

ASPECTS OF THE LIFE CYCLE OF Protostrongylus stilesi (Nematoda, Protostrongylidae) IN BIGHORN SHEEP WITH EMPHASIS ON ENVIRONMENTAL INFLUENCES ON EXCRETION OF LUNGWORM LARVAE IN FECES.

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Abstract: The effect of experimentally altered environmental conditions on excretion of first stage larvae (L<sub>1</sub>) of Protostrongylus stilesi in feces of bighorn sheep (Ovis c. canadensis) was investigated. Bighorns brought into captivity maintained highly elevated but rather variable L<sub>1</sub> excretions compared to age-matched free-ranging sheep. Water and food deprivation of 2 sheep for 8 days resulted in no change in L<sub>1</sub> excretion, but water and food deprivation and crowding of 5 sheep in a 1.4 x 3 m pen for 15 days resulted in a 2.5 fold increase in fecal larval output above the pre- and post-stress levels. Four sheep (2 lungworm-free sheep born in captivity and 2 captive wild bighorns) were experimentally infected with Protostrongylus lungworms. Wild sheep were treated several times with ivermectin. Sixty-three days after the last treatment, third stage Protostrongylus larvae (L<sub>3</sub>), grown in the snail (Vallonia pulchella) were inoculated into the rumen. One sheep was infected with a mixture of P. rushi and P. stilesi while the other 3 received only P. stilesi. The prepatent period was 45 days for the mixed infection and 48, 51, and 54 days, respectively for P. stilesi. Quantitation of mature parasites in the lungs of two sheep 11 and 234 days post-patency indicated 10% and 30% of inoculated L<sub>3</sub> molted into adults.

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Virtually all wild bighorn sheep are infected with Protostrongylus lungworms. From data of necropsies and quantitation of lungworms, it is apparent that larvae excretion in the feces does not reflect the extent of worm burden in the lung (Onderka and Wishart 1984). This suggests a host controlled regulatory mechanism, but besides seasonal variations, little is known about factors that influence excretion of lungworm larvae in bighorn sheep feces. Furthermore, the prepatent period for Protostrongylus lungworms is unknown in pure-bred bighorns.

This paper summarizes results of a study to influence fecal lungworm larvae excretion and to determine the prepatent period of P. stilesi in bighorn sheep.

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## METHODS

### Animals

Seven bighorn lambs were captured at 3 locations in autumn in corral traps baited with salt or by the administration of 150 mg xylazine (Haver-Lockart Laboratories, Shawnee, Kansas) delivered by a projectile dart (Cap-Chur gun, Palmer Chemical and Equipment Co. Ltd., Douglasville, Georgia). They were transported to the laboratory in individual crates under xylazine sedation (4 mg/kg intramuscularly). They were housed individually on straw in wiremesh-covered indoor stalls measuring 1.4 x 3 m and 1.8 m high. The animals were each fed free-choice hay supplemented daily with 250 g of alfalfa pellets and 2 g cobalt-iodized stock salt sprinkled on the hay every other day. Four additional bighorn sheep were acquired from zoos.

### Fecal Analyses

Fecal samples were collected from the rectum after the sheep have been blind-folded to keep them calm. The Baermann technique as described by Samuel and Gray (1982) was used to determine larvae per gram of dry feces (LPG). The same method was used to obtain LPG counts from the wild herds at the Sheep River Sanctuary and Mt. Allan in southwestern Alberta and from Cadomin in the northwestern Alberta Rocky Mountains from where the experimental sheep originated. Data were also compared with those of age-matched sheep from the Ram Mountain herd in westcentral Alberta.

### Experiments

#### A. Environmental Influences on Fecal Larval Output

1. Captivity.--Two lambs from the Sheep River Sanctuary were held for two months indoors and were then transferred to an outdoor pen measuring 7 x 7 m where they remained from mid-October to mid-January. The temperatures were as low as -35C. Fecal samples were collected twice a month from each sheep. The number of samples from age-matched wild sheep at the Sanctuary varied from 1 to 11 per month.

2. Food and water deprivation.--Two male lambs from Mt. Allan and 1 male lamb from Cadomin were acclimatized to captivity from mid-June to the end of July. In August, the 2 Mt. Allan sheep were deprived of food and water for 8 consecutive days but remained on straw bedding. The third lamb served as control. The frequency of fecal sampling before, during and after the experiment is given in Figure 2. The degree of dehydration was assessed using packed cell volume determined on day 1, 5 and 8. Since no data were available from wild Mt. Allan sheep, LPG was compared to age-matched bighorn lambs from Ram Mountain. The number of samples from June through August were 23, 17 and 8, respectively.

3. Food and water deprivation with crowding.--Two wild female lambs from Cadomin were acclimatized to captivity from June to October before they were housed together with 3 male lambs used in experiment 2 in a stall measuring 1.4 x 3 m. All 5 sheep were deprived of food and water for 15 days, except for a little water on day 7. Only the 2 female lambs

were monitored. The frequency of fecal sampling before, during and after the experiment is given in Figure 3. Packed cell volume was determined on day 4, 8, 11 and 15. The LPG of age-matched wild sheep from Ram Mountain is the same as in experiment 2 except for October, which is based on only one sample.

## B. Prepatency Period

Trial 1.--Two zoo born bighorn lambs found to pass no lungworm larvae in the feces were housed in separate indoor stalls on concrete floors covered with straw. One lamb was inoculated via a rumen tube with 1004 third stage larvae (L<sub>3</sub>) of P. stilesi and P. rushi grown in 49 Vallonia pulchella snails as described by Samson and Holmes (1985). The other lamb was sham inoculated and served as control.

Trial 2.--A zoo born lamb found to pass no lungworm larvae in the feces was inoculated with 240 L<sub>3</sub> of P. stilesi, contained in 20 snails. To assure single genus infection, snails were infected with larvae extracted from typical P. stilesi nodules from lungs with no evidence of P. rushi after careful dissection of the airways.

Trial 3.--The 2 Mt. Allan sheep used earlier in experiment A-2 and one zoo born male yearling sheep were used in this trial. All 3 sheep excreted lungworm larvae and had to be treated with ivermectin (Ivomec, Merck-Sharp-Dome, Kirkland, Quebec) given at 200 ug/kg subcutaneously in the neck. Several treatments were necessary before the sheep no longer passed L<sub>1</sub> in the feces. The zoo born sheep served as control while the other 2 received 222 L<sub>3</sub> and 575 L<sub>3</sub> of P. stilesi in 31 and 49 snails, respectively.

Rectal fecal samples were collected weekly up to 40 days post inoculation and from then on every 2nd day until patency was established. The sheep from trail 1 and 2 were euthanized 11 and 24 days post-patency, respectively, using an overdose of intravenous Pentobarbital (M.T.C. Pharmaceuticals, Mississauga, Ontario). To recover adult nematodes from the lungs, nodular tissue was minced and digested in a solution of 3.6 mg Papain in 50 ml water for 1.5 h at 22 C. For the recovery of larvae, the nodular tissue was further digested using .45 g pepsin and .7 ml 37% HCl in 100 ml water for 16 h at 37 C. A thin segment of tissue was fixed in 10% buffered formalin for histologic examination.

## RESULTS

### Excretion of Larvae

Excretion of lungworm in sheep feces increased dramatically when bighorn sheep were brought into captivity as compared to age-matched animals in the wild herds from which they were caught (Figure 1). The normal seasonal increase in LPG during the winter months was observed in the wild herds but not in captive sheep. Exposure of the lambs to severe weather conditions had no effect on the LPG. Stressors such as food and water deprivation had no appreciable influence on LPG (Figure 2), although it was reflected in a rise in packed cell volume and loss of elasticity of the skin. In a second experiment, when the sheep were crowded in addition

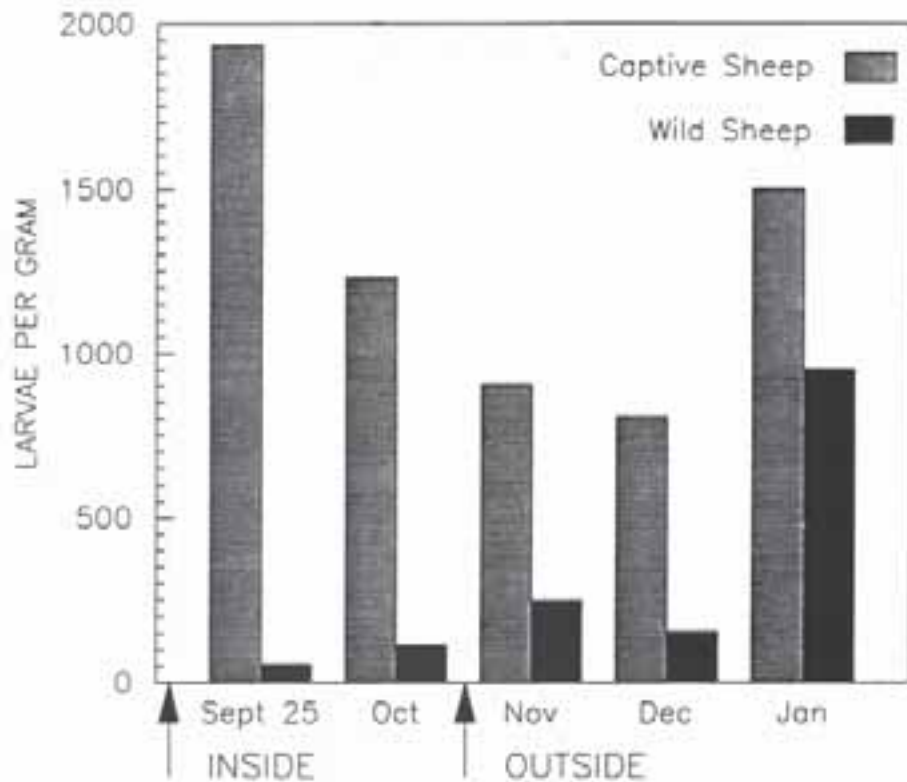


Figure 1. Mean lungworm larvae per gram in feces of two bighorns brought into captivity compared to age-matched wild sheep from the herd of origin.

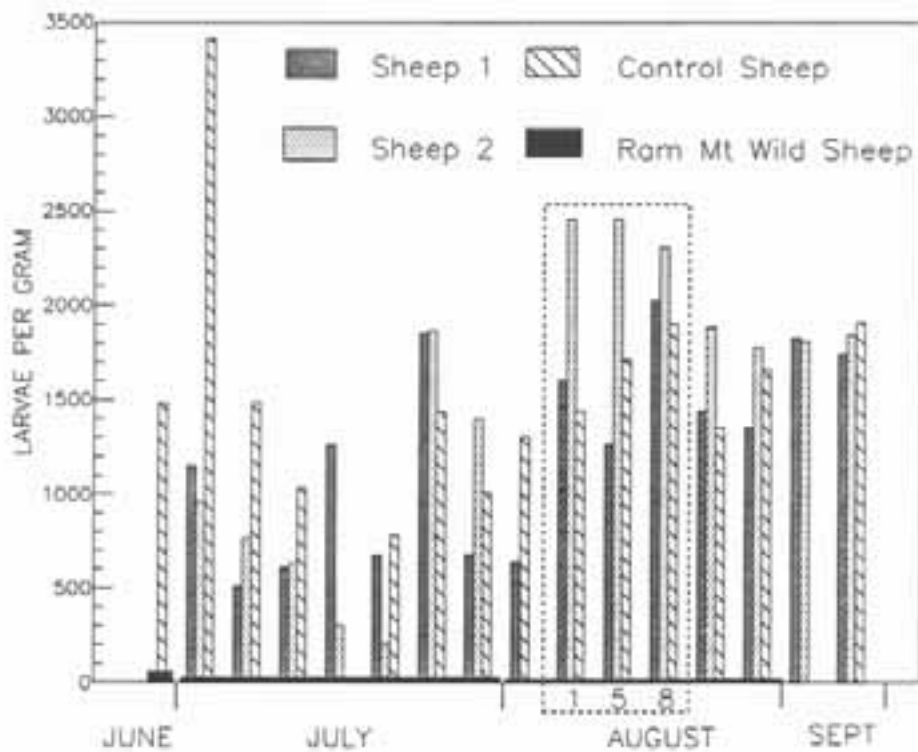


Figure 2. Mean numbers of lungworm larvae in feces of wild, captive bighorns compared to age-matched wild sheep. The time frame within the dotted lines refers to 8 days of food and water deprivation for sheep 1 and 2.

to a longer period of food and water deprivation, a distinct increase in LPG was noticed followed by a decline after the experiment was terminated (Figure 3).

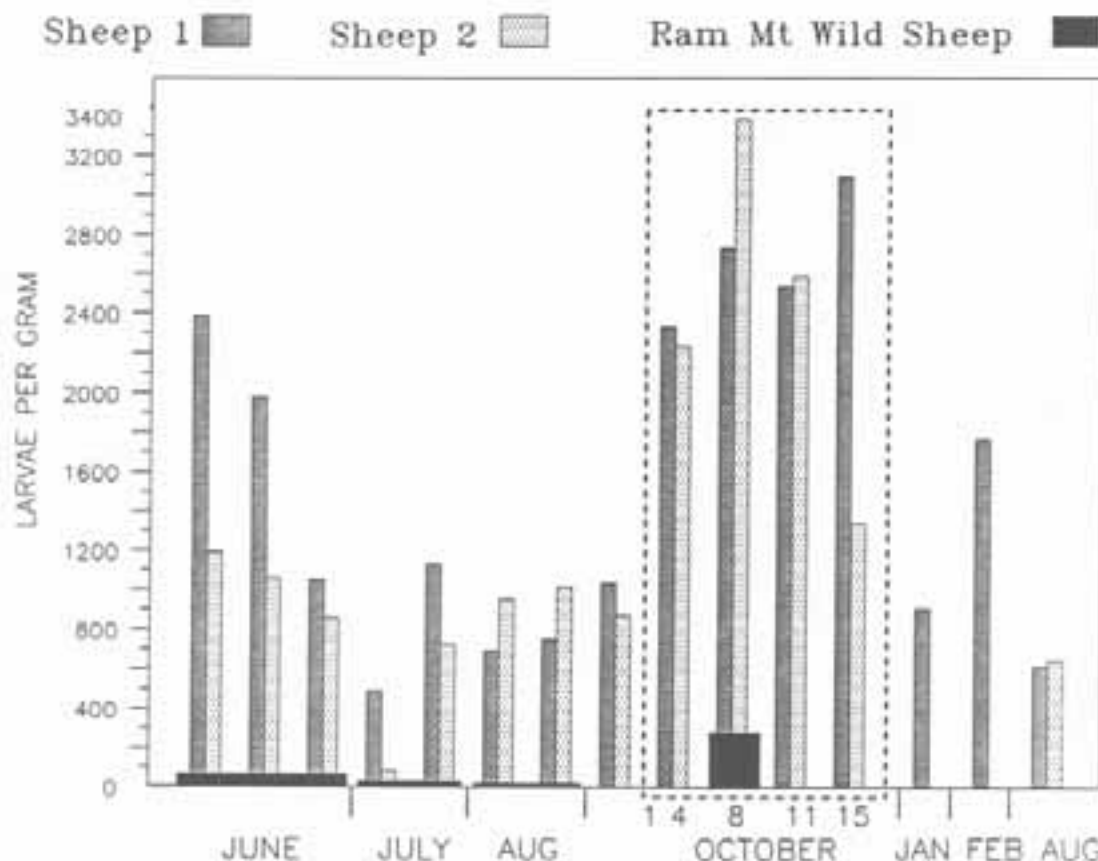


Figure 3. Mean numbers of lungworm larvae in feces of wild captive bighorns compared to age-matched wild sheep. The time frame within the dotted line refers to 15 days of food and water deprivation and crowding with 3 additional bighorn sheep.

#### Treatment Against Lungworms

A single injection (day 0) of Ivermectin to 3 bighorns resulted in a 97% reduction of LPG after 23 days. A second treatment was given on day 43. At this time, one sheep had 0 LPG but from day 83 on (40 days after second treatment) all 3 sheep again excreted lungworm larvae. Six months later, these 3 sheep were again treated with ivermectin. Injections were given on day 0 and day 13. Fecal samples taken on day 119 were negative but a third treatment was given at this time. The sheep were monitored until day 182 (63 days after last treatment) when two of the animals were used for experimental lungworm infection. The third sheep served as

control and maintained 0 LPG for over 260 days at which time the experiment was terminated.

#### Prepatent Period

The mixed infection of *P. stilesi* and *P. rushi* resulted in a prepatent period of 43 days. The prepatent period for the *P. stilesi* infection was 48, 51 and 54 days respectively. In the sheep inoculated with 1004 mixed species L<sub>3</sub>, the LPG started very low with a steep rise to 950 after 11 days and 1200 after 24 days. At necropsy, 24 grey, raised, firm lungworm nodules consisting of adult nematodes, embryonating eggs, L<sub>1</sub> and lymphocytes contained 294 adults of *P. stilesi* (126 females and 168 males). In addition, there were 35 adult *P. rushi* in airways. This suggests that after 24 days 30% of L<sub>3</sub> developed into adults. The control sheep had no evidence of lungworms. The sheep from trail 2 was necropsied 11 days post-patency. It had received 240 L<sub>3</sub> of only *P. stilesi*. Six dark-red, firm nodules were found in the posterior margin of the caudal lung lobes. Microscopic examination showed a fairly acute reaction with hemorrhage, neutrophil and some eosinophil infiltrations in response to the reproductive activity of the lungworms. Twenty-two adult nematodes (10 females and 12 males) were found. The LPG was only 6 although an average of 15000 L<sub>1</sub> per female were found. At this time 10% of third stage larvae had developed into adults.

#### DISCUSSION

The dramatic increase of LPG in bighorns brought into captivity suggests that environmental changes have a marked influence on the excretion of lungworm larvae in feces. This could be further enhanced by crowding stress. These data support the value of long-term monitoring of LPG in bighorns to assess the stress level of a herd. It does not, however, reflect the actual extent of lungworm infection in individual animals.

Treatment of wild sheep with anthelmintics has been of interest in the management of small, poor doing herds. Our results, using ivermectin, show that at least two treatments 14 days apart are necessary for long term lungworm control. If the treatments were spaced further apart sheep again began to shed larvae. The interval between treatment and resumption of shedding was 40 days. This is similar to results reported by McCraw and Menzies (1986) in the treatment of *Muellerius capillaris* in goats. Our results agree with those of Miller et al. (1987) working with bighorns and Gregory et al. (1985) working with goats showing that ivermectin causes significant reduction of LPG even down to 0 but differ, in that in our study, long term monitoring showed resumption of fecal larvae excretion while in the other two studies follow up samples were obtained only for 29 days (Miller et al.) and 14 days (Gregory). The large difference in the number of L<sub>3</sub> developed into adults after oral inoculation between 11 days and 24 days post-patency lends support to suggestions by McGraw and Menzies (1986) that this development is gradual and ivermectin may only be effective against adult stages of lungworms.

The prepatent period of *P. stilesi* infection varied from 48-54 days. A shorter period was found when a mixed infection with *P. rushi* was given.

These data differ considerably from those reported for 3 bighorn sheep by Monson and Post (1972) (63,119 and 122 days). They likely used a mixed infection as their source of L<sub>1</sub> was fecal pellets from wild sheep that were inoculated into bighorn sheep-mouflon crosses.

The gradual development of L<sub>3</sub> into adults will be investigated further in view of the possible storage of L<sub>3</sub> in the lung of the ewe for transplacental transmission to the fetus.

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