Bulgarian Journal of Agricultural Science, 28 (No 3) 2022, 521–525

Infestation with protostrongylids of the Balkan chamois (*Rupicapra rupicapra balcanica*, Bolkey 1925) in Rila National Park, Bulgaria: preliminary data

Y. Yankov[1,3*], D. Georgiev[1], P. Iliev[2], A. Tonev[2], S. Arangelov[3], K. Valchev[3], D. Panayotov[1] and S. Peeva[1]

[1]*Trakia University, Faculty of Agriculture, 6000 Stara Zagora, Bulgaria*
[2]*Trakia University, Faculty of Veterinary Medicine, 6000 Stara Zagora, Bulgaria*
[3] *Balkani Wildlife Society, 1000 Sofia, Bulgaria*
[*]*Corresponding author:* qnkoph@abv.bg

Abstract

Yankov, Y., Georgiev, D., Iliev, P., Tonev, A., Arangelov, S., Valchev, K., Panayotov, D. & Peeva, S. (2022). Infestation with protostrongylids of the Balkan chamois (*Rupicapra rupicapra balcanica*, Bolkey, 1925) in Rila National Park, Bulgaria: preliminary data. *Bulg. J. Agric. Sci., 28 (3)*, 521–525

A total of 109 fecal samples were collected from May to September, 2020 in the area of Rila National Park, Bulgaria. The samples were processed by flotation technique using saturated sodium chloride (sp. gr. 1.20), routine sedimentation method, Baermann's method and larval cultivation. In a total of 59 samples (54.12%) representatives of the family Protostrongylidae were found. The larvae found belong to four genera. The predominant infestations were with *Muellerius* spp. presented in 35.78% of the studied samples followed by *Cystocaulus* spp.– in 34.86%, respectively. The genus *Protostrongylus* was present in 7.34% of the samples, and *Neostrongylus* – 3.67%. Research on protostrongylid infestation of the Balkan chamois in the area of Rila National Park was carried out for the first time in Bulgaria.

Keywords: Protostrongylidae; pastures; *Rupicapra rupicapra;* environmental factors

Introduction

The Rila National Park was established in 1992 and nowadays, with its area of 81 046 ha, it is the largest among the three Bulgarian national parks. It is one of the most significant and safe places for preservation of mountain fauna in Europe. There are more than 100 mountain peaks with over 2000 m a.s.l. The highest parts of the mountain represent some of the best habitats for the Balkan chamois (*Rupicapra rupicapra balcanica*, Bolkey, 1925), sheltering one of the subpopulations of the species.

The Balkan chamois is a mountain antelope, perfectly adapted to high-mountain environment. Like other small ruminants, the species is susceptible to protostrongylid infections.

Parasitic diseases and in particular lung nematodes, were reported to contribute considerably the morbidity rate of chamois (Kotrly, 1958; Erhardova-Kotrla & Rysavy, 1967; Dollinger, 1974; Balbo et al., 1975; Homing, 1975; Hugonnet & Euzeby, 1980; Genchi et al., 1984; Svarc, 1984; Bidovec et al., 1985; Cancrini et al., 1985; Nocture, 1986; Sattlerova-Stefancikova, 1987; Diez et al., 1987, 1990; Stefancıkova, 1994; Ciberaj et al., 1997; Panayotova-Pencheva, 2009).

In this paper are presented preliminary data on protostrongylid infestation in chamois inhabiting the Rila National Park, Bulgaria.

Material and Methods

A total of 109 chamois excrements were sampled from May to September 2020 in the area of Rila National Park, Bulgaria, at a minimum required quantity of 30 g per sample. Owing to the conservation status of the study area and its altitude, possible errors of sampling goats' (*Capra hircus*) excrements were excluded. All samples were examined under a microscope to determine the presence of first stage larvae (L_1) of protostrongylids, through a flotation technique using saturated sodium chloride; routine sedimentation method; Baermann's method, and larval cultivation (Zajac & Conboy, 2012; Koinarski et al., 2014).

The infection descriptors correspond to the definitions given by Bush et al. (1997).

In order to find whether there are differences in the occurrence of the two most frequently found parasites genera (*Muellerius* and *Cystocaulus*) in the fecal samples across the months, a Two-Sample T-test for independent variables was applied. To define the strength of the influence of each month on the chamois infestation with protostrongylids, Pearson's Chi-square test was calculated, as well as the Cramer's coefficient using IPM SPSS Statistics ver. 26.

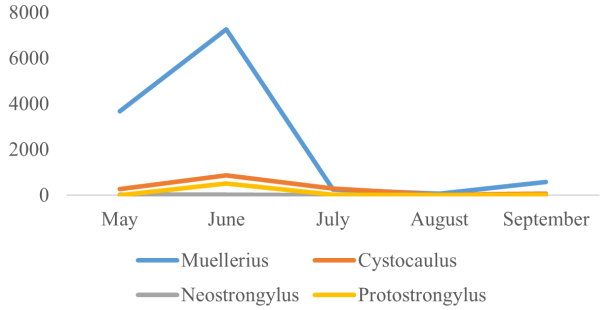

Fig. 1. Monthly distribution of the LPG values of protostrongylids found in chamois' fecal samples in the Rila National Park, Bulgaria in 2020

Table 1. Nematode infection parameters: number of the samples (N); number of infected samples (Ninf); prevalence of L_1 (P%); intensity of infestation(I); mean intensity of infestation (Iav) and mean abundance (MA) of protostrongylid larvae in the chamois' fecal samples, in the area of Rila National Park, Bulgaria, monthly

	Nematode infection parameters					
Genera	N	Ninf	P %	I	Iav	MA
Month	May					
Muellerius	11	9	81.81	19-1410	406.88	332.91
Cystocaulus	11	3	27.27	6-59	88.33	24.09
Neostrongylus	11	1	9.09	6	6	0.55
Protostrongylus	11	0	0	0	0	0
June						
Muellerius	13	10	76.92	16-3798	725.2	557.85
Cystocaulus	13	9	69.23	30-211	86.5	66.54
Neostrongylus	13	1	7.69	15	15	1.15
Protostrongylus	13	3	23.08	52-252	171.67	39.62
July						
Muellerius	40	9	22.50	3-62	25.56	5.75
Cystocaulus	40	17	42.50	1-100	16.53	7.03
Neostrongylus	40	0	0	0	0	0
Protostrongylus	40	2	5.00	2	7.00	0.35
August						
Muellerius	11	4	36.36	10-21	16.75	6.09
Cystocaulus	11	3	27.27	4-5	4.33	1.18
Neostrongylus	11	2	18.18	4-5	4.50	0.82
Protostrongylus	11	1	9.09	2	2	0.18
September						
Muellerius	34	7	20.59	5-217	80.57	16.58
Cystocaulus	34	6	17.65	3-29	60.00	1.76
Neostrongylus	34	0	0	0	0	0
Protostrongylus	34	2	5.88	3-11	7	0.41

Results

The results from the fecal samples analyses revealed a high level of infestation in chamois with protostrongylids in the area of Rila National Park (Figure 1).

The microscopic analyses revealed the L_1 found in the chamois' fecal samples belonged to four genera from the family Protostrongylidae. The predominant infestations were with *Muellerius* spp. present in 35.78% of the samples, and *Cystocaulus* spp. – 34.86%, respectively. *Protostrongylus* spp. were established in 7.34% of the samples, while *Neostrongylus* spp. in 3.67% (Table 1, Figure 2).

Significant differences of LPG values in favor of *Muellerius* in May and August were observed (Table 2). A weak but insignificant predominance of *Cystocaulus* was registered only in July.

The Pearson's Chi square coefficient value of 26.210[a] showed the month most reliably influenced the presence of *Muellerius* spp. in wild goat samples (Table 3) taking into account the moderate influence of the factor (Cramer's V = 0.490). Significant but slight influence of the month is reported on the presence of *Cystocaulus* spp. and *Neostrongylus* spp. The month did not affect the presence of *Protostrongylus* spp. in chamois' fecal samples in the Rila National Park.

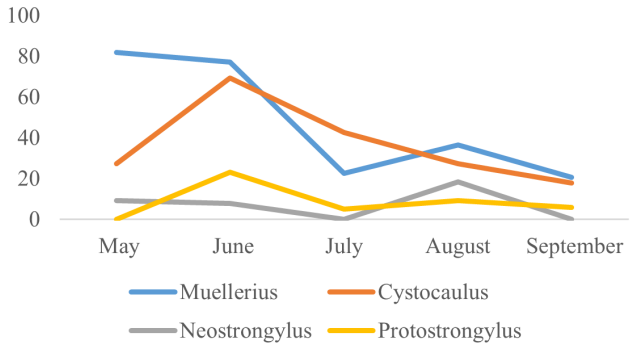

Fig. 2. Prevalence of protostrongylids L1 in the chamois' fecal samples in the area of Rila National Park, Bulgaria in 2020

Discussion

According to our observations, chamois migrates to lower altitudes during the severe winter seasons. As a result, chamois frequently graze on pastures also used by domesticated sheep and goats. It's possible these pastures to serve as a reservoir for the parasites common for chamois and domesticated goats and to be potential places for infestation of intermediate hosts, thus resulting in infestation of the chamois. In our opinion, the most likely reason for the protostrongylids infection observed peak in the beginning of summer was due to the intermediate hosts starting activity. Another reason for the summer peak found in protostrongylids infection in chamois in Rila National Park could be the mobility of L_1 of *Muellerius* spp.

The climate conditions could influence directly the survival of L_1 in the nature (Cabaret, 1984; Samson & Holmes, 1985). At 23-25°C L_1 can survive up to 8 weeks, and at 3-6°C up to 36 weeks, respectively (Rose, 1957). According to Egorov (1960) and Trushin (1973) L_1 of *Muellerius capillaris* are considerably mobile during the humid seasons and could migrate out of the feces in environment, thus easily infecting intermediate hosts.

In the studied area, the *Muellerius* spp. larvae form half or more than half of the number of the protostrongylids lar-

Table 2. „Two Sample T-test" results for the monthly differences in LPG values of the protostrongylids Muellerius and Cystocaulus in the studied chamois' fecal samples in Rila National Park, Bulgaria

Month	N	Mean *Muellerius*	Mean *Cystocaulus*	Sig. (2-tailed)
May	10	366.2000	26.5000	0.048
June	11	659.2727	78.6364	0.107
July	22	10.4545	12.7727	0.735
August	5	13.4000	2.6000	0.045
September	11	51.2727	5.4545	0.095

Table 3. Pearson Chi-Square and Cramer's coefficient values characterizing influence of the month on protostrongylids' presence in the chamois excrements in National Park Rila, Bulgaria

	Pearson Chi- Square	Cramer's V	Sig.
Month * *Muellerius*	26.210[a]	0.490	0.000
Month * *Cystocaulus*	12.785[a]	0.342	0.012
Month * *Neostrongylus*	10.882[a]	0.316	0.028
Month * *Protostrongylus*	6.083[a]	0.236	0.193

vae found. This result corresponds to the data by Cabaret (1984), who reported M. *capillaris* with a higher reproductive capacity than the other protostrongylids in sheep and goats. Additionally, this statement is in accordance with the data from previous studies on nematodes affecting the lungs of sheep and goats in Bulgaria, which reveal M. *capillaris* as a species with the most wide distribution and higher parameters of infestation, compared to other protostrongylids (Zurliyski, 1994).

The decrease in the protostrongylids density during the summer period could be explained with the low survival rate of L_1 under direct sunlight (Rose, 1957). It's notable the LPG values for the *Muellerius* spp. and *Cystocaulus* spp. remain higher than these of the other nematode species. Some studies outline the high survival rate of *M. capillaris* L_1 even after drying when remain infectious (i.e. Solomon et al., 1998 r.). The survival rate of *M. capillaris* larvae is higher than that of *N. linearis* (Cabaret et al., 1991, Morrondo-Pelayo et al., 1992), *C. nigrescens* (Cabaret et al., 1991) and *C. ocreatus* (Reguera-Feo et al., 1986). Reguera-Feo et al. (1986) reported the highest survival capacity of first stage larvae of *M. capillaris*, followed by *C. ocreatus* and *N. linearis*. Species of the latter genus show an overall low distribution and average intensity and can be considered rare in the study area, while species of the genera *Muellerius* and *Cystocaulus* follow that reported by Reguera-Feo et al. (1986) a trend with a proven influence of the month on their occurrence.

The second summer peak found in August was formed of the larvae of two nematode species - *Muellerius* and *Cystocaulus*, as described by their reproduction scale (*M. capillaris* > *N. linearis* > *C. ocreatus* > *Protostrongylus rufescens*, i.e. Cabaret 1984). The total differences in the four nematode species distribution in the fecal samples studied were significant. The order based on their frequency was (*Muellerius* spp.> *Cystocaulus* spp. > *Protostrongylus* spp. > *Neostrongylus* spp.). This possibly reflects the various types of survival and infection at the time of the transfer. Similar tendency was described by Georgiev et al. (2003) in domesticated sheep and goats in Bulgaria.

The lack or poorly defined autumn peak is possibly due to the warm and dry autumn of 2020. Thus additional studies are needed, as the data available covered the beginning of the autumn. About half of the park's territory has not been studied and there is a lack of malacological information about the Skakavishki and Arizmanishki ridges (Hubenov, 2007). At the time when the samples in the studied areas were taken, no land snails were found. If the minimal density of the population of the intermediate hosts explains the summer minimum in the distribution of the protostrongylids populations, then remains the question of what is the reason for the increase in frequency of both nematode species, while the tendency in the rest remains the same.

Conclusions

According to the preliminary data, protostrongylides infestations occur in chamois in the area of Rila National Park, Bulgaria.

Larvae of *Muellerius* spp. were the most frequent among protostrongylids found.

The highest risk of infection is in winter and early spring, when chamois winter habitats are partially overlapped with the grazing pastures of domestic animals.

References

Balbo, T., Costantini, R. & Peracino, V. (1975). Indagini sulla diffusione dei nematodi polmonari nello stambecco *(Capra ibex)* e nel camoscio *(Rupicapra rupicapra)* del Parco Nazionale del Gran Paradiso e della Riserva di Valdieri. *Parasitologia, 17*, 65-68 (It)

Bidovec, A., Valentinic, S. & Kuses, M. (1985) Parasitic pneumonia in chamois *(Rupicapra rupicapra* L.) in Slovenia pp. 240-242 *in* Lovary, S. *(Ed.) The biology and management of mountain ungulates.* London, Croom Helm.

Bush, A. O., Lafferty, K. D., Lotz, J. M. & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *Journal of Parasitology*, *83*, 575.583.

Cabaret, J. (1984). Sheep and goats epidemiology of protostrongylid lungworm infections. *Int. Goats and sheep Res., 2*, 142-152

Cabaret, J., Risye Risyaeni, S. & Baeza, E. (1991). Survival of sheep and goat first stage protostrongylid larvae in experimental condition: influence of humidity and temperature. *Journal of Helminthology*, *65*, 201-207.

Cancrini, G., Iori, A., Rossi, L. & Fico, R. (1985). Occurrence of pulmonary and gastrointestinal nematodes in the Abruzzo chamois. 256–257. In: Lovary S. (*Ed*.) *The Biology and Management of Mountain Ungulates*. London, Croom Helm.

Ciberej, J., Letkova, V. & Kacur, M. (1997). Helminth fauna of alpine chamois (*Rupicapra r. rupicapra*) in the territory of the Slovak Paradise National Park. *Slovensky´ Veterina´rsky C˘asopis, 22*, 301–302 (Slo).

Diez, P., Diez, N., Anton, A. & Morrondo, M. P. (1987). Principales problemas parasitarios del rebeco en la Cordillera Cantabrica. *Adas de las Jornadas de Estudio sobre la Montana.* Edita URZ, asociation para el estudio y proteccion de la naturaleza, 337—350 (Sp).

Diez, P., Diez, N., Morrondo, P. & **Cordero, M.** (1990). Broncho-pulmonary helminths of chamois *(Rupicapra rupicapra parva)* captured in north-west Spain: assessment from first stage larvae in faeces and lungs. *Annales de Parasitologie Humaine et Compare'e, 2,* 74-79.

Dollinger, P. (1974). Contribution to the knowledge of the endopar-

asite faune of chamois *(Rupicapra rupicapra)* in Switzerland. *Zeitschrift fur jagdwissenschschaft. 20*, 115-118.

Egorov, G. (1960). On the biology of the pulmonary helminth parasite *Muellerius capillaris. Trudy Nauchno-Issledovatelskogo Veterinarnogo Instituta,* Minsk, 1, 160 - 170 (Ru).

Erhardova-Kotrla, B. & Rysavy, B. (1967). Species varieties and specificity of lungworms and enterohelminths in domestic and free-living animals in CSSR. *Veterindmi Media'na, 12*, 697-702 (Cz).

Georgiev, D., Kostadinova, A. & Georgiev, B. (2003). Land snails in the transmission of protostrongylids on pastures in Southern Bulgaria: variability of infection levels related to environmental factors. *Acta Parasitologica*, *48(3),* 208–217.

Genchi, C., Manfredi, M. T. & Sioli, C. (1984). Les infestation naturelles des chevres par les strongles pulmonaires en milieu Alpin. *Les maladies de la chevre, Niort (France),* 9 - 11 Octobre, 1984, *INRA 28*, 347-352. (Fr)

Homing, B. (1975). Die Rolle des Parasitenbefals in den Wildbestanden. *Schweizerische Zeitschrift fur Forstwesen 5,* 361-372. (De)

Hubenov, Z. (2007). Distribution and zoogeographical characteristics of mollusks (Mollusca), from Bulgarian national parks. *Historia Naturalis Bulgarica, 18*, 127-159.

Hugonnet, L. & Euzeby, J. (1980). Le parasitisme chez les jeunes chamois de la reserve naturelle des Bauges. *Bulletin de I'Academie Veterinaire de France, 53,* 77-85. (Fr)

Koinarski, V., Ivanov, A., Prelezov, P. & Kirkova, Z. (2014). Guide of Veterinary Parasitology. 2nd edn. *Kontrast,* Bogomilovo, Bulgaria (Bg).

Kotrly, A. (1958). Lungworm fauna of hoofed animals in CSR. *Ceskoslovenska Parazitologie, 2,* 101-110 (Cz).

Morrondo-Pelayo, M. P., Diez-Banos, P. & Cabaret, J. (1992). Influence of desiccation of feces on survival and infectivity of first-stage larvae of Muellerius capillaris and Neostrongylus linearis. *Journal of Helminthology*, *66*, 213-219.

Reguera-Feo, A., Rojo-Vazquez, F. A. & Cordero-Del-Campillo, M. (1986). On first-stage larval resistance under controlled conditions of three species of nematodes of the family Protostrongylidae Leiper, 1926. *Annales de Parasitologie Humaine et Comparée*, *61*, 297-301.

Nocture, M. (1986). Etude de l'infestation des pasturages l'altitude par les strongles des chamois. These Doctor Veterinaire, Lyon, 1-109. (Fr)

Rose, J. H. (1957), Observations on the bionomics of the free-living first stage larvae of the sheep lungworm, *Muellerius capillaris*. *Journal of Helminthology*, *31*, 1728.

Panayotova-Pencheva, M. (2009). Etiological, epizootological and pathomorphological studies on protostrongylidosis in domestic and wild ruminants in Bulgaria. PhD Thesis, Bulgarian Academy of Sciences, Institute of Experimental Pathology and Parasitology, Sofia (Bg).

Samson, J. & Holmes, J. C. (1985). The effect of temperature on rates of development of larval *Protostrongylus* spp. (Nematoda: Metastrongyloidea) from bighorn sheep, *Ovis canadensis canadensis*, in the snail *Vallonia pulchella*. *Canadian Journal of Zoology*, *63*, 1445-1448.

Sattlerova- Štefaničova, A. (1987). Ecological conditions for lungworm infections of chamois in the Tatra National Park. *Bioldgia (Bratislava), 42*, 113-119 (Slo).

Štefaničova, A. (1994). Lung nematodes of chamois in the Low Tatra National Park, Slovakia. *Journal of Helminthology, 68*, 347–351.

Štefaničova, A., Covancova, B., Hajek, B., Dudinak, V. & Šnabel, V. (2011). Revision of chamois infection by lung nematodes under ecological conditions of national parks of Slovakia with respect to ongoing global climate changes. *Helmintholo-gia, 48(3),* 145 – 154.

Svarc, R. (1984). Pulmonary nematodes of the chamois *Rupicapra rupicapra tatrica,* Blahout, 1971.1. Pathomorphological picture of lungs during the development of worms into the adult stage. *Helminthologia, 21*, 141-149.

Trushin, I. N. (1973). The role of ecological factors for the natural infestation of molluscs with larvae of Muellerius. In: Problemy Obshcheiȼ i Prikladnoiȼ Gel'mintologii (Ed. V. G. Gagarin). *Nauka,* Moscow, 344-347 (Ru).

Zajac, A. & Conboy, G. (2012). Veterinary Clinical Parasitology 8th edn. *Blackwell Publishing,* Chichester, West Sussex, UK.

Zurliyski, P. (1994). Study on the intermediate hosts of the family Protostrongylidae (Leiper, 1926). *Veterinarna Sbirka, 1*, 26 - 27 (Bg).

Received: **April, 21, 2022,** *Accepted*: **May, 21, 2022**, *Published*: **June, 2022**