony jars where they were retained for one month and later re-examined. This would allow development of any larvae which might have been present, but not detected, at the time of collection.

Snail colony jars were similar to those used by Pillmore (1958a). Three-inch standard flower pots were fitted into the mouths of quart jars. Gauze, wedged into the hole in the bottom of the pot, was allowed to extend to the bottom of the quart jar. The jar was filled about one quarter full with tap water and capillary movement of water up the gauze provided proper humidity for the flower pot "habitat." Mollusks were placed in the flower pot and a large Petri dish was inverted over the top of the pot in order to retain both the

snails and the moisture. The colonies were kept at a temperature of 15° ± 2°C. Mollusks survived under these conditions for at least four months. The summer of 1959 seemed drier than usual and living mollusks apparently aestivated and were unusually difficult to locate (Brunson, pers. comm.).

The living snails and slugs collected at Kootenai Falls, Many Glacier, Rock Creek, Sun River, and Ural-Tweed all proved negative to lungworm infections. Indications of infection were obtained from three mollusks of 30 that were collected on Wildhorse Island (Table IV). A nematode larva, apparently not a protostrongylid, was found in the foot of a snail, Vitrina alaskana, and possible remnants of a larval sheath were found in the foot of a slug, Deroceras laeve, and in the foot of a snail, Oxyloma decampi gouldi.)

Artificial Infection of Land Mollusks

Living mollusks which were found to be uninfected were used in artificial infection experiments. Over 100 active first stage protostrongylin larvae, obtained by the Baermann Technique from fresh bighorn droppings, were collected in a 2cc. hypodermic syringe and then sprayed on and around the active mollusks in each culture. After the initial spraying the mollusks were sprayed periodically with a water mist to activate any that may have aestivated and to activate any inactive larvae. Apparently the mollusks as well as the larva must be active in order for a penetration and subsequent infection to occur (Matekin et al., 1954).

Table IV. Land mollusks collected on bighorn sheep ranges in Western Montana and examined for lungworm infections during 1959

Mollusk	Date Collected	Total Number Examined
Kootenai Falls		
Euconulus fulvus	9/17	2
Microphysula ingersolli	9/17	2
Oreohelix strigosa	9/17	3
Triodopsis mullani	9/17	2
Zacoleus idahoensis	9/17	2
Many Glacier		
Euconulus fulvus	9/19	6
Microphysula ingersolli	9/19	6
Oreohelix subrudis	9/19	1
Rock Creek		
Deroceras laeve	10/31	1
Zacoleus idahoensis	10/31	3
Sun River		
Discus sp.	4/11	1
Vallonia cyclophorella	4/11	1
Ural-Tweed		
Deroceras laeve	9/16	1
Magnipelta mycophaga	9/16	1
Microphysula ingersolli	9/15	3
Oreohelix strigosa	9/15, 9/16	8
Wildhorse Island		
Deroceras laeve	9/11	5
Oreohelix subrudis	9/11	5
Oxyloma decampi	9/11	1
Retinella sp.	6/20, 6/21	3
Vitrina alaskana	4/10	1
	5/14, 8/29	2
Zonitoides arboreus	6/6, 6/7, 6/21	13
TOTAL		82

No infections were established in species of Allogona, Deroceras, Euconulus, Oreohelix, Retinella, Triodopsis, Vertigo, Vitrina, Zacoleus, and Zonitoides, which were exposed to larvae for periods of time ranging from 28 to 151 days (Table V). Mollusks which died in the colonies after exposure were examined microscopically and the remains were treated in the Baermann apparatus to recover any active larvae present.

From these limited experiments it appears that these mollusks might be refractive to protostrongylin larvae.

Table V. Land mollusks exposed to first stage protostrongylin larvae and number of days of exposure

Mollusk	Number of Mollusks Exposed	Length of Exposure (Days)
Allogona ptychophora	1 (adult)	86
	1 (juvenile)	86
Deroceras laeve	1	89
Euconulus fulvus	1	75
	2	76
	1	85
	2	89
Oreohelix strigosa	2	89
Oreohelix subrudis	1	88
	5	89
Oreohelix sp.	1 (juvenile)	85
	2 (juvenile)	89
Retinella sp.	2	50
	1	77
	1	85
	2	89
Triodopsis mullani	1	88
Vertigo sp.	1*	28
Vitrina alaskana	1	87
	1	88
Zacoleus idahoensis	1	89
Zonitoides arboreus	2	50
" "	6**	60
" "	9	74
" "	1	88
" "	1	89
" "	1	151
TOTAL NUMBER	51	

*Exposed to pellets containing larvae and retained in Stender dish

**Snails retained in Stender dish

LUNGWORM OCCURRENCE IN BIGHORN SHEEP OF MONTANA

Review of the Literature

Rush (1927) reported a die-off of bighorns at Sun River in 1924-25 and described five cases of congested lungs which he attributed to a "virulent form of pneumonia." No lungworms were found at this time, although the descriptions of the lung pathology are very similar to that ascribed to lungworm damage. The first published report of lungworm occurrence in bighorns in Montana was in 1935 when Dikmans (1935) identified several adult Protostrongylus stilesi from the lungs of a bighorn from Yellowstone National Park. Mills (1937) examined lungs from a five-year old ewe and a four-year old ram from Yellowstone and found them also infected with P. stilesi. In 1938, Marsh reported losses of bighorns in Sun River, Glacier National Park, Yellowstone National Park, and the National Bison Range and attributed adult deaths to verminous pneumonia caused by P. stilesi and lamb deaths on the Bison Range to acute pneumonia without any concurrent lungworm infections. Brink's (1941) report contained an account of an autopsy performed by Dr. W. L. Jellison of the Rocky Mountain Laboratory in which Protostrongylus rushi adults were found in the bronchial tubes of the lungs of a yearling ram from the Ural-Tweed herd. From 1942 to 1944 Couey (1950) examined droppings from Ural-Tweed, Thompson Falls, Gallatin, Yellowstone, Stillwater, Rosebud, Tobacco Root, and Sun River bighorn herds and found infections in all but the Tobacco Root herd. He concentrated

mainly on the Sun River sheep and found 85 percent of the sheep to be infected. Several autopsies revealed adult protostrongylin lungworms in the lung tissue. In June of 1953 Ogren (1954) determined by fecal analysis that one-third of a population of 83 bighorns on Wildhorse Island contained light infections of protostrongylin lungworms. In 1954 the lungs of 19 hunter-killed mature bighorn rams from the Sun River, Stillwater, and Gallatin herds were examined by Marquardt and Senger (1956). Seventeen were found to be infected by the lungworms P. stilesi and P. rushi. Both uninfected lungs were from the Gallatin herd.

Analysis of Bighorn Sheep Fecal Samples from Herds in Montana

Droppings less than one day old were collected every month from April 1959 to March 1960, from a herd of bighorn sheep on Wildhorse Island, Flathead Lake, Montana (Figure 4). Collections were made several times each month during the spring and summer, whereas only one collection was made each month of the fall and winter. Between 1500 and 2000 mg. of wet fecal material from each sample were treated by the Baermann technique. Final results were expressed in dry weights of feces because it was felt that dry weights were more comparable than wet weights.

The seasonal larval output was determined by grouping the results according to season; summer (July, August, and September), fall (October, November, and December), winter (January, February, and March), and spring (April, May, and

June). The larval output, expressed in number of larvae per 100 mg. dry fecal material, increased from 2.2 in summer to 3.3 in fall, 4.7 in winter, and 7.5 in the spring (Figure 5). Detailed results concerning these monthly collections are given in Appendix F. The increase in shedding of larvae in the winter and spring and decrease in summer and fall agree with information reported by Couey (1950) for bighorns in Sun River, Montana, and Pillmore (1955) for Colorado bighorns.

Fecal samples were collected from nine other bighorn herds in Western Montana (Figure 4) and were analyzed in the same manner as the Wildhorse samples (Table VI). Sampling may in some cases be biased due to collecting methods. Seasonal variation in larval output in droppings must also be considered as this will influence the results of fecal analyses considerably.

The overall average for 24 samples from Stillwater was 90.2, for five Ural-Tweed samples was 80.8, for one Rosebud sample was 79.8, for 48 Sun River samples was 37.9 and for eight Yellowstone samples was 25.6 larvae per 100 mg. dry feces. Averages for Many Glacier, Gallatin, Rock Creek, Wildhorse, and Kootenai Falls samples were below ten larvae per 100 mg. dry feces.

Of approximately 500 different bighorn fecal samples examined in this study, 96 percent were found to contain protostrongylin lungworm larvae. A sample collected on October 31, 1959 from a ewe in the Rock Creek Herd was found to be negative, but the rest of the 19 samples were positive.

Figure 5. Seasonal occurrence of protostrongylin lungworm larvae in feces of bighorn sheep on Wildhorse Island during 1959-1960

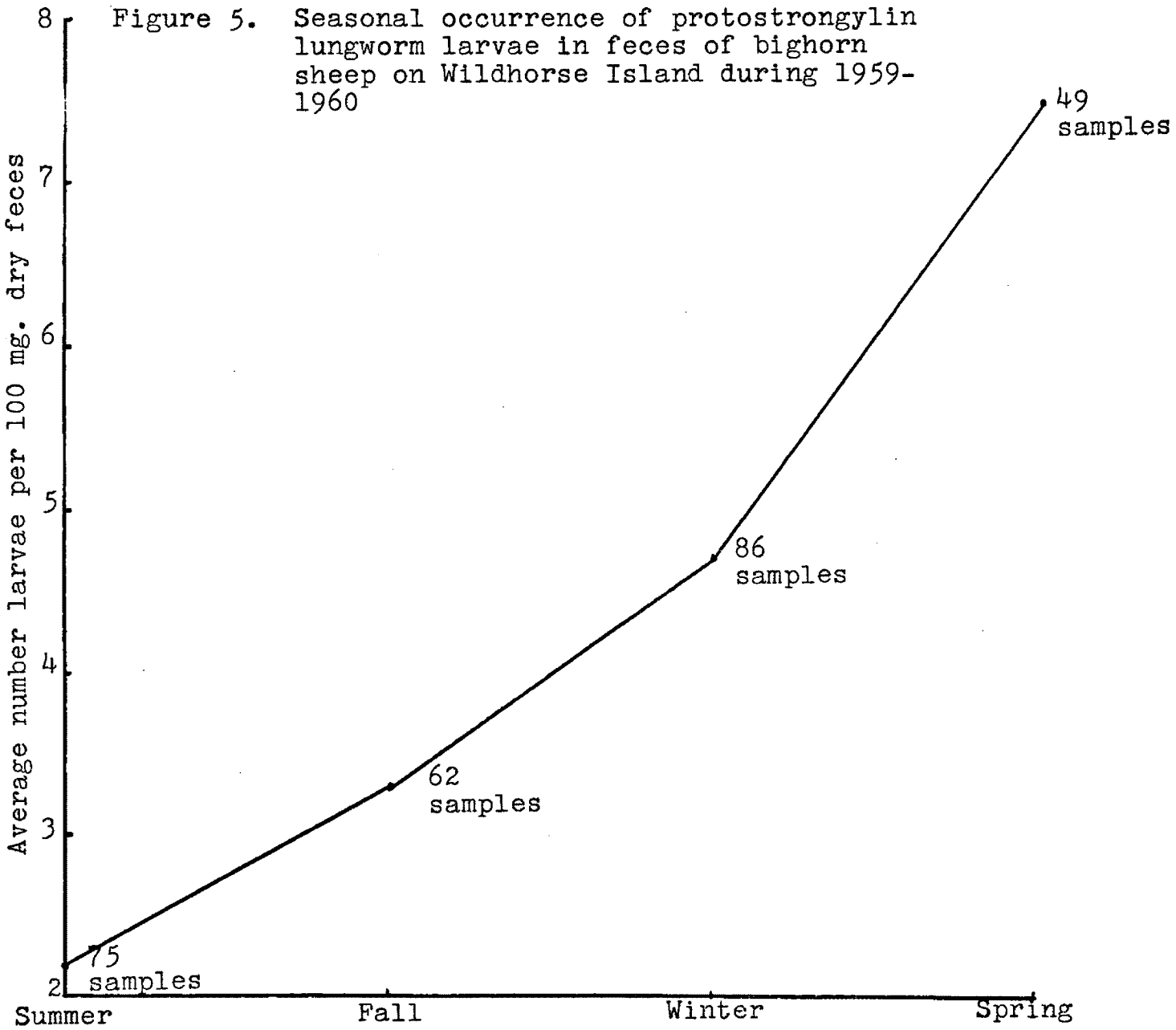

Table VI. Occurrence of protostrongylin larvae in feces from bighorn sheep in Western Montana during 1959

	Average Number of Larvae per 100 mg. dry weight of feces				
Herd	Overall	Fall	Winter	Spring	Summer
Stillwater	90.2(24)	30.8(1)	--	90.8(23)	--
Ural-Tweed	80.8(5)	129.2(1)*	--	--	--
		68.7(4)	--	--	--
Rosebud	79.8(1)	79.8(1)	--	--	--
Sun River	37.9(48)	68.5(17)	20.6(2)**	27.3(29)	--
Yellowstone	25.6(8)	--	--	25.6(8)	--
Many Glacier	8.0(7)	--	--	5.8(2)	8.9(5)
Gallatin	6.1(32)	11.0(6)	--	5.0(26)	--
Rock Creek	5.7(19)	9.8(3)*	--	--	5.4(2)
		6.9(14)	--	--	--
Wildhorse	4.6(272)	3.3(62)	4.7(86)	7.5(49)	2.2(75)
Kootenai Falls	0.9(50)	0(1)*	--	--	--
		0.5(48)	--	--	--
		10.8(1)	--	--	--

*Collected during 1958

**Collected during 1960

Mean values are followed by numbers of samples in parentheses

Eight samples out of 50 collected on September 17, 1959 from ewes in the Kootenai Falls herd were also negative. On Wildhorse Island a mature ram sample collected on June 6, 1959, a mature ram and three unidentified samples collected on October 17, 1959, and one unidentified sample collected on December 14, 1959, four on January 28, 1960, and one on March 22, 1960, were found to be devoid of larvae. Many of the samples from young lambs were negative as will be discussed later.

The data obtained in this study are very similar to those reported by Couey (1950) for Montana bighorn lungworm infections. Couey found that the Gallatin and Yellowstone samples were 100 percent infected and the Sun River, Ural-Tweed, Stillwater, and Rosebud samples were between 75 and 85 percent infected.

Fecal samples collected from Wildhorse Island ewes during May, July, September, and November contained larger quantities of larvae than samples collected from rams at the same time, whereas ram samples during June and August contained more larvae than ewe droppings (Table VII). Identified samples from nine other herds showed that ram droppings contained a mean number of 39.3 larvae per 100 mg. dry feces, yearling samples had 27.5, and ewe samples had 23.2 larvae per 100 mg. dry feces (Table VIII). These data are probably not comparable because collections were made at various times of the year when seasonal fluctuations in larval output occur.

Table VII. Occurrence of protostrongylin larvae in feces from identified bighorn sheep on Wildhorse Island during 1959 to 1960

	Average Number Larvae/100 mg. Dry Feces	
Month	Rams	Ewes
April	--	--
May	1.9 (2)	6.7(5)
June	17.1 (7)	3.9(18)
July	1.6 (9)	3.1(28)
August	3.4 (3)	2.1(4)
September	1.1 (1)	4.4(5)
October	0.05(2)	--
November	2.4 (1)	4.0(1)
December	0.7 (1)	--
January	6.6 (4)	--
February	--	--
March	--	--
MEAN	3.9(30)	4.0(61)

Table VIII. Occurrence of protostrongylin larvae in ram, ewe, and yearling fecal samples from nine herds in Western Montana during 1958 to 1960

		Average Number Larvae per 100 mg. Dry Feces		
Herd	Date	Rams	Ewes	Yearlings
Stillwater	March, 1959	--	88.7(8)	--
	October, 1959	30.8(1)	--	--
Ural-Tweed	November, 1958	129.2(1)	--	--
	November, 1959	68.7(4)	--	--
Rosebud	October, 1959	79.8(1)	--	--
Sun River	April, 1959	47.6(1)	20.8(4)	79.5(2)
	October-November, 1959	68.5(17)	--	--
	February, 1960	--	--	27.3(1)
Yellowstone	March, 1959	--	25.8(6)	--
Many Glacier	March, 1959	2.4(1)	--	--
Gallatin	March, 1959	7.7(8)	--	3.0(1)
	September-October, 1959	11.0(6)	--	--
Rock Creek	November, 1958	9.8(3)	--	--
	July, 1959	--	10.7(1)	0.1(1)
	October, 1959	16.4(1)	4.1(12)	--
Kootenai Falls	November, 1958	0(1)	--	--
	September, 1959	--	1.3(34)	--
	November, 1959	--	10.8(1)	--
MEAN		39.3(45)	23.2(66)	27.5(5)

Numbers in parentheses indicate the number of samples examined

Acquisition of Lungworm by Bighorn Lambs

In the spring of 1959, seven lambs on Wildhorse Island varying in age from one day to two months were examined for presence of lungworm in order to determine when bighorns first acquire protostrongylin infections. Pellets were collected when possible, but sometimes only cotton swabs of the rectum or mouth were taken and later baermannized. No larvae were detected from these examinations (Table IX). Throughout the year lamb droppings were collected on Wildhorse Island and baermannized to determine larval incidence. Results from examination of 34 lambs indicate that shedding of larvae may be sporadic and therefore result in many negative tests (Table IX). Pillmore (1959) indicates that two captive lambs shed larvae consistently although not in the same quantity.

On Wildhorse Island larvae in lamb droppings were first found in 1959 on June 21, at which time a lamb of unknown age shed 2.1 larvae per 100 mg. dry weight, or six larvae in 289 mg. dry feces (Table IX). The exact date of the first lambing was known from careful field observations to be May 5, 1959, so the unknown lamb could not have been older than 47 days. Pillmore (1958) gives the prepatent period of protostrongylin lungworms as from 30 to 60 days. This lamb, therefore, must have acquired infective larvae very early in life or possibly prenatally through the placenta.

A week-old orphan ram lamb was removed from Wildhorse Island on May 29, 1959 and subsequently bottle-fed and raised by Montana Fish and Game personnel. On September 13, 1959 the

Table IX. Results of examination of bighorn lambs on Wildhorse Island during 1959 to 1960

Date	Estimated Age of Lamb	Type of Sample	Results
13 May 1959	4 days	Pellets	Negative
15 May 1959	2 or 3 days	Pellets	Negative
27 May 1959	8 or 9 days	Pellets	Negative
7 June 1959	1 week	Rectal swab	Negative
7 June 1959	4 days	Rectal swab	Negative
10 June 1959	2 weeks	Rectal and mouth swabs	Negative
21 June 1959	Unknown	Pellets	2.1 larvae/ 100 mg. dry feces
9 July 1959	Unknown (4 lambs)	Pellets	Negative
10 July 1959	3 or 4 days	Pellet, rectal and mouth swabs	Negative
10 July 1959	Unknown (7 lambs)	Pellets	Negative
25 July 1959	Unknown (5 lambs)	Pellets	Negative
5 Aug. 1959	Unknown	Pellets	Negative
9 Aug. 1959	Unknown	Pellets	Negative
10 Aug. 1959	2 months	Rectal and mouth swabs	Negative
11 Aug. 1959	3 mos.11 days*	Pellets	3.3 larvae/ 100 mg. dry feces
6 Sept.1959	Unknown	Pellets	91.9 larvae/ 100 mg. dry feces
6 Sept.1959	Unknown (4 lambs)	Pellets	Negative
11 Sept.1959	Unknown	Pellets	0.1 larvae/ 100 mg. dry feces
11 Sept.1959	3 mos.20 days*	Pellets	Negative
11 Sept.1959	3 mos.29 days	Pellets	Negative
28 Nov. 1959	6 mos.22 days	Pellets	Negative
14 Dec. 1959	Unknown	Pellets	Negative
28 Jan. 1960	Unknown	Pellets	1.0 larvae/ 100 mg. dry feces

*Same lamb (blue tag in left ear)

lamb was returned to the Montana Cooperative Wildlife Research Unit and was kept by the author in an outdoor pen through the winter. Droppings were collected periodically from the lamb from May 1959 through March 1960. The first shedding of larvae was detected on October 25, 1959 when the lamb was five months old. Forty-four larvae were found in a 1,503 mg. dry weight fecal sample giving a larval abundance of 2.9 larvae/100 mg. dry feces. No further shedding of larvae was noted until November 16, 1959, when one larva was found in 326 mg. dry weight of fecal material, 0.3 larvae/100 mg. dry feces. From November 16, 1959 to March 13, 1960 no larvae were found in fecal samples. The low and sporadic occurrence of larvae probably indicates a low infection. The lamb apparently either acquired the infection prenatally or during his first week of life, since there was little chance of infection after he was removed from the island.

Prenatal transmission has also been observed with other nematodes. Toxocara canis, the dog ascarid, has been found to infect puppies prenatally (Sprent, 1954). Douglas and Baker have shown that T. canis "larvae may remain at least a year in the tissues of the bitch without intercurrent infections, and during pregnancy larvae become mobilized and migrate into the fetus as well as completing their normal migration into the intestine of the bitch" (Douglas, letter of March 7, 1960).

Eveleth and Eveleth (1943) found lungworm larvae in

amniotic and allantoic fluids of domestic ewes and in fetal lambs. Pillmore (1959) has presented evidence for prenatal infection of bighorn lambs in Colorado and states that "the infective larvae passed through the placenta, entered the fetus, but failed to develop before the lungs became functional." Pillmore recovered larvae from two lambs, born in captivity and kept free from exposure to lungworms, within a month after birth. The delay in the occurrence of larvae in the droppings coincides with the observed prepatent period reported previously by Pillmore (1958b).

The occurrence of a delay in migration of infective larvae might help explain the widespread infection of bighorns by protostrongylin lungworms. One can speculate that bighorn ewes could obtain infective larvae by ingesting snails and the larvae could remain inactive until the stress of pregnancy instigates migration into the fetus. Ogren (1954), working on Wildhorse Island bighorns, reported that 48 percent of the ewes which produced lambs shed lungworm larvae in their droppings, whereas only 22 percent of the ewes currently without lambs shed larvae. These data seem to support the delayed migration theory.

Bighorn Lung Analyses

During 1958 and 1959 43 lungs from mature rams and two from mature ewes were examined for lungworm infections. Of these, 23 were from the Sun River herd, seven from the Gallatin herd, six from the Ural-Tweed herd, four from the Rock Creek herd, two from the Kootenai Falls herd, and one

each from the Wildhorse, Rosebud, and Stillwater herds. No lungs from lambs or yearlings were examined.

In most cases lungs were collected from successful bighorn hunters by Montana Fish and Game Commission personnel. Both lungs and fecal samples were stored frozen until examined. Upon examination the lengths and widths of lungworm lesions were measured in millimeters, charted on prepared lab sheets (Appendix H), and photographed. Lesions usually resembled off-color nodules on the lung surface and were positively identified by checking for larvae in a smear from the suspicious spot. Lungs were then dissected and any adult P. rushi encountered were counted and preserved. Scrapings of the trachea, bronchi, and bronchioles were examined with a stereo-microscope under a magnification of 15 diameters. When dropping samples accompanied the lungs, these were baermannized and the number of larvae per 100 mg. dry feces was determined.

All of the lungs examined were found infected with protostrongylin lungworms. The approximate areas of lesions were computed by multiplying the lengths of each lesion by its width and adding up the areas on each lung. Infections varied from a total P. stilesi lesion area of 57mm.^{2} to 4,247mm.^{2} Nineteen of the 45 lungs contained P. rushi adults in the air passages, varying in number from one to 89 per lung (Tables X and XI).

Most of the lesions were found on the dorsal surfaces of the lungs and were located mostly along the obtuse margin

and the posterior periphery of the diaphragmatic lobes. Frequently the right diaphragmatic lobe was more infected than the left. Similar observations were made for syntheto-caulosis (protostrongylosis) infections in domestic sheep in Russia (Shults and Boev, 1940).

Scrapings of the air passages revealed larvae in almost all the lungs examined, larvae being found in largest quantities in the bronchioles.

Mean lesion areas for 26 lungs collected from six herds in 1959 are presented in Table XII. One lung from Stillwater had over 2,800 mm.^{2} of lesions and one from Rosebud had over 1,700 mm.^{2} Sixteen lungs from the Sun River herd had a mean total lesion area of 1,727 mm.^{2} and four from the Gallatin herd had a mean total area of over 1,400 mm.^{2} Three lungs from the Ural-Tweed herd averaged 1,293 mm.^{2} of lesions. Lungs from Rock Creek showed low to moderate infections (Table XI), but lesions were not measured and so no comparison can be made with the 1959 collection. Small infections were found in the lungs of a lactating ewe killed accidentally on Wildhorse Island and in the lungs of a ewe killed by a fall in the Kootenai Falls area.

It would seem desirable to be able to examine fecal samples from a herd of bighoms and to determine the approximate degree of lungworm pathology in lungs from the larval content of the feces. Twenty-five sets of bighorn lungs and matching fecal samples were analyzed in this study (Table X). Results of these analyses suggest that there is no apparent

Table X. Protostrongylin lungworm infections in 33 lungs from bighorn rams killed by hunters in Western Montana during the fall of 1959

Hunting Area	Lesion Areas (mm^2)			Number *P. rushi* Adults Found in air Passages	Number Larvae per 100 mg. Dry Feces
	Dorsal	Ventral	Total		
Sun River	41	16	57	20	5.0
Sun River	143	70	213	2	52.9
Sun River	260	0	260*	0	--
Sun River	260	0	260*	3	--
Sun River	184	254	438	1	39.1
Sun River	486	90	576	33	244.4
Sun River	449	315	764*	0	9.6
Ural-Tweed	377	440	817	3	15.7
Sun River	824	28	852*	2	--
Ural-Tweed	682	341	1,023*	13	94.4
Gallatin	863	165	1,028	0	22.0
Gallatin	713	363	1,076	0	29.2
Sun River	525	659	1,184	0	43.2
Gallatin	1,201	0	1,201*	0	--
Sun River	678	598	1,276	0	6.9
Sun River	761	663	1,424*	10	--
Sun River	1,156	383	1,539*	5	58.3
Gallatin	1,413	147	1,560	0	1.2
Sun River	1,346	420	1,766	9	--
Rosebud	1,329	457	1,786	1	79.8
Ural-Tweed	1,629	410	2,039*	28	119.3
Sun River	1,163	877	2,040	89	14.5
Sun River	1,493	567	2,060*	4	14.4
Gallatin	774	1,345	2,119	1	4.5
Sun River	1,305	987	2,292*	0	14.2
Sun River	2,120	324	2,444*	0	154.9
Ural-Tweed	1,269	1,178	2,447*	0	--
Sun River	1,336	1,220	2,556*	0	5.0
Sun River	1,655	1,137	2,792	0	92.1
Stillwater	1,808	1,007	2,815	0	195.2
Sun River	2,525	436	2,961	11	--
Sun River	2,774	395	3,169*	7	162.1
Sun River	2,286	1,961	4,247*	0	234.5

*Lung damaged or partly missing

Table XI. Results of autopsies of miscellaneous bighorn lungs not included in Table X

Date of Kill	Herd	Number of *P. stilesi* lesions	Number of *P. rushi* adults	Number of Larvae per 100 mg. dry feces	Remarks
11/3/58	Rock Creek	3	0	24.39	Low infection
11/7/58	Rock Creek	0	0	0	Lungs were rotten; illegal kill found in field
11/8/58	Rock Creek	7	0	4.19	Moderate infection
11/13/58	Rock Creek	5	0	0.91	Low-moderate infection
11/30/59	Kootenai Falls	4	0	10.8	$5\frac{1}{2}$ year-old ewe
11/2/58	Kootenai Falls	3	0	0	Low infection
11/2/58	Ural-Tweed	10	7	256.51	High infection
Fall 59	Ural-Tweed	-	0	45.60	Bloodshot infection areas contained lungworm larvae
5/29/59	Wildhorse Island	4	0	3.17	Low infection; lactating ewe killed accidentally
9/20/59	Gallatin	-	-	4.00	Only two small chunks of the lung were obtained; larvae found in lung scrapings
9/27/59	Gallatin	18	0	5.20	-----
11/19/59	Sun River	2	0	13.70	Half of lung missing

Table XII. Mean lungworm lesion areas of 26 bighorn ram lungs collected from six ranges in Western Montana during the fall of 1959

Herd	Number of Lungs	Mean Lesion Areas (mm^2)			Mean Number *P. rushi* Adults	Mean Number Larvae 100 mg. Dry Feces
		Dorsal	Ventral	Total		
Stillwater	1	1,808.0	1,007.0	2,815.0	0	195.2
Rosebud	1	1,329.0	457.0	1,786.0	1.0	79.8
Sun River	16	1,112.1	615.8	1,727.9	10.0	71.9
Gallatin	4	940.7	505.0	1,445.7	0.2	14.2
Ural-Tweed	3	896.0	397.0	1,293.0	14.6	76.4
Kootenai Falls	1*	37.0	56.0	93.0	0	10.8
ALL ANIMALS	26	1,054.5	560.9	1,615.5	7.9	66.3

*Ewe

relationship between numbers of larvae in droppings and degrees of lung pathology. There appear to be daily and seasonal variations in larval shedding within a single animal (Pillmore, 1958a) and between individual animals. These variations would have to be considered if larval content of feces is to be correlated with lung pathology. Perhaps a trend between herds or groups of sheep might be found if an adequate sample of lungs and fecal material were analyzed.

DISCUSSION

Protostrongylin lungworms apparently have a very well adapted life cycle which is completed by a series of intricate events. A summary of Pillmore's (1958a) description of this sequence follows:

1. Feces containing the first stage larvae must be deposited on the range by infected animals.

2. The intermediate host must be present where the feces are deposited.

3. Suitable conditions of moisture and temperature must be present to instigate migration of larvae from the feces and to activate the molluskan hosts.

4. The larvae must penetrate the foot of a suitable mollusk.

5. The larvae must develop to the infective stage in the living mollusk. This development, which takes from 40 to 60 days, is influenced considerably by temperature.

6. The mollusk containing infective larvae must be ingested by the bighorn host. Plants which provide a habitat for the mollusks should be those eaten by the bighorns.

7. At least one male and one female lungworm must succeed in reaching the lungs where they mature, mate, and the female lays eggs.)

The resistance of these protostrongylin lungworm larvae to extremes in temperature and moisture seems to be a definite advantage in the completion of the life cycle.

Hobmaier and Hobmaier (1930) feel certain that a direct cycle is not involved in protostrongylosis. Also, the excellent work done by Boev and Wolf (1940), Shults and Boev (1940), Davtian (1947), Gerichter (1948; 1950; 1951), and Pillmore (1956; 1957; 1958a; 1958b) provides a tremendous amount of evidence to support the mollusk intermediate host theory. However, it must be stated that it has never been proven that the protostrongylin lungworms infecting bighorn sheep require land mollusks for intermediate hosts. A protostrongylin infection has never been artificially established by the feeding of infective mollusks to a bighorn. Until this is done, the possibility of some other organism serving as an intermediate host should not be eliminated.

If it is desirable to control protostrongylin lungworm infections, one of the more probable ways would be to eliminate or control the intermediate host. Pillmore (1956; 1957) found that metaldehyde and aerosol OT-B were successful molluscicides for pupillid snails in laboratory experiments. Such molluscicides might be used to control snail populations in critical winter concentration areas.

Eveleth and Eveleth (1943) found that injections of phenothiazine in alcohol and glycerin effectively killed lungworm larvae in the digestive tracts of domestic sheep. However, this treatment did not decrease the infection, but only seemed to decrease numbers of migrating larvae while production of larvae continued. Monnig (1940) reports a temporary decrease in egg laying by lungworms, Dictyocaulus filaria,

in domestic sheep as a result of intratracheal iodine injections. The use of salts containing iodides or phenothiazine has not been experimentally tested with bighorn sheep.

Approximately 96 percent of the 500 bighorn sheep fecal samples examined in this project contained protostrongylin lungworms. Although the number of larvae varied among individuals and herds, the data indicate that a high percentage of the bighorn sheep in Montana are infected with lungworm. However, because the effect of protostrongylosis on bighorn sheep in Montana is not yet well established, it seems unlikely that control measures are necessary at this time. If bighorn populations are properly managed and range depletion and competition are prevented, the effect of lungworm on the bighorn sheep might be kept at a minimum.

Upon completion of this preliminary study, I would like to make the following recommendations:

1. The monthly dropping collections should be continued on Wildhorse Island in order to obtain additional comparable data on lungworm infections.

2. Further work should be undertaken to establish definitely the intermediate hosts of protostrongylin lungworms which infect bighorn sheep in Montana.

3. Further controlled experiments should be conducted to investigate the effects of temperature and humidity on the survival of first stage larvae. Results of these experiments should be correlated with actual field conditions.

4. The effect of protostrongylosis on bighorn sheep

as well as other game animals should be further investigated in Montana and more quantitative data on the relative infections in Montana bighorn herds and among sex and age classes should be obtained.

5. The possibility of prenatal or early postnatal infections in lambs should be studied further.

SUMMARY

1. A preliminary investigation of the protostrongylin lungworm-bighorn sheep relationships in Montana was conducted from September, 1958, to March, 1960. The study consisted of three major phases: the effects of temperature and humidity on the first stage larvae of protostrongylin lungworms infecting bighorn sheep, the intermediate host bionomics, and the occurrence and status of protostrongylin lungworm infections in bighorn sheep, Ovis canadensis, in Montana.

2. First stage protostrongylin lungworm larvae that had migrated from fecal material were found to survive slightly better in 0.9 percent saline solutions than on dry slides when incubated at 36° ± 2°C for one, two, four, and eight days.

3. Larvae survived longer in dried than in wet feces when samples were incubated at 27°C. for logarithmically increasing periods of time from one day to one year. At 16 weeks some larvae were still alive in dried pellets and all larvae were dead in wet ones.

4. Survival of larvae in wet feces was higher than in dry feces at temperatures of -24°, -10°, 9°, 22°, and 32°C., but at 42° and 52°C. survival was highest in dried droppings which had been incubated for one, two, and four days. It appears that maximum survival of larvae in dried and wet pellets over a four-day period occurred at 22° and 32°C.

5. Survival of larvae incubated at 52°, 62°, and 72°C. for one, two, and four days was higher in dried pellets while

survival in both dried and wet pellets decreased with time and increasing temperature.

6. Larvae in fecal material and suspended in distilled water were observed to survive freezing as low as -22°C. for five months. Alternate thawing and freezing appeared to increase mortality of larvae suspended in water.

7. Experimental observations indicated that first stage larvae suspended in distilled water are positively phototropic.

8. Land mollusks were collected from ten bighorn ranges in Western Montana: the National Bison Range, Gallatin, Kootenai Falls, Many Glacier, Rock Creek, Stillwater, Sun River, Ural-Tweed, Wildhorse, and Yellowstone. Collecting efforts were extensive on the Wildhorse Island range and considerably less extensive on the other ranges.

9. Mollusks of the families Valloniidae and Pupillidae, which have been proven by other workers to serve as intermediate hosts of protostrongylin lungworms, were found on all except the Bison Range and Rock Creek ranges.

10. No natural protostrongylin infections were observed in 82 snails and slugs collected on bighorn ranges during 1959.

11. Attempts to infect 51 mollusks belonging to the genera Allogona, Deroceras, Euconulus, Oreohelix, Retinella, Triodopsis, Vertigo, Vitrina, Zacoleus, and Zonitoides were unsuccessful.

12. Fresh droppings collected every month for a year from a herd of bighorns on Wildhorse Island showed the number of larvae per 100 mg. dry feces to increase from 2.2 in summer

to 3.3 in fall, 4.7 in winter, and 7.5 in the spring.

13. In an analysis of fresh droppings collected from the major bighorn herds in Western Montana during 1958-1960, 96 percent of almost 500 individual fecal samples from bighorn sheep contained protostrongylin lungworm larvae.

14. The average number of larvae per 100 mg. dry feces for the Stillwater, Ural-Tweed, Rosebud, Sun River, and Yellowstone fecal samples was 90.2, 80.8, 79.8, 37.9, and 25.6 respectively. Averages for Many Glacier, Gallatin, Rock Creek, Wildhorse, and Kootenai Falls samples were below ten larvae per 100 mg. dry feces.

15. In 1959 lungworm larvae were first detected in lamb droppings from Wildhorse Island on June 21st, 47 days after the first lamb was born.

16. An orphaned bighorn lamb shed larvae for the first time on October 25, 1959, at five months of age and apparently was infected either prenatally or during its first week of life before it was removed from the sheep range.

17. Forty-three lungs from mature rams and two from mature ewes were examined from 1958 to 1960. Lesions caused by Protostrongylus stilesi were found on all lungs with total lesion areas as low as 57 $mm.^2$ and as high as 4,247 $mm.^2$

18. Adult Protostrongylus rushi were found in the air passages of 19 of the 45 lungs examined with the number per lung ranging from one to 89.

19. No evident correlation was found between the degree of lung pathology and the numbers of larvae in the droppings.

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A P P E N D I C E S

LIST OF APPENDICES

Appendix A. Detection and counting of first stage larvae of protostrongylin lungworms in droppings of bighorn sheep

The Baermann technique (Baermann, 1917) has been used by parasitologists as a relatively simple way to recover nematodes from fecal material. The principle of the technique is that larvae will become active and migrate out of fecal material soaked in water. Being heavier than water, larvae will settle to the bottom of a container. If fecal material is retained above a screen in a glass funnel, larvae can readily be collected at the bottom of the water-filled stem after a certain soaking period.

The following technique was used in this study to determine the numbers of first stage protostrongylin larvae found in fecal droppings of bighorn sheep:

1. Dropping samples were collected in the field and stored in glass jars under refrigeration at about 9°C. until examined.

2. Samples were weighed to the nearest milligram by use of a Roller-Smith Balance and the weights recorded as "wet" weights. Between 1500 and 2000 mg. of feces were used in each sample.

3. Warm tap water was added to 75 mm., long-stem funnels with stems closed off by rubber tubing and pinchcock clamps. Funnels were suspended in numbered racks.

4. Fecal samples were supported inside of the funnels on 60 mesh copper wire screens.

5. Samples were soaked for at least 48 hours to allow the majority of the living larvae to migrate out of the feces and sink to the bottom of the funnel stems. Some larvae may come down after 48 hours (Pillmore, 1958a), but this time period was utilized on the assumption that if the soaking period was kept constant comparable results would be obtained.

6. After soaking the feces for 48 hours an aliquot was taken from the bottom of the funnel stem. The larvae in the sample were counted in a 60 mm. Stender dish over a black line grid at a magnification of 15 diameters.

7. Each fecal sample was oven dried at $95^{o} \pm 5^{o}$C. for at least 48 hours to drive off the unbound water.

8. "Dry" fecal weights were obtained and recorded.

9. The numbers of larvae per 100 mg. of "wet" and "dry" feces were computed.

Appendix B. Detailed results of Desiccation Experiment 1. Survival of liberated lungworm larvae, Protostrongylus stilesi, subjected to dry and humid conditions at a temperature of 36° ± 2°C.

Time (Days)	Calcium Chloride (Drier)			Calcium Sulfate (Drier)			Control (Water)		
	No. Alive	No. Dead	Percent Survival	No. Alive	No. Dead	Percent Survival	No. Alive	No. Dead	Percent Survival
1	94	54	63.5	185	88	67.6	297	15	94.9
2	88	61	59.0	105	71	59.7	294	39	88.3
4	132	126	51.2	142	223	38.9	105	95	52.5
8	57	54	51.4	66	58	53.3	123	39	76.0

Appendix C. Detailed results of Desiccation Experiment 2. Survival of first stage lungworm larvae, Protostrongylus stilesi, incubated in dry and wet fecal pellets at 27°C. for up to a year

	Number larvae per 100 mg. feces			
	Dry		Wet	
Time	Pellet #1	Pellet #2	Pellet #1	Pellet #2
1 day	3.5	5.5	13.1	17.3
2 days	2.5	8.0	6.0	8.3
4 days	2.8	4.8	5.1	14.6
8 days	3.3	3.2	3.5	2.5
2 weeks	3.6	2.0	13.8	10.6
4 weeks	0.8	2.7	2.1	8.0
8 weeks	3.5	2.3	0.2	0.2
16 weeks	2.5	1.0	0	0
32 weeks	0	0	0	0
1 year	0	0	0	0

Appendix D. Detailed results of Desiccation Experiment 3. Survival of protostrongylin lungworm larvae in wet and dry feces incubated at -24°, -10°, 9°, 22°, 32°, 42°, and 52°C. for one, two, and four days

		Number larvae per 100 mg. feces			
		Dry		Wet	
Temperature	Time (Days)	Pellet #1	Pellet #2	Pellet #1	Pellet #2
-24°C.	1	8.2	11.6	15.8	13.1
	2	10.8	19.9	23.1	20.4
	4	16.4	8.4	24.5	23.5
-10°C.	1	10.8	19.4	23.3	18.0
	2	20.9	18.8	22.3	23.7
	4	14.3	19.6	13.5	37.4
9°C.	1	15.0	20.7	19.3	27.7
	2	23.7	17.5	21.6	23.3
	4	26.6	6.7	27.1	22.4
22°C.	1	15.2	28.3	30.3	33.7
	2	6.4	5.8	26.3	13.3
	4	23.8	28.5	19.8	49.8
32°C.	1	15.2	15.0	57.4	38.4
	2	35.7	15.3	30.6	39.8
	4	8.0	7.6	27.3	22.5
42°C.	1	8.0	24.3	3.0	24.0
	2	14.2	17.8	14.0	2.5
	4	17.2	12.3	0	2.6
52°C.	1	0.3	19.1	0.3	0
	2	21.4	12.4	0.2	0.7
	4	13.3	5.5	0	0

Appendix E. Detailed results of Desiccation Experiment 4. Survival of protostrongylin lungworm larvae in wet and dry feces incubated at 52°, 62°, and 72°C. for one, two, and four days

		Number larvae per 100 mg. feces							
		Dry				Wet			
Temp.	Time (Days)	Pellet #1	Pellet #2	Pellet #3	Pellet #4	Pellet #1	Pellet #2	Pellet #3	Pellet #4
52°C.	1	21.5	29.5	5.1	11.8	33.4	3.8	2.6	2.8
	2	17.3	11.1	3.1	4.2	1.0	5.4	0.4	2.9
	4	2.9	1.5	1.6	0.8	0	0.3	0	0
62°C.	1	2.2	16.8	2.3	19.7	2.1	5.2	24.4	0.7
	2	0.7	3.0	5.6	0	0	0	0.4	0
	4	1.7	0.8	0	0	0	0	0	0
72°C.	1	0	17.5	0.3	3.2	10.9	0.6	0.3	1.2
	2	0.3	0	1.6	0.2	0.3	0	0.8	0
	4	4.0	0.8	0	0.3	0	0	0	0

Appendix F. Detailed results of lungworm analyses of fresh droppings collected monthly from bighorn sheep of Wildhorse Island during 1959-1960

Bighorn	Number Samples	Number Larvae	Dry wt. of Feces (mg.)	Ave. No. Larvae per 100 mg. dry wt. Feces
April, 1959				
Unknown	6	156	1,848	11.6
May, 1959				
Rams	2	11	547	1.9
Ewes	5	122	2,046	6.7
Lambs	3	0	970	0
Yearlings	1	44	254	16.0
Unknown	6	340	2,329	11.3
TOTAL	17	517	6,146	7.1
June, 1959				
Rams	7	1,116	4,918	17.1
Ewes	18	463	11,916	3.9
Lambs	1	6	289	2.1
TOTAL	26	1,585	17,123	7.2
July, 1959				
Rams	9	142	8,307	1.6
Ewes	28	753	24,132	3.1
Lambs	16	0	--	0
TOTAL	53	895	32,439	2.2
August, 1959				
Rams	3	8	2,270	3.4
Ewes	4	65	4,007	2.1
Lambs	2	0	2,645	0
TOTAL	9	73	8,922	2.0

Appendix F. (Continued)

Bighorn	Number Samples	Number Larvae	Dry wt. of Feces (mg.)	Ave. No. Larvae per 100 mg. dry wt. Feces
September, 1959				
Rams	1	9	802	1.1
Ewes	5	185	4,517	4.4
Lambs	6	1	5,123	0.02
Unknown	1	86	902	9.5
TOTAL	13	281	11,344	2.5
October, 1959				
Rams	2	1	1,419	0.05
Unknown	16	602	14,142	4.5
TOTAL	18	603	15,561	4.0
November, 1959				
Rams	1	27	1,111	2.4
Ewes	1	32	807	4.0
Lambs	1	0	765	0
Unknown	32	719	22,357	3.2
TOTAL	35	778	25,040	3.1
December, 1959				
Rams	1	5	673	0.7
Lambs	1	8	723	1.1
Unknown	7	223	6,175	3.2
TOTAL	9	236	7,571	2.7
January, 1960				
Rams	4	232	3,627	6.6
Lambs	1	6	601	1.0
Unknown	28	916	25,481	3.3
TOTAL	33	1,154	29,709	3.9

Appendix F. (Continued)

Bighorn	Number Samples	Number Larvae	Dry wt. of Feces (mg.)	Ave. No. Larvae per 100 mg. dry wt. Feces
February, 1960				
Unknown	15	1,065	11,790	8.5
March, 1960				
Unknown	38	1,450	34,728	4.1
OVERALL TOTAL	272	8,793	202,221	4.6

Appendix G. Detailed results of lungworm analysis of fresh droppings collected from the major bighorn ranges in Western Montana

Animals	Number Samples	Number Larvae	Dry wt. of Feces (mg.)	Ave. No. Larvae per 100 mg. dry wt. Feces
			Stillwater	
March, 1959				
Mat. ewes	8	7,999	8,483	88.7
Unknown	15	16,563	18,706	93.0
Sub-total	23	24,562	27,189	90.8
October, 1959				
Mat. rams	1	574	719	30.8
OVERALL	24	25,136	27,908	90.2
			Ural-Tweed	
November, 1958				
Mat. rams	1	2,551	1,626	129.2
November, 1959				
Mat. rams	4	2,365	3,362	68.7
OVERALL	5	4,916	4,988	80.8
			Rosebud	
October, 1959				
Mat. rams	1	574	719	79.8
			Sun River	
April, 1959				
Mat. rams	1	761	1,597	47.6
Mat. ewes	4	937	4,285	20.8
Yearlings	2	2,385	2,972	79.5
Lambs	1	293	2,525	11.6
Unknown	21	8,339	33,671	23.7
Sub-total	29	12,715	45,050	27.3

Appendix G. (Continued)

Animals	Number Samples	Number Larvae	Dry wt. of Feces (mg.)	Ave. No. Larvae per 100 mg. dry wt. Feces
February, 1960				
Yearlings	1	182	666	27.3
Lambs	1	104	742	14.0
Sub-total	2	286	1,408	20.6
October-November, 1959				
Mat. rams	17	8,253	11,208	68.5
OVERALL	48	21,254	57,666	37.9
		Yellowstone (Mt. Everts)		
March, 1959				
Mat. ewes	6	2,449	9,622	25.8
Lambs	2	811	2,776	25.0
OVERALL	8	3,260	12,398	25.6
		Many Glacier		
March, 1959				
Mat. rams	1	17	712	2.4
Unknown	1	95	1,026	9.3
Sub-total	2	112	1,738	5.8
August, 1959				
Lambs	1	0	--	0
Unknown	4	884	7,900	11.1
Sub-total	5	884	7,900	8.9
OVERALL	7	996	9,638	8.0
		Gallatin		
March, 1959				
Mat. rams	8	503	6,977	7.7
Yearlings	1	38	1,268	3.0
Unknown	17	656	17,000	3.8
Sub-total	26	1,197	25,245	5.0

Appendix G (Continued)

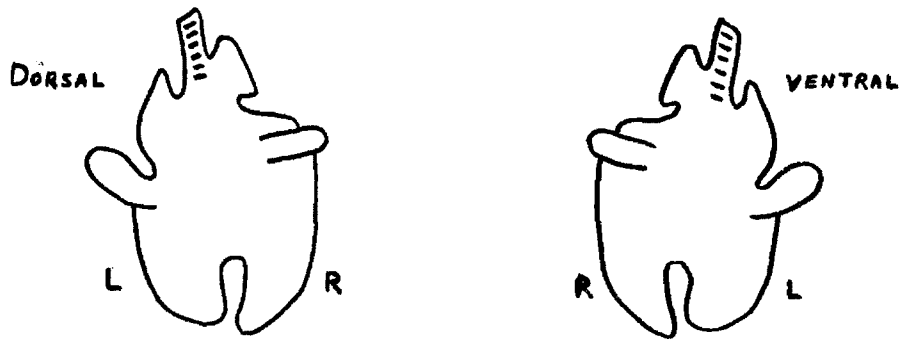
Animals	Number Samples	Number Larvae	Dry wt. of Feces (mg.)	Ave. No. Larvae per 100 mg. dry wt. Feces
September-October, 1959				
Mat. rams	6	419	3,948	11.0
OVERALL	32	1,616	29,193	6.1
			Rock Creek	
November, 1958				
Mat. rams	3	1,553	6,626	9.8
July, 1959				
Mat. ewes	1	150	1,572	10.7
Yearlings	1	2	1,592	0.1
Sub-total	2	152	3,164	5.4
October, 1959				
Mat. rams	1	119	726	16.4
Mat. ewes	12	420	9,783	4.2
Lambs	1	0	835	0
Sub-total	14	539	11,344	6.9
OVERALL	19	2,244	21,134	5.7
			Kootenai Falls	
November, 1958				
Mat. rams	1	0	507	0
September, 1959				
Mat. ewes	34	226	23,361	1.3
Lambs	9	1	6,860	0.01
Unknown	5	5	3,340	0.12
Sub-total	48	232	33,561	0.5
November, 1959				
Mat. ewes	1	158	1,460	10.8
OVERALL	50	390	35,528	0.9

Appendix H. Sample lab form used for lung autopsies.

BHS #_____
Sex_____
Locality_____
Shot by_____
Date of kill_____
Date Examined_____

I. Lung (external)

A. Nodule diagram:



B. Lung photographed on_____(date)
C. Number nodules found_____
D. Measurements of nodules_____
_____

II. Lung (internal)

	Larvae in scrapings?		P. rushi Male	P. rushi Female	P. rushi Total
A. Trachea	_____	-------	____	_____	_____
B. Bronchi	_____	-------	____	_____	_____
C. Bronchioles	_____	-------	____	_____	_____

D. Tissue sample
1. Fixed on_____in_____.
2. Preserved on_____in_____.

III. Droppings

A. Wet weight.....
B. Dry weight.....
C. Number larvae.....
D. Number larvae/100 mg. wet weight.....
E. Number larvae/100 mg. dry weight.....

IV. Other comments:

_____

_____