

EFFICACY OF ORALLY ADMINISTERED IVERMECTIN ON LUNGWORM INFECTION IN FREE-RANGING BIGHORN SHEEP

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Infection with parasitic lungworm (Nematoda: Protostrongylidae) is common among Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) in North America (Buechner 1960, Forrester 1971, Stelfox 1971). *Protostrongylus* spp., the most frequently reported lungworm, may lead to the development of pneumonia and increased levels of adult and juvenile mortality (Forrester 1971, Woodard et al. 1974, Hibler et al. 1982). Rocky Mountain bighorn sheep in the Black Hills of South Dakota appear uniquely free of *Protostrongylus* spp., but they contain *Muellerius capillaris*, a lungworm common in domestic sheep and goats (Pybus and Shave 1984). Of 9 adult sheep examined in Custer State Park (CSP), South Dakota, all lungs contained adult *M. capillaris* and lesions indicative of chronic bronchitis and pneumonia (Pybus and Shave 1984). Additionally, Demartini and Davies (1977) implicated *M. capillaris* as an important pathologic agent present during a die-off of captive bighorn sheep moved from CSP to Colorado. Although epizootic lungworm outbreaks are generally considered symptomatic of more fundamental population-limiting processes (Risenhoover et al. 1988), anthelmintic drugs may be the only practical means of managing lungworm infec-

tion in many populations (Hibler et al. 1976, Schmidt et al. 1979).

Several anthelmintic drugs have been used to reduce abundance of *Protostrongylus* spp. in bighorn sheep, including the benzimidazoles (cambendazole, fenbendazole, and albendazole; Schmidt et al. 1979, Foreyt and Johnson 1980) and injectable ivermectin (22,23-dihydroavermectin B₁; Miller et al. 1987). Oral administration of cambendazole and fenbendazole successfully reduced *Protostrongylus* spp. levels in free-ranging bighorn sheep in Colorado (Schmidt et al. 1979), but disadvantages of using those drugs included potential toxicity, as well as logistic problems associated with delivering effective dosages (Miller et al. 1987).

Injectable ivermectin has also been used to reduce *Protostrongylus* spp. infections in both captive and transplanted bighorn sheep (Miller et al. 1987, Miller and Hobbs 1988). Though proven effective over the short term, injection of ivermectin requires capturing and handling individual animals, which is difficult with free-ranging bighorns. Furthermore, frequent reappearance of lungworm following treatment with anthelmintic drugs indicates that repeated administrations may be necessary for long-term control of *Protostrongylus* spp. in free-ranging populations (Schmidt et al. 1979, Miller and Hobbs 1988). Practical means of administering ivermectin to free-ranging wildlife populations have not been evaluated.

We conducted a management experiment to determine if repeated oral administration of ivermectin was effective in reducing levels

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of *M. capillaris* infection in free-ranging bighorn sheep in CSP, South Dakota. Preliminary testing helped establish effective doses and techniques for administering ivermectin orally but did not evaluate long-term efficacy of oral treatments (Layne and McCabe 1986). While attempting to develop a practical field method of administering ivermectin to free-ranging populations, we tested the null hypothesis that there were no differences in concentrations of first-stage lungworm larvae (L1) in feces of bighorn ewes given 0, 1, or 2, 2-day oral treatments with ivermectin during winters of 1987 and 1988.

STUDY AREA AND METHODS

CSP encompasses 29,150 ha in the southern Black Hills in southwestern South Dakota (43°37.5'N, 103°22.5'W). Twenty-two Rocky Mountain bighorn sheep were released in CSP in 1964 to replace the extinct Audubon's bighorn sheep (*O. canadensis auduboni*), which were indigenous to the Black Hills until the early 1900's. The present population is comprised of 3 subpopulations of ewe groups consisting of ewes, lambs, and subadults (Brundige 1985) and 3 groups of adult rams (Layne 1987). Although the ranges of ewe groups overlap slightly and intermingling among subpopulations has occasionally been observed (Brundige 1985), the ewe groups maintain spatially segregated activity patterns and group membership.

Ewe groups in CSP inhabit Grace Coolidge and French Creek canyons. Elevations range from approximately 1,160 m in the canyon bottoms to 1,530 m on the canyon rims. Bighorns occupy steep, rocky cliffs on the canyon walls bordered by a mosaic of ponderosa pine (*Pinus ponderosa*) and midgrass prairie on the canyon rims. Understory vegetation consists primarily of sedges (*Carex* spp.), bluegrasses (*Poa* spp.), buffalo grass (*Buchloe dactyloides*), grama grasses (*Bouteloua* spp.), and little bluestem (*Schizachyrium scoparium*). Bighorn sheep ranges are shared with bison (*Bison bison*), elk (*Cervus elaphus*), white-tailed deer (*Odocoileus virginianus*), and mule deer (*O. hemionus*).

Drug Administration

We used previously marked adult ewes from 3 ewe groups to compare fecal concentrations of L1 among ewes given 0 (TRT0), 1 (TRT1), and 2 (TRT2) oral treatments with ivermectin. Treatments were assigned to ewe groups, so all ewes within a group received the same treatment. Each treatment consisted of giving ivermectin on 2 successive days. TRT1 ewes received a single 2-day treatment each year in February 1987

and 1988. TRT2 ewes received 2, 2-day treatments each year in February and March 1987 and January and February 1988.

Free-ranging ewes were given ivermectin orally by feeding them fresh alfalfa treated with the drug. Control and experimental ewe groups were prebaited with untreated alfalfa for 2 weeks prior to treatment each year and intermittently between treatments. Prebaiting accustomed bighorns to accepting alfalfa and allowed us to determine an amount of alfalfa that was consumed completely and ensured prompt ingestion of the drug. Based on prebaiting trials, treated alfalfa was subsequently fed at a rate of approximately 0.5 kg per bighorn present at the time of treatment, an amount generally consumed within 20–30 minutes.

We administered injectable ivermectin (Merck and Co., Inc., Rahway, N.J., 10 mg/ml) by diluting it with 2 parts distilled water and spraying the mixture on alfalfa immediately before feeding. Injectable ivermectin was chosen over an oral formulation (4 mg/ml) because the higher concentration helped ensure that individual ewes consumed effective doses. Recognizing the wide margin of safety (30X) in administering ivermectin to domestic livestock (Campbell et al. 1983), we administered ivermectin at the rate of 2 ml per ewe or subadult ram and 1 ml per lamb present during treatment, i.e., approximately twice the manufacturer's recommended dose (1 ml/50 kg body weight). Alfalfa treated with ivermectin was spread in a line approximately 15 m long to allow all ewes present to feed without interference. Ear-tagged ewes present on both days of treatment comprised the experimental groups of treated ewes. Although we could not measure specific doses received by individual ewes within a group, liberal doses of ivermectin offered on 2 successive days helped to ensure that all individuals present received an effective dose (Layne and McCabe 1986). Ewes in the control group were fed alfalfa at the same frequency as ewes receiving ivermectin.

Fecal Analysis

To monitor changes in output of L1 following treatment, fecal samples were collected from ear-tagged ewes known to have received ivermectin (TRT1 and TRT2) and known control animals (TRT0) by following them until they defecated. Fecal samples were collected weekly prior to treatment in January, following treatment until the end of March, and from June to August of each year. All fecal samples were stored frozen at -20 C to prevent emigration of L1 and to minimize variability associated with sample storage. Although freezing may affect viability of L1, that effect was presumed constant across treatments and should not affect our interpretations (Beane and Hobbs 1983).

Concentrations of L1 in fecal samples were determined using a modification of Baermann's technique (Beane and Hobbs 1983), in which a Buchner funnel was not used. Samples were oven-dried at approximately 30 C for 3 days to ensure constant temperature

and humidity conditions. Approximately 5 g of dried fecal material was weighed, placed in cheesecloth gauze, and submerged in 50 ml of water for 3 days. After soaking, the gauze-wrapped sample was removed from the water, squeezed dry, and discarded. The remaining liquid was centrifuged at 6,000 rpm for 15 minutes. The supernatant was discarded, leaving a sediment of fecal material and L1, to which 1–4 ml of water was added depending upon concentration of L1 in the resulting solution. Five 0.05-ml aliquots were withdrawn, placed on microscope slides, and viewed microscopically at 10X magnification. The number of L1 counted in 0.25 ml (i.e., 5×0.05 -ml aliquots) was used to compute total abundance of L1 based on the known volume of solution. L1 concentration was computed as the total abundance of L1 divided by the initial dry weight of feces.

Data Analysis

Concentrations of L1 in sheep feces were compared among treatments and over time with a 2-way (treatment \times month) analysis of variance each year. Years were analyzed separately because drug administration schedules differed between years and because different individually marked sheep were available for study each year. Data were analyzed as a repeated-measures design with mean L1 output of individual ewes serving as replicates for each treatment-month combination (SAS Inst. 1987:602). Because it was not possible to assign treatments independently of herd membership, individual ewes within a herd were actually pseudo-replicates of each treatment (Hurlbert 1984). If the treatment \times month interaction was significant, Fisher's protected least significant difference tests were used to test for significant differences between treatment means within months.

RESULTS

Bighorn sheep readily ate alfalfa that had been treated with ivermectin, indicating no adverse taste. Four and 6 individually marked ewes received 1 dose of ivermectin (TRT1) in 1987 and 1988, respectively. Seven and 14 marked ewes comprised TRT2 in 1987 and 1988, respectively, and 5 and 9 marked ewes comprised the control group each year, respectively.

L1 outputs from bighorn sheep differed among treatment groups in 1987 ($F = 4.62$; 2,13 df; $P = 0.03$) but not in 1988 ($F = 1.63$; 2,26 df; $P = 0.21$). Seasonal patterns of L1 output, however, differed among treatments both years indicating a significant seasonal ef-

fect of treatment (treatment \times month interaction; $F = 4.17$ – 5.04 ; $P < 0.01$).

All fecal samples collected prior to treatment with ivermectin contained L1, with mean concentrations ranging from 1,199–2,403 L1/g feces (Fig. 1, January 1987 and 1988). There were no differences in L1 output among treatment groups and the control group prior to administration of ivermectin in 1987 (Fig. 1). Although L1 output differed between TRT1 and TRT2 in January 1988, neither treatment group differed from the control group at that time (Fig. 1).

Ivermectin reduced output of L1 for approximately 1 month following treatment (Fig. 1). L1 output, however, did not differ among treatment groups by the summer following treatments (Fig. 1).

DISCUSSION

Oral administration of ivermectin reduced short-term output of *M. capillaris* larvae, which corroborates the pattern produced by subcutaneous injections of ivermectin on *Protostrongylus* spp. in captive bighorn sheep (Miller et al. 1987, Fougere-Tower and Onderka 1988) and on *M. capillaris* in domestic goats (McCraw and Menzies 1986). We did not, however, observe a lasting influence of ivermectin on L1 concentrations of free-ranging bighorn sheep, contrary to findings of Miller and Hobbs (1988). Reappearance of L1 in as little as 30 days in our study (Fig. 1A, TRT1; Fig. 1B, TRT2) indicates that not all third-stage larvae or adult lungworm were killed by the treatment and that surviving individuals produced additional L1 (Miller and Hobbs 1988, Samuel 1988). Because L1 do not appear in the feces until 52–59 days after *M. capillaris* lungworm are ingested (Boev 1984), we rejected the possibility that reappearance of L1 resulted from reinfection of lungworm from the environment.

Because ivermectin treatments were assigned to individual sheep on the basis of ewe

group, demonstrated treatment effects may not connote causation (Hurlbert 1984). Differences in L1 output among ewe groups could either be related to experimental treatment or to underlying nutritional or physiological differences among ewe groups. However, mean levels of 2,6-diaminopimelic acid (DAPA), a residue of rumen bacterial fermentation and an indicator of dietary quality (Davitt and Nelson 1984), did not differ among treatment and control groups either during winters (mean DAPA = 0.35–0.41 mg/g feces; $F = 1.56$; 2,12 df; $P = 0.25$) or subsequent summers (mean DAPA = 0.69–0.84 mg/g feces; $F = 0.30$; 2,12 df; $P = 0.75$) in 1987 and 1988 (Easterly 1989). Additionally, mean lamb:ewe ratios did not differ among ewe groups either during summer 1987 (mean lambs:100 ewes = 37–50, $\chi^2 = 0.55$, 2 df, $P = 0.76$) or summer 1988 (mean lambs:100 ewes = 61–74, $\chi^2 = 0.88$, 2 df, $P = 0.64$) (Easterly 1989). Consequently, differences in L1 outputs among ewe groups were most likely a result of treatment with ivermectin.

Several factors may have contributed to differences in long-term efficacy of ivermectin as reported previously (Miller et al. 1987, Miller and Hobbs 1988) and in this study. First, oral administration of ivermectin, as reported in this study, may be less effective than subcutaneous injection. Marriner et al. (1987), for example, reported that efficacy of ivermectin was more persistent following subcutaneous injection than oral drenching. Additionally, we have no way of assessing actual dosages of ivermectin received by individual ewes or the influence of ruminal fermentation on efficacy of the drug. Secondly, the effect of ivermectin on *M. capillaris* may not be as potent as on *Protostrongylus* spp., although this factor is also difficult to evaluate. Several authors have reported varying levels of efficacy of ivermectin against both *M. capillaris* in domestic sheep and goats (Gregory et al. 1985, Denev et al. 1986, Dorchies and Ducos de Lahitte 1986, McCraw and Menzies 1986) and against *Pro-*

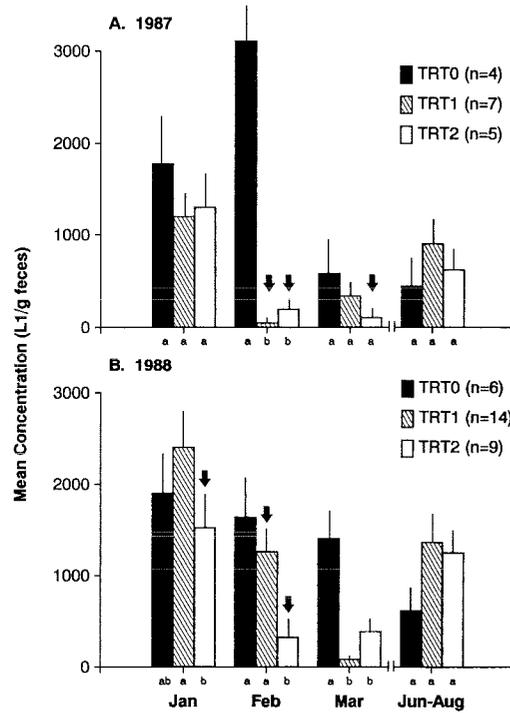


Fig. 1. Monthly mean concentration of lungworm larvae (L1/g feces \pm SE) in feces of bighorn sheep ewes receiving 0 (TRT0), 1 (TRT1), and 2 (TRT2) treatments of ivermectin in Custer State Park during winters 1987 and 1988. Bold arrows indicate months of treatment with ivermectin. Ivermectin was administered at the beginning of months in 1987 and at the end of months in 1988. Therefore, all January samples represent pretreatment periods in 1987 and 1988. Different lowercase letters beneath bars indicate differences between treatments within a month ($P < 0.05$, Fisher's protected least significant difference test).

tostrongylus spp. in bighorn sheep (Miller et al. 1987, Miller and Hobbs 1988). Lastly, differences between our results and those of previous studies could reflect differences in initial levels of lungworm infection. Pretreatment output of *M. capillaris* from bighorn sheep in CSP (1,199–2,403 L1/g dry feces) was higher than output of *Protostrongylus* spp. in previously studied herds in Colorado (94 L1/g dry feces, Schmidt et al. 1979; <350 L1/g dry feces, Miller et al. 1987). Levels of L1 observed in CSP corresponded to the heavy infection level designated by Uhazy et al. (1973).

Our results clearly indicated that the experimental regime of administering ivermectin to free-ranging sheep orally was ineffective in controlling *M. capillaris* infection levels under the conditions tested. Drug-treatment programs treat only a symptom of habitat loss and degradation that affects many isolated bighorn populations in North America (Miller and Hobbs 1988, Risenhoover et al. 1988). Until those ultimate limiting factors can be enhanced, we believe that drug treatment programs will have limited success in controlling lungworm infection in free-ranging populations. Higher dosages, more frequent treatments, and formulations of ivermectin developed since our study (e.g., Benz et al. 1989) may have utility in specific circumstances and warrant additional research.

SUMMARY

We evaluated influences of 0 (TRT0), 1 (TRT1), and 2 (TRT2) oral administrations of ivermectin on lungworm infection levels in 3 free-ranging ewe groups of bighorn sheep in CSP, South Dakota. Although ivermectin reduced output of L1 for approximately 1 and 2 months in TRT1 and TRT2 ewes, respectively, L1 output increased to TRT0 levels by the summer following treatment. We concluded that broad-scale oral administration of ivermectin was not an effective tool for managing lungworm infections in a free-ranging population of bighorn sheep.

Acknowledgments.—This study was funded by South Dakota Department of Game, Fish and Parks, and Federal Aid to Wildlife Restoration Funds, Project W-75-R, Study No. 7531. We thank W. Fairbanks for assistance in the field; W. Winter for field assistance and providing logistical support; H. Shave for help in establishing laboratory procedures; L. Tucker for helping with statistical analyses; and N. R. Holler, K. L. Risenhoover, and the anonymous reviewers for reviewing previous drafts of the manuscript.

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Received 27 August 1990.

Accepted 26 August 1991.

Associate Editor: Holler.

