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NATURAL INFECTION BY GASTROINTESTINAL AND BRONCHOPULMONARY NEMATODES IN MOUFLONS (*OVIS MUSIMON*) AND THEIR RESPONSE TO NETOBIMIN TREATMENT

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ABSTRACT: Gastrointestinal and bronchopulmonary nematode infections and the efficacy of netobimin (Hapasil®) were analyzed by way of fecal examination in 10 female mouflons (*Ovis musimon*), in central Spain, February 1993. Before treatment all 10 mouflons had *Trichostrongylus axei, Teladorsagia circumcincta* and *Marshallagia* spp.; six had *Nematodirus* spp., two had *Trichuris* sp., one had *Capillaria* sp., seven had bronchopulmonary *Dictyocaulus filaria* and 10 mouflons had protostrongylid lungworms (*Muellerius capillaris, Protostrongylus rufescens, Cystocaulus ocreatus* or *Neostrongylus linearis*). Netobimin (7.5 mg/kg) was 100% effective against *T. axei, T. circumcincta, Marshallagia* spp., and *D. filaria* infections whereas one animal continued eliminating *Nematodirus* spp. eggs. The drug also was effective against *Capillaria* spp. but not against *Trichuris* spp. or protostrongylid infections.

Key words: Nematode, mouflon, *Ovis musimon,* trichostrongylids, protostrongylids, *Nematodirus, Dictyocaulus filaria,* netobimin.

INTRODUCTION

Nematode infections in domestic small ruminants have been widely analyzed, but reports from mouflon (*Ovis musimon*) and other game animals are limited. High prevalences of gastrointestinal and pulmonary nematodes have been reported in mouflons in Poland (Kozakiewicz and Maszewska, 1984; Soltysiak and Bartczar, 1991) and other game species (chamois, *Rupicapra rupicapra*; roe-deer, *Capreolus capreolus*) in a reserve of Spain (Díez-Baños et al., 1987, 1990). We wanted to know the anthelmintic efficacy in this and other wild ruminant species since responses can differ considerably between domestic ruminants and phylogenetically-related wild ruminants. Our objectives were to determine the species of gastrointestinal and bronchopulmonary nematodes present in mouflons coexisting with Spanish red deer (*Cervus elaphus hispanicus*) and fallow-deer (*Dama dama*), and to evaluate their response to netobimin treatment.

MATERIALS AND METHODS

The adult mouflons analyzed came from the El Hosquillo National Wildlife Reserve (Cuenca) in central Spain (2°00′N, 40°30′W), where they coexisted on 400 ha with Spanish red deer (*Cervus elaphus hispanicus*) and fallow-deer (*Dama dama*). In February 1993, ten female mouflons (five 1- to 2-yr-olds and five 3- to 4-yr-olds) were isolated from the other animals and maintained indoors in a sand-floor stable, 200 m^2 in size, and fed a controlled quantity of a complete ration (Ovinanta®, Nanta S. A., Griñon-Madrid, Spain) supplemented with barley grain, barley straw, and dry alfalfa. Samples of rectal feces were taken on day 0, before treatment, and 3 wk after anthelmintic treatment. All animals received an oral dose of 7.5 mg/kg live weight of netobimin (Hapasil®, Schering-Plough, Union, New-Jersey, USA). Anthelmintic efficacy of netobimin was calculated on the basis of the following formulae:

$$\text{Per cent change} = \Big[\{[\text{Number of eggs (or larvae) on day 0}] - [\text{Number of eggs (or larvae) on day 21}]\} \div [\text{Number of eggs (or larvae) on day 0}]\Big] \times 100$$

Per cent cure animals

$$= \left[\{[\text{Number of positive animals on day } 0] - [\text{Number of positive animals on day } 21]\} \div [\text{Number of positive animals on day } 0]\right] \times 100$$

Counts of eggs per gram (EPG) in rectal feces were determined by the McMaster modified method (sodium chloride in saturated solution at density 1.22 as flotation liquid) according to recommendations of the Ministry of Agriculture, Fisheries and Food (1977). The eggs were identified to genus (*Marshallagia* spp. *Strongyloides* sp., *Trichuris* spp., *Capillaria* spp. and *Nematodirus* spp.) or classified as trichostrongylid eggs (Family Trichostrongylidae) (Thienpont et al., 1979). Fecal samples from all infected animals were collectively incubated for 20 days at 25 C and kept moist to identify the trichostrongylids through the specific morphological characteristics of the third-stage larvae (Ministry of Agriculture, Fisheries and Food, 1977). For each determination 100 larvae were observed; whenever more than 300 larvae were found, approximately 30% of all larvae were identified. First-stage larvae of bronchopulmonary nematodes were extracted from 5 to 10 g of feces with a Baermann funnel (left overnight) and then counted and classified as *Dictyocaulus filaria* or protostrongylid first-stage larvae (L1) in McMaster chambers following the Baermann-Wetzel method (Ministry of Agriculture, Fisheries and Food, 1977). When less than five larvae were found in the first 1 ml aliquot from the original 10 ml suspension, the remaining 9 ml were centrifuged, and the sediment pipetted onto slides for counting of all remaining larvae. Protostrongylid first-stage larvae were identified by their size and specific tail morphology (Kotlán, 1960), at 250× or 400× magnification. The identification of all larvae which migrated from 5 to 10 g of feces (or 100 larvae whenever more were detected), once calculated as percentage, determined the species present in each animal. The Mann-Whitney *U*-test was used for statistical analysis of data (Sokal and Rohlf, 1986).

RESULTS

On the pretreatment fecal examinations (day 0), all 10 animals were infected. Mean (±SE) number of trichostrongylid eggs shed before treatment was 230 ± 115 EPG and the species involved were *Trichostrongylus axei, Teladorsagia circumcincta* (syn. *Ostertagia circumcincta*) and *Marshallagia* spp.. Six mouflons (four 1 to 2-yr and two 3 to 4-yr-old) also were shedding *Nematodirus* spp. eggs with intensities ranging from 5 to 100 EPG (mean ± SE, 63 ± 15).

All mouflons were shedding protostrongylid larvae and seven animals also were shedding *D. filaria.* The number of larvae per gram of feces ranged from seven to 271 (mean ± SE, 97 ± 27) for Protostrongylidae and from two to 58 (mean ± SE: 22 ± 3) for *D. filaria.* Protostrongylid larvae output was higher in 3- to 4-yr-old than in 1- to 2-yr-old mouflons (126 ± 43 L1 per gram *versus* 68 ± 25 L1 per gram); however, differences were not statistically significant ($P > 0.1$). *Muellerius capillaris, Protostrongylus rufescens, Cystocaulus ocreatus* and *Neostrongylus linearis* L1, were identified in both age groups. All four protostrongylid genera were identified in 80% of the mouflons. Two 1- to 2-yr-old mouflons had a very low intensity of infection (seven and 13 L1 per gram of feces, respectively) of three lungworm genera. Differences among the percentages of lungworm genera in animals of the two age groups were not statistically significant ($P > 0.1$). The predominant lungworm was *M. capillaris,* with a mean of 53% of total larval observed for the 10 animals and a 100% prevalence. It was followed by *P. rufescens,* with a mean of 32% of all lungworm of the 10 mouflons and a 100% prevalence. Finally, *C. ocreatus* had a mean of 13% of all lungworms and a 90% prevalence. Although *N. linearis* was detected in nine of the animals, it composed a mean of only 2% of all the lungworm larvae among infected animals.

Netobimin was 100% effective against *T. axei, T. circumcincta, Marshallagia* spp., and *D. filaria* infection, whereas one of six animal continued eliminating *Nematodirus* spp. eggs. Netobimin was effective against *Capillaria* spp. but not against *Trichuris* spp.

All mouflons maintained protostrongylid infections after treatment. Larval counts were similar before and after treatment in 3- to 4-yr-old mouflons (mean ± SE, 126 ± 43 and 130 ± 40 L1 per gram, respectively) but were reduced by a mean (±SE) of 85% (±2%) in mouflons less than 3 yr old (mean intensity ± SE, 68 ± 25 and 12 ± 5, respectively). Differences among the intensity of infection after treatment in the two age groups were statistically significant ($P < 0.01$). In both age groups the four lungworm genera were detected after netobimin treatment. Although four animals were free of larvae of *N. linearis* and three of them also of *C. ocreatus*, the most important effect of treatment was against *P. rufescens*. Larvae of this genus were greatly reduced in both age groups. After netobimin treatment two young mouflons were free of larvae of *P. rufescens* and in the other three shedding was reduced by 85% to 99.9%, leading to an average reduction of 93% (Table 1). In older mouflons *P. rufescens* shedding was reduced by 58%. *M. capillaris* was unaffected by netobimin treatment in five 3- to 4-yr-old mouflons but was reduced by 0 to 82% in the 1- to 2-yr-olds (72% of average reduction in young mouflons) (Table 1). Two and four weeks later all mouflons had intensities of protostrongylids similar to those of the initial post-treatment counts and stools remained negative for eggs of gastrointestinal nematodes and larvae of *D. filaria*.

DISCUSSION

We found a high prevalence of both gastrointestinal and bronchopulmonary nematode infections in mouflons involving trichostrongylid species. *Nematodirus* spp., *Dictyocaulus filaria*, and protostrongylid species. These gastrointestinal nematodes detected also were reported frequently in mouflons in Poland (Kozakiewicz and Maszewska, 1984) and in chamois (*Rupicapra rupicapra parva*) in northwestern areas of Spain (Díez-Baños et al., 1987).

In contrast to our observation in mouflon, *D. filaria* infection was not detected in chamois older than 2 yr from a moister

TABLE 1. Effect of netobimin on average number of larvae of protostrongylid nematodes from 10 female mouflons (five 1 to 2-yr-old and five 3 to 4-yr-old) before and after treatment.

Parasite	1 to 2-yr-old				3 to 4-yr-old			
	Before treatment	After treatment	Percent change	Percent cure	Before treatment	After treatment	Percent change	Percent cure
Muellerius capillaris	32.3 ± 12.3[a]	8.9 ± 3.5[a]	(−) 72[b]	0	76.3 ± 28[a]	97.6 ± 32.2[a]	(+) 28[b]	0
Protostrongylus rufescens	12 ± 2.53	0.8 ± 0.5	(−) 93	40	37.6 ± 21.9	15.9 ± 10.9	(−) 58	20
Cystocaulus ocreatus	21.7 ± 12	1.7 ± 1.5	(−) 92	40	8.9 ± 3.7	6.4 ± 3.2	(−) 28	20
Neostrongylus linearis	1.9 ± 0.7	0.2 ± 0.1	(−) 89	50	2.2 ± 0.8	9.7 ± 6.1	(+) 341	40

[a] Mean ± SE larvae per gram of feces based on one sample for each mouflon.
[b] (−), decrease; (+), increase.

area in northwestern Spain (Díez-Baños et al., 1990). The incidence of *D. filaria* decreases while that of protostrongylids increases with age of the host (Gaafar et al., 1985). Based on our results, we believe that protostrongylid incidence could be as high in 1- to 2-yr-old mouflons as in 3- to 4-yr-old animals but the intensity of infection is lower. Differences in protostrongylid intensity related to age also have been reported for other host species. Some authors have found a higher level of larvae in lungs of chamois less than 3 yr old in spite of showing larvae output lower than in older ones (Díez-Baños et al., 1990). Whether the higher intensity of infection in old animals comes from an acumulative parasitism by reinfection (McCraw and Menzies, 1986) or the arrest of larvae in the lungs of younger animals (Díez-Baños et al., 1990) should be investigated.

Muellerius capillaris also is predominant in multiple lungworm infections in mouflons in Poland (Soltysiak and Bartczak, 1991) and in domestic sheep and goats in Spain (Rojo-Vázquez, 1973; Cordero del Campillo et al., 1980). This lungworm species is the only one recovered from dairy-goats in western France (Kulo et al., 1994), and is considered the most common lungworm of sheep and goats. Of interest could be the low output of *Neostrongylus linearis* larvae detected in mouflon in contrast to a report of this parasite in chamois (Díez-Baños et al., 1990); *N. linearis* occurs less frequently in domestic sheep in Spain (Rojo-Vázquez, 1973) and has not been detected in mouflons in different areas of Poland (Kozakiewicz and Maszewska, 1984; Soltysiak and Bartczak, 1991).

As reported for domestic sheep (Sanz et al., 1986; Richards et al., 1987), netobimin given orally at a dose rate of 7.5 mg/kg is highly effective against *Trichostrongylus axei, Teladorsagia circumcincta, Marshallagia* spp., and *D. filaria* infection in mouflon sheep. Our limited results against whipworms and *Nematodirus* are in agreement with those reported in domestic sheep (Richards et al., 1987). These intestinal nematodes and the protostrongylid lungworms are considered the dose-limiting parasites for many anthelmintics in domestic small ruminants. Netobimin did not eliminate protostrongylid infection in mouflon and only *P. rufescens* seemed to be affected by the recommended dose. Cordero del Campillo et al. (1980) reported a reduction of 86 to 92% in parenchymal *M. capillaris* and *P. rufescens* lungworms in sheep treated with 5 mg/kg of albendazole. However, most of the investigations, mainly in *M. capillaris* infection in goats, found poor efficacy of pro- and benzimidazoles against protostrongylid infection at the normal therapeutic dosages for gastrointestinal nematodes (Cabaret, 1991; Kulo et al., 1994).

Based on our results, we believe that gastrointestinal and bronchopulmonary infection in mouflons have a similar response to netobimin treatment as domestic sheep and goats. This pro-albendazole seems to be a useful anthelminthic for trichostrongylid and *D. filaria* infections in mouflon sheep at a dosage of 7.5 mg/kg live weight. However, the poor efficacy of netobimin against protostrongylid infection, highly prevalent in mouflons, limits its application to the integral control of helminths in this ovine species.

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