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GASTROINTESTINAL PARASITES AND LUNG WORMS OF WILD RUMINANTS FROM SOUTHWESTERN BULGARIA. II. MOUFLON. BOVIDAE: *OVIS ARIES MUSIMON* (PALLAS, 1811)

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ABSTRACT

A parasitological examination of fecal samples, liver, intestine, and lungs of 36 mouflons was done. The materials were obtained after regular hunting in South-West Bulgaria. The materials are from the DGS "Blagoevgrad" (n = 1), DGS "Mesta" (n = 3), DLS "Dikchan" (n = 31), and DLS "Iskar" (n = 1). In a partial helminthological autopsy of the lungs and examination of fecal samples with Baerman's method, invasion by lung nematodes of the family Protostrongylidae were found. In a partial helminthological autopsy of the liver and intestine, trematodes of the genus *Dicrocoelium*, cestode larvae (*Cysticercus tenuicollis*) and nematodes of the genus *Haemonchus* were isolated. After examination of fecal samples by the method of Fulleborn, eggs of nematodes of the genera *Nematodirus*, *Trichuris*, and *Eimeria* oocysts were found. After morphological examination of cultured larvae, invasions with representatives of the families Trichostrongylidae Leiper, 1912 and Chabertiidae Lichtenfels, 1980 were discovered. Ovoscopically examined fecal samples, after sedimentation showed the presence of eggs belonging to parasites from the genera *Dicrocoelium* and *Paramphistomum*.

Key words: *Ovis aries musimon*, endoparasites.

Introduction

During research on Mouflon's parasites in Europe, a large number of species have been found in territories outside of Bulgaria. Some of them show that in the former Czechoslovakia, mouflons are infected with *Dictyocaulus viviparus*, *Mullerius capillaris*, and *Protostrongylus kochi* (Jansen & van Haaften, 1968). Later, the list of Mouflon's parasites was supplemented by 17 more names of nematodes found after studies conducted in Germany (Boch & Horchner, 1962). These finds have been identified as: *Bunostomum trigonocephalum*, *Capillaria bovis*, *Chabertia ovina*, *Haemonchus contortus*, *Moniezia expansa*, *Muflona podjapolskyi*, *Nematodirus filicollis*, *Oesophagostomum venulosum*, *Ostertagia circumcincta*, *O. ostertagi*, *O. trifurcata*, *Spiculopteragia bohmi*, *Trichostrongylus capricola*, *T. vitrinus*, *Trichuris globulosa*, *Tr. ovis*, *Tr. skrjabini*, and some finds have been identified as members of the genera *Eimeria* spp., *Ostertagia* sp. and *Protostrongylus* sp. Studies in the Netherlands on the same host have found infestations with eight species of nematodes, as follows: *Ostertagia leptospicularis*, *Rinadia mathevossiani*, *Skrjabinagia lasensis*, *Skrjabinema ovis*, *Spiculopteragia spiculoptera*, *Stadelmannia circumcincta*, *S. trifurcate*, and *Trichoformlus colubriformis* (van den Broek & Jansen, 1964). Later, in the Netherlands, in mouflon, there were reported invasions by *Nematodirus roscidus* (Jansen & van Haaften, 1968), *Cooperia onchophora*, and *Cysticercus tenuicollis*-larval stage of *Taenia hydatigena* (van den Broek & Jansen, 1969). Following subsequent studies in Austria (Kutzer, 1971) and the former Czechoslovakia (Vanek et al, 1971) where parasite infestations were discovered, the list of mouflon parasites in Europe was expanded to include *Cooperia pectinate*, *Dicrocoelium dendriticum*, *Neostrongylus linearis*, *Trichostrongylus axei* (Austria), *Cooperia bisonis*, *C. curticei*, *Oesophagostomum columbianum*, and *Trichocephalus ovis* (Former Czechoslovakia).

Observations on wildlife parasites continue to be relevant due to the presence of changes resulting from human activity. Some of them create suitable conditions for the exchange of parasites between domestic and wild animals. Studies on parasites of mouflon continue to be conducted in a number of European countries, including Poland (Balicka et al., 2017; Bartczak et al., 2014) where the presence of numerous gastrointestinal parasite infestations in mouflons is monitored, and Italy (Varcasia et al., 2017), where for the first time in Europe, an invasion of wild ruminants with *Gongylonema nepalensis* and other species of parasites was established.

Invasion of mouflons from four game farms (Devin, Kardzhali, Momchilgrad, and Ropotamo) with lung nematodes from the genera *Muellerius*, *Cystocaulus*, *Protostrongylus*, and *Neostrongylus* was described by Panajotova-Pencheva et al. (2004) in Bulgaria. In farms with different natural and climatic characteristics, and helminth infestations with representatives of 13 genera: *Bunostomum*, *Capillaria*, *Chabertia*, *Cooperia*, *Dicrocoelium*, *Dictyocaulus*, *Gongylonema*, *Haemonchus*, *Muellerius*, *Nematodirus*, *Oesophagostomum*, *Ostertagia*, *Protostrongylus*, *Trichostrongylus*, and *Trichuris* have been found (Todev et al., 2004). The authors have identified six species: *Chabertia ovina*, *Cooperia oncophora*, *Nematodirus spathiger*, *Ostertagia circumcincta*, *O. leptospicularis*, and *Trichuris vitrinus*. Investigating nematodes of the family Dyctiocaulidae in wild ruminants, in mouflons from DLS "Devin" invasions with *Dictyocaulus* sp. (Panayotova-Pencheva et al., 2005), and from DLS "Izvora" and DLS "Balchik"-*Cystocaulus ocreatus, Muellerius capillaris, Neostrongylus linearis, Protostrongylus hobmaieri*, and *Protostrongylus rufescens* have been established (Panayotova-Pencheva, 2006; 2011). Infections with gastrointestinal nematodes of the genera *Haemonchus*, *Ostertagia*, and *Oesophagostomum* have been found in the same host in DLS Devin (Radev et al., 2011). Recent extensive studies on mouflon parasites in Bulgaria have shown the presence of infestations with trematodes of 2 genera: *Dicrocoelium* and *Paramphistomum*; lung nematodes of 4 genera: *Dictyocaulus, Muellerius*, *Neostrongylus*, and *Protostrongylus*; gastrointestinal nematodes-*Bunostomum, Capillaria, Chabertia*, *Gongylonema, Haemonchus, Marshallagia*, *Nematodirus, Oesophagostomum*, *Ostertagia*, *Strongyloides*, *Teladorsagia*, *Trichostrongylus*, and *Trichuris*; and protozoa of the genus *Eimeria* (Dakova, 2020). The author has identified six species: *Gonylonema pulchurum*, *Muellerius capillaris*, *Protostrongylus rufescens*, *Nematodirus abnormalis*, *Teladorsagia circumcincta, T. davtiani*, and *Dicrocoelium dendriticum*.

Remarks: Ostertagia kolchida Popova, 1937 is synonymous with Muflonagia podjapolskyi; Stadelmannia circumcincta and Ostertagia circumcincta are synonymous with Teladorsagia circumcincta (Stadelman, 1894); Stadelmannia trifurcate is synonymous with Teladorsagia trifurcata (Ransom, 1907), and Skrjabinagia lasensis is synonymous with Ostertagia lasensis Asadov, 1953; Protostrongylus kochi, Rinadia mathevossiani, and Trichocephalus ovis are now known as Protostrongylus rufescens (Leuckart, 1865), Spiculopteragia mathevossiani Ruchliadev, 1948, and Trichuris ovis (Abildgaard, 1795), respectively (https://fauna-eu.org/).

The purpose of the research is to obtain up-to-date information on the presence of infestations with gastrointestinal parasites and lung worms in mouflons from Southwestern Bulgaria in order to protect their health and population size.

Materials and methods

A parasitological examination of fecal samples and internal organs of 36 mouflons from Southwestern Bulgaria, collected during the period September 2020–February 2021, was performed. They

were from DGS* "Blagoevgrad" (n = 1), DGS "Mesta" (n = 3), DLS "Dikchan" (n = 31), and DLS "Iskar" (n = 1).

The majority of the mouflons studied, twenty-five, were between the ages of one and three years. A total of nine mouflons, three from each age group, were found-up to one year old, up to three years old, or over seven years old.

For detection of gastrointestinal nematode eggs, the coproovoscopical method of sedimentation, or Fulleborn's flotation method, has been applied. A partial autopsy was performed to detect adult gastrointestinal nematodes, or lungworms. A partial autopsy was performed by examining a specific area or organ. This method has been proposed by Skrjabin and acquired by large numbers of researchers and is often mentioned in many literature sources. Both methods concerning detection of gastrointestinal nematode eggs as well as finding of adult gastrointestinal nematodes or lung worms according to methods described by several authors were done (Kamenov & Radev, 2002; Koinarski et al., 2014).

To demonstrate lung worm larvae, a modified Berman's method was used. The larvae obtained were identified by their morphological characteristics and biological features according to criteria suggested by Kamenov (2016) and Beugnet et al. (2008).

After a cultivation procedure according to Kanchev et al. (2016), gastrointestinal nematode larvae at the 3rd stage were obtained. For identification of 3-rd stage larvae, morphological characteristics and biological features accepted as taxonomically significant by Hubert and Kerboeuf (1984), Anderson (2000), and van Wyk and Mayhew (2013) were used.

Results and discussion

All tested samples of mouflons from Southwestern Bulgaria were positive for invasion by gastrointestinal or lung parasites. In a partial helminthological autopsy of the lungs, infestations with lung nematodes from the families Protostrongylidae (Figures 9, 11) and Dictyocaulidae were established, and after larvoscopical examination of fecal samples, their larvae were found (Figure 7). From the liver, isolated trematodes of the genus *Dicrocoelium* were detected on the omentum, *Cysticercus tenuicollis* (Figure 10) – larvae of *Taenia hydatigena* were detected, and from the small intestine, nematodes of the genus *Haemonchus* were also obtained. After ovoscopical examinations of fecal samples using Fuleborn's flotation method, eggs of nematodes in the genera *Nematodirus* (Figure 2), *Trichuris* (Figure 3), of representatives in the family *Trichostrongylidae* Leiper, 1912 (*Teladorsagia*/*Ostertagia*, Figure 4), of the family *Chabertiidae* Lichtenfels, 1980 (*Oesophagostomum*, Figure 5) and eimeria oocysts were found. After ovoscopic examination of fecal samples by the sedimentation method, eggs of representatives of the genera *Dicrocoelium* (Figure 6) and *Paramphistomum* (Figure 1) were found. Results of morphological examination of cultured larvae confirm the presence of members from the families Trichostrongylidae Leiper, 1912 (*Teladorsagia*/*Ostertagia*) and Chabertiidae Lichtenfels, 1980 (*Oesophagostomum* Figure 8).

A single invasion with *Paramphistomum* sp. was detected in only two mouflons from Satovcha. In all other cases, mixed infestations with more than one type of parasite were found. Predominant mixed invasions with representatives of different species of parasites were observed in mouflon from the same region in which the samples with single invasions were found.

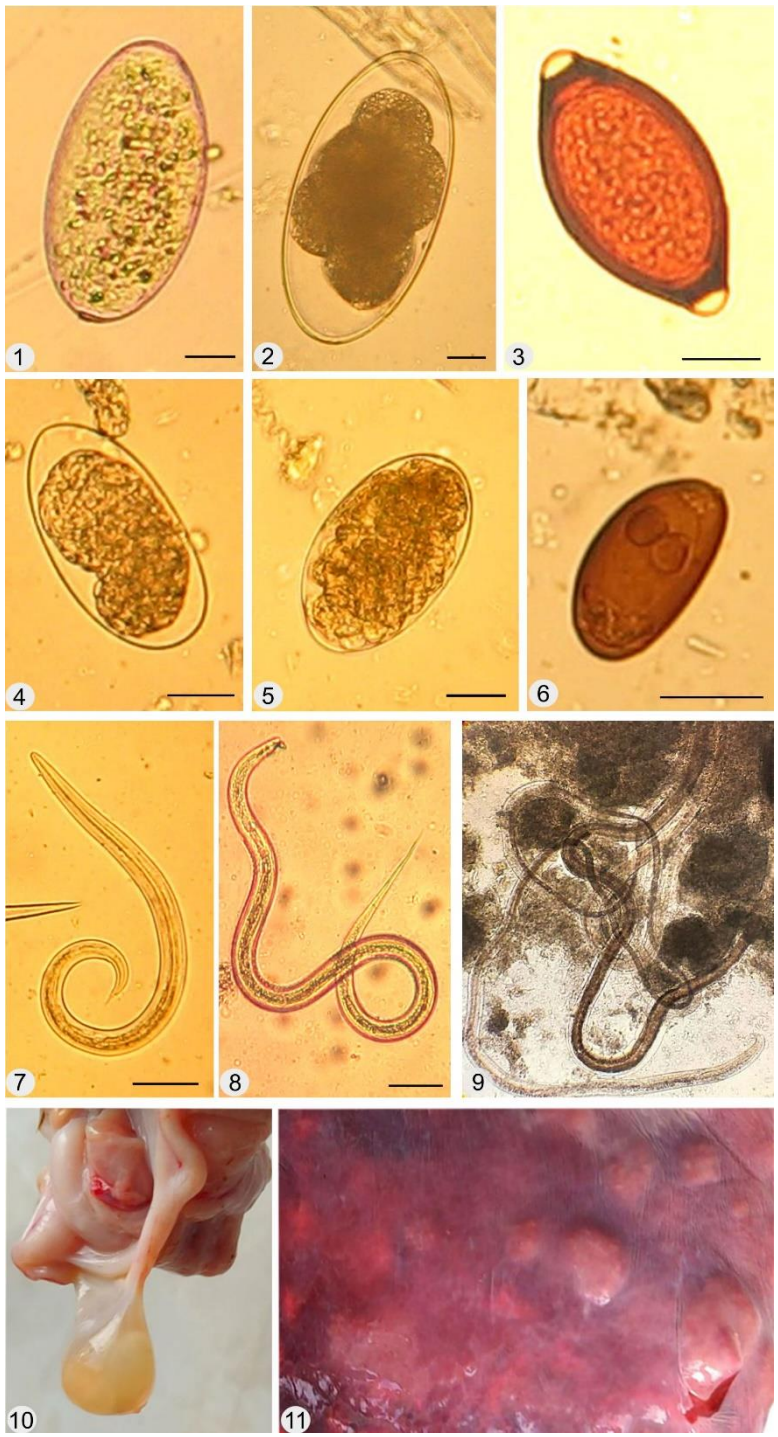

Figure 1-11: Results of ovoscopic, larvoscopic and helmintoscopic examinations of mouflon's samples: 1 – *Paramphistomum* sp., egg; 2 – *Nematodirus* sp., egg; 3 – *Trichuris* sp., egg; 4 – Trichostrongylidae, *Teladorsagia* egg; 5 – Chabertiidae, *Oesophagostomum* egg; 6 – *Dicrocoelium* sp., egg; 7 – Protostrongylidae, larva; 8 – Dictyocaulidae, larva; 9 –Protostrongylidae, adults; 10 – *Cysticercus tenuicollis*, larva of *Taenia hydatigena*; 11 – Changes in the lung surface with larvae of protostrongylids and adults included.

The largest part of the positive results are the invasions by gastrointestinal nematodes (38.75%) (Figure 12). The morphology of the findings in ovoscopic, helminthoscopic, and larvoscopic examinations showed the presence of nematodes in 7 genera. The highest share of gastrointestinal nematodes has representatives of the genus *Chabertia* (28%), with an extent of invasion (EI) of 38.9%, followed by those of *Trichostrongylus* (18%), EI 25%, *Trichuris* and *Oesophagostomum* with an equal share of 14%, and EI 19.44% each. The share of infestations with representatives of the genera *Haemonchus* was lower (10%), with EI 13.9%, *Nematodirus* and *Teladorsagia*/*Ostertagia* with an equal relative share of 8% and EI-11.10% each (Figure 13, Table 1).

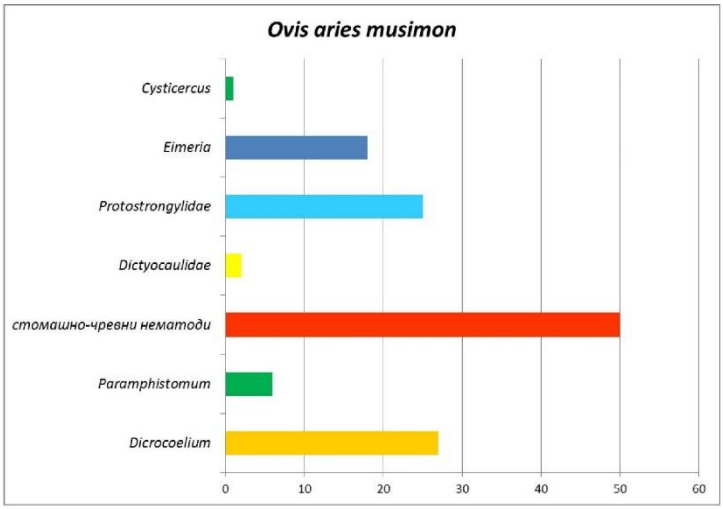

Figure 12: Relationship between gastrointestinal and lung parasites numbers from different systematic groups found in mouflons from southwestern Bulgaria.

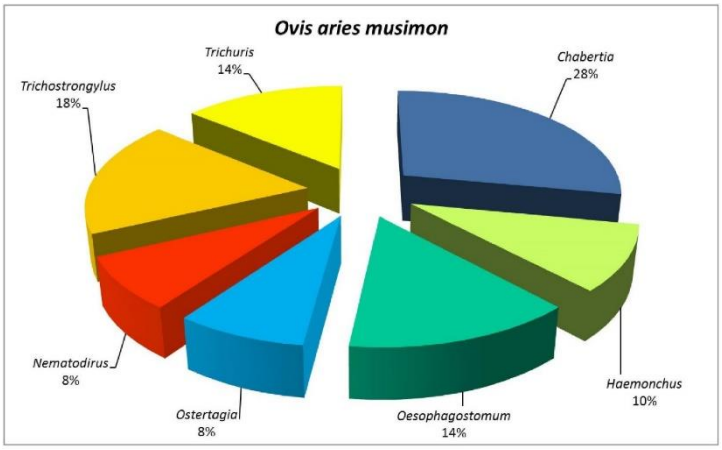

Figure 13: Percent correlation between gastrointestinal nematodes detected in *Ovis aries musimon* from southwestern Bulgaria.

Infections with trematodes represent 25.58% of the total number of positive results. Of these, 20.93% were positive for invasion with members of the genus *Dicrocoelium*, EI 75%, and 4.65% with members of the genus *Paramphistomum*, EI 16.66% (Figures 12, Table 1).

Table 1: Percentage ratio between the total number of positive findings and the established invasions. Extent of invasion

Kind of infestation	European mouflon (*Ovis aries musimon*)		
	Positive results – number	Percentage of the total positive results number	Invasion extent (%)
Gastrointestinal nematodes			
Chabertia	14	10.85	38.9
Haemonchus	5	3.88	13.9
Nematodirus	4	3.1	11.1
Oesophagostomum	7	5.43	19.44
Teladorsagia/Ostertagia	4	3.1	11.1
Trichostrongyus	9	6.98	25
Trichuris	7	5.43	19.44
Total	50	38.75	
Trematoda			
Dicrocoelium	27	20.93	75
Paramphistomum	6	4.65	16.66
Cestoda			
Cisticercus tenuicollis	1	0.77	2.78
Lung worms			
Dictyocaulidae	2	1.55	5.56
Protostrongylidae	25	19.38	69.44
Protozoa			
Eimeria	18	13.95	50

Infestations with pulmonary nematodes account for 20.93% of the total positive results. Cases of invasion with representatives of the family Protostrongylidae (29.38%) and EI 69.44% were found more often. The positive results for invasion with members of the family Dictyocaulidae are less and represent 1.55% of the total number of positive results, 5.56% (Figures 12, Table 1). The presence of invasions by eimerial protozoans accounted for 13.95% of the total number of positive results, with an EI of 50% (Figures 12, Table 1).

Conclusion

The results obtained are in agreement with those obtained in other studies (Dakova, 2020; Panayotova-Pencheva, 2006, 2011; Panajotova-Pencheva et al., 2004, 2005; Todev et al., 2004) on mouflon's helminth fauna. All age groups of mouflons investigated have gastrointestinal or pulmonary nematodes. Young animals were shown to have more trematodes and cestodes than adult animals. Eimeria oocysts were identified in greater quantities in younger mouflons, but only in a few in adults. The established parasitic infestations in mouflon from Southwestern Bulgaria, as well as the results of similar studies in the same or other regions of our country, show that as a final host, he is an active participant in parasite-host relationships. This requires the parasitological status of mouflons to be systematically monitored. An aim, which could be achieved through periodic examination of fecal samples after the end of each season, as well as mandatory examination of materials from internal organs (lung, liver, rennet, and colon) of found dead animals, or after hunting. It would be appropriate to systematically carry out therapeutic treatments depending on the results obtained concerning the parasitological status of the population.

*Abbreviation is according http://www.iag.bg/struct/lang/2/type/R/id/17/unit_single

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