New records of protostrongylid lungworms from wild ruminants in Bulgaria

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ABSTRACT: A necropsy of lungs from mouflons, chamois, red deer and fallow deer from Bulgaria was performed aiming at determining the species composition of protostrongylids. For the first time in the country *Muellerius capillaris, Cystocaulus ocreatus, Neostrongylus linearis, Protostrongylus rufescens, P. hobmaieri* are reported as part of the helminth fauna in the mouflon, *M. capillaris, N. linearis* and *P. rupicaprae* are reported from the chamois, and *Varestrongylus sagittatus* is reported from the red deer. This is the first record of *P. hobmaieri, P. rupicaprae* and *V. sagittatus* for the fauna of Bulgaria. Some morphological data of males and first stage larvae of *P. hobmaieri* on materials from the present study are presented.

Keywords: *Protostrongylus hobmaieri*; *Protostrongylus rupicaprae*; *Varestrongylus sagittatus*; first records; Bulgaria

According to the National Forestry Management, 95 800 wild ruminants inhabit the territory of Bulgaria. These animals are mainly located in State Wildlife-Breeding Stations installed in ecologically suitable geographic regions. Due to their way of life in a comparatively restricted area, the wild ruminants are infected by and are transitory recipients of a great number of helminths. The parasitoses cause a delay in fur shedding, a lowered fecundity and loss of weight, poor formation of the trophy parts and a low resistance to infectious diseases and deficient alimentation. Lung strongylatoses including protostrongylidoses represent some of the most wide-spread parasitic diseases in wild ruminants. The knowledge of the etiology of these disorders is not only of fundamental importance, but is also a decisive factor of their correct prevention and treatment. The data on the species composition of protostrongylids in wild ruminants in Bulgaria are scarce. Svilenov et al. (1985) have reported a case of infection of roe deer with *Muellerius capillaris*. In trials for the treatment of strongylatoses in game, Moutafov et al. (1989) have marked *M. capillaris* and *Cystocaulus* sp. as agents of lung helminthosis, solely based on larval diagnostics. In our previous studies, parasitosis of chamois with *M. tenuispiculatus* was reported (Panayotova-Pencheva and Mutafova, 2005). Due to the above-mentioned reasons, the purpose of the present study was to obtain more data about the species composition of protostrongylids in the country, based on a helminthoscopic diagnosis.

MATERIAL AND METHODS

Helminthological necropsy of the lungs of 28 wild ruminants from different regions of the country was carried out during the period from 2003 to 2006. Eleven of the lungs were from mouflons (*Ovis musimon* L.), 10 from chamois (*Rupicapra rupicapra* L.), 4 from fallow deer (*Dama dama* L.) and 3 from red deer (*Cervus elaphus* L.).

The animals come from the territories of wildlife-breeding stations situated and fenced in hunting regions comprising habitats typical of these spe-

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cies. Three of the stations are situated in the Balkan Mountains ("Vitinia", "Svoge", "Mediket"), one is situated in the Rhodope Mountains ("Izvora") and one is located near the Black Sea ("Balchik").

The lungs under study were taken mainly from male animals. Only two of the chamois were female. The age of the animals varied from 1 to 11 years.

The adult helminths found in the tracheas and bronchi during the necropsy of the lungs were collected in Barbagalla solution. For detection of protostrongylids located in the small bronchi and bronchioles, lung tissue parts were observed by compression following their boiling in lactic acid in a water bath (Panayotova-Pencheva and Mutafova, 2005). The Baermann technique was used for establishing the first stage protostrongylid larvae (L1). The species of nematods were identified according to the data in Boev's (1975) monograph.

The morphological structures of the helminths under study were measured using the classic methods of parasitology. All measurements are in micrometers. Drawings were made using a light microscope "Opton", supplied with a camera "Lucida" and pictures were taken using a light microscope "Amplival", supplied with a web camera. The adult specimens were deposited in the collection of the Institute of Experimental Pathology and Parasitology, at the Bulgarian Academy of Sciences in Sofia, Bulgaria.

RESULTS

The results from the necropsy of the lungs are presented in Table 1. They show that eight protostriongylid species have been found. The most common species was *M. capillaris*, which was detected in 75 percent of the parasitized lungs. The rest of the species are ranked according to the degree of infection in animal populations as follows: *N. linearis* (50%), *M. tenuispiculatus* and *P. rufescens* (12.5%) and finally *C. ocreatus*, *P. hobmaieri, P. rupicaprae* and *V. sagittatus* (6.25%) each. More than half of the parasitized lungs had multispecies infections (56.25%). No protostrongylid infections were found in fallow deer. Obviously this was due to their young age.

M. capillaris and *N. linearis* have been established in mouflons and chamois, *M. tenuispiculatus* and *P. rupicaprae* in chamois, *P. rufescens* and *C. ocreatus* in mouflons, *V. sagittatus* in red deer. It is evident that these species have been found in their typical hosts.

The lungworms listed above are distributed in different wild ruminants in different European countries: Austria, the Czech Republic, Slovenia, Germany, Italy, Switzerland, Poland, Slovak Republic, Spain, France and Hungary (Kutzer and Hinaidy, 1969; Dyk and Chroust, 1973; Brglez et al., 1974; Balbo et al., 1975; Polley and Hoerning, 1977; Schutze, 1978; Rezac, 1990; Soltysiak and Bartczak, 1991; Stefancikova, 1994; Meana et al., 1996; Lamka et al., 1997; Nocture et al., 1998; Takacs, 2003 and others).

P. hobmaieri however, is recorded mainly on the Asian continent, in domestic ruminants (Asadov, 1960; Azimov, 1962; Wu et al., 1963; Gubaidulin, 1964) and more rarely in wild ruminants (in argali mouflons in Turkmenistan – Kibakin et al., 1964, in Siberian goats in Mongolia – Tazieva et al., 1981). In Europe, *P. hobmaieri* has been found in domestic sheep in Moldova (Zgardan et al., 1966) and in chamois in Italy (Balbo et al., 1975).

By reason of the extremely rare registration of this species in the territory of Europe, and due also to the fact that it was detected in a mouflon and not in his typical host – domestic sheep – we supply brief morphological data on the tail end of males and first stage larvae yielded by the present study.

The sexual bourse of the specimens of *P. hobmaieri* is typical of the species (Figure 1, Figure 2). The dorsal rib is hemispheric in shape. The outer-dorsal ribs are free and do not reach the edge of the bourse. The lateral ribs stem from a common source. The posterior and middle-lateral ribs are confluent in their stem and reach the edge of the bourse, and

Figure 1. Sexual bourse of *P. hobmaieri* (dorsal view)

Table 1. Protostrongylid findings in the lungs of wild ruminants (Bulgaria)

Host	Sex of the host	Age of the host	Origin of the host	L1 obtained by Baermann teqnique	Adult helminths in the bronchi	Adult helminths obtained by compression
Mouflon	male	7	Izvora	Muellerius Protostrongylus	*P. rufescens* *P. hobmaieri*	*M. capillaris* *P. hobmaieri*
Mouflon	male	9	Izvora	Muellerius	negative	*M. capillaris*
Mouflon	male	6	Izvora	negative	negative	negative
Mouflon	male	6	Izvora	Muellerius	negative	*M. capillaris*
Mouflon	male	9	Izvora	Muellerius Neostrongylus	negative	*M. capillaris* *N. linearis*
Mouflon	male	7	Izvora	negative	negative	negative
Mouflon	male	7	Balchik	Muellerius Neostrongylus Protostrongylus	*P. rufescens*	*M. capillaris* *N. linearis* *C. ocreatus*
Mouflon	male	1	Izvora	Muellerius	negative	*M. capillaris*
Mouflon	male	6	Izvora	Muellerius Neostrongylus	negative	*M. capillaris* *N. linearis*
Mouflon	male	6	Izvora	Muellerius Neostrongylus	negative	*M. capillaris* *N. linearis*
Mouflon	male	4	Mediket	Muellerius	negative	*M. capillaris*
Chamois	female	7	Izvora	Muellerius Neostrongylus	negative	*M. capillari* *M. tenuispiculatus* *N. linearis*
Chamois	male	3	Izvora	Muellerius Neostrongylus	negative	*M. tenuispiculatus* *N. linearis*
Chamois	male	3	Izvora	negative	negative	negative
Chamois	male	6	Izvora	Muellerius	negative	*M. capillaris*
Chamois	male	6	Izvora	negative	negative	negative
Chamois	male	11	Izvora	negative	negative	negative
Chamois	male	6	Izvora	Muellerius	negative	*M. capillaris*
Chamois	female	9	Izvora	negative	negative	negative
Chamois	male	4	Izvora	Neostrongylus	*P. rupicaprae*	*N. linearis*
Chamois	male	6	Izvora	Muellerius Neostrongylus	negative	*M. tenuispiculatus* *N. linearis*
Red deer	male	4	Svoge	negative	negative	negative
Red deer	male	1	Vitinia	Elaphostrongylus	negative	negative
Red deer	male	8	Vitinia	Elaphostrongylus Varestrongylus	negative	*V. sagittatus*
Fallow deer	male	1	Balchik	negative	negative	negative
Fallow deer	male	1	Balchik	negative	negative	negative
Fallow deer	male	1	Balchik	negative	negative	negative
Fallow deer	male	1	Balchik	negative	negative	negative

Figure 2. Sexual bourse of *P. hobmaieri* (lateral view)

Table 2. Measurements (in µm) of six male specimens of *P. hobmaieri* from a mouflon (*Ovis musimon* L.) in Bulgaria

Specimen No.	1	2	3	4	5	6	Min.–Max.	Average
Body length	20 000	17 000	20 000	20 000	–	18 000	17 000–20 000	19 000
Maximum body width	118.58	–	135.52	104.72	146.3	115.2	104.72–146.3	124.06
Body width before the spicules	110.78	106.28	135.52	104.72	126.28	111.36	104.72–135.52	115.82
Spicule length	280.32	253.4	233.44	257.12	241.92	249.6	233.44–280.32	252.64
Gubernaculum length	111.36	120.12	100.1	118.58	121.66	92.4	92.4–21.66	110.7
Length of crura of gubernaculum	53.9	67.76	72.38	66.22	69.3	53.9	53.9–72.38	63.91
Length of ears of gubernaculum	46.2	–	43.12	47.74	43.12	46.2	43.12–47.74	45.28
Maximum distance between the ears	40.04	–	46.2	46.2	30.8	41.58	30.8–46.2	40.96

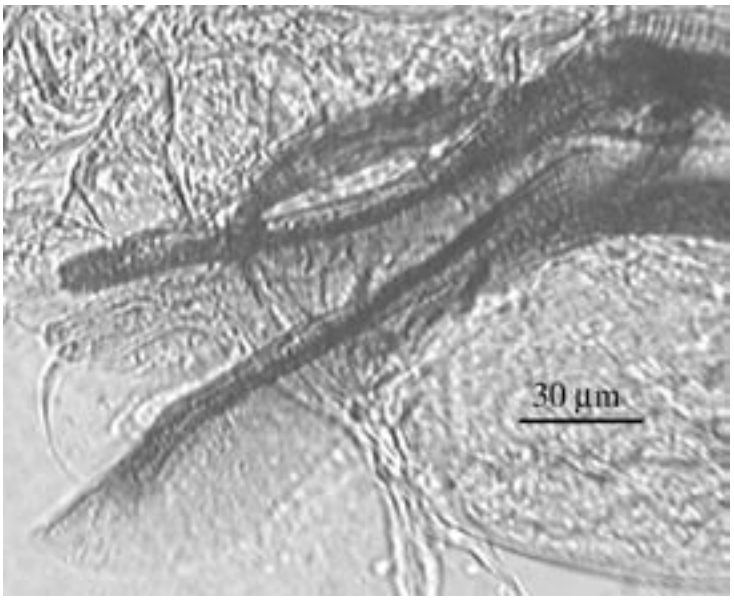

Figure 3. End of the spicules of *P. hobmaieri*

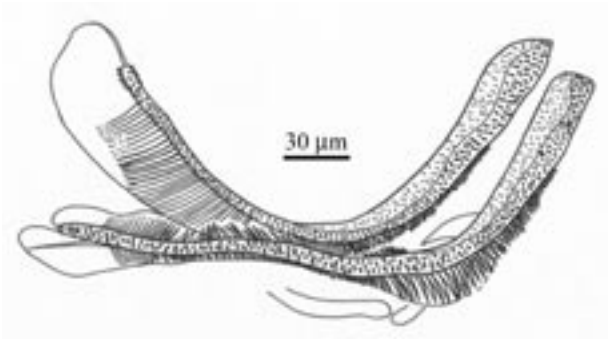

Figure 4. Spicules of *P. hobmaieri* (drawing)

the anterior lateral ribs do not extend to the edge of the bourse. The ventral ribs are directed to the anterior and fused at their stemming. The spicules consist of a spongiform trunk and comb-like wings. At their beginning the wings are narrower and gradually widen. Towards the distal end of the spicules, the comb-shape becomes ever less noticeable and disappears at the end, while the wings end in a transparent rounded membrane (Figure 3, Figure 4). The gubernaculum has a caput, corpus

Table 3. Measurements (in µm) of ten first stage larvae of *P. hobmaieri* from a mouflon (*Ovis musimon* L.) in Bulgaria

No.	Body length	Maximum body width	Oesophagus length	AE-IFG	AE-EO	PE-A	Tail length
1	318.72	20.02	134.4	184.8	89.32	47.74	23.04
2	299.52	20.02	117.04	184.8	84.7	46.2	18.48
3	299.52	17.36	115.2	184.32	72.96	42.24	19.2
4	311.04	19.2	134.4	222.72	80.64	46.08	19.2
5	307.2	19.2	134.4	211.2	80.64	42.24	23.04
6	318.72	18.48	141.68	184.8	80.08	46.2	20.02
7	318.72	18.48	138.24	207.9	84.48	46.08	19.2
8	307.2	18.48	134.4	192	88.32	43.12	18.48
9	326.4	18.48	134.4	188.16	88.32	42.24	19.2
10	307.2	20.02	122.88	195.84	88.32	47.74	18.48
Min.–Max.	299.52–326.4	17.36–20.02	115.2–141.68	184.8–211.2	72.96–89.32	42.24–47.74	18.48–23.04
Average	311.42	19	130.7	195.6	83.8	45	19.8

AE-IFG – distance between anterior end and the initial formation of genitals
AE-EO – distance between anterior end and excretory outlet
PE-A – distance between posterior end and anus

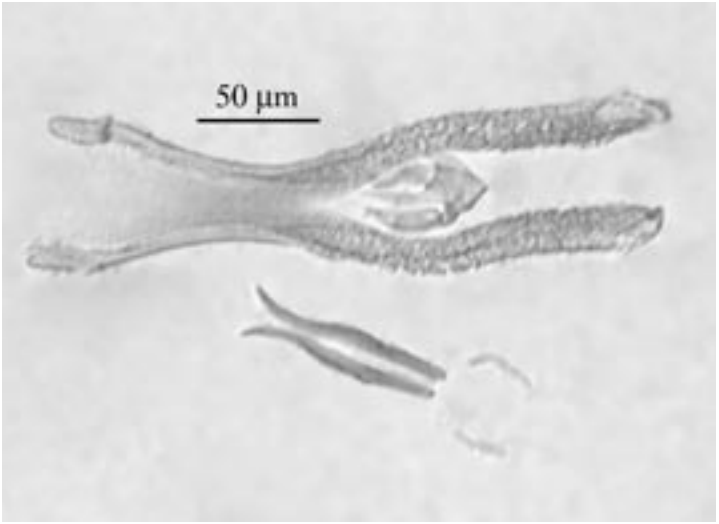

Figure 5. Spicules and gubernaculum of *P. hobmaieri* (under compression)

Figure 6. Gubernaculum of *P. hobmaieri*

and crura. It is bright brown with a paler corpus (Figure 5). The caput has the shape of an avian thoracal bone (Figure 5), its middle is attached to the corpus, and the ears are pointed and dorsally-caudally directed (Figure 6). The crura are ventrally bent, smooth, without teeth and shaped like sledge runners (Figure 5, Figure 6). The metric data about these structures are presented in Table 2.

First stage larvae are illustrated in Figure 7. The metric data about them are shown in Table 3.

DISCUSSION

Comparing our results concerning the spreading of species with those reported in the local literature

Table 4. Measurements (in µm) of male *P. hobmaieri* – comparative data

Authors	Host	Body length	Maximum body width	Spicule length	Gubernaculum length	Length of crura of gubernaculum	Maximum distance between the ears
Boev (1975)	Sheep (*Ovis ammon f. domestica* L.)	16 800–38 400	76–207	208–258	102–152	44–81	45–48
Boev (1975)	Argali mouflon (*Ovis ammon* L.)	–	–	253–257	123–158	–	–
Our data	Mouflon (*Ovis musimon* L.)	17 000–20 000	104.72–146.3	233.44 –280.32	92.4 –121.66	53.9 – 72.38	30.8 – 46.2

Table 5. Measurements (in µm) of first stage larvae of *P. hobmaieri* – comparative data

Authors	Body length	Maximum body width	Oesophagus length	AE-IFG	AE-EO	PE-A
Davtian (cit. Boev, 1975)	260–320	15–18	110–140	170–190	65–95	40–50
Our average data	311.42	19	130.7	195.6	83.8	45

AE-IFG – distance between anterior end and the initial formation of genitals
AE-EO – distance between anterior end and excretory outlet
PE-A – distance between posterior end and anus

Figure 7. First stage larva of *P. hobmaieri* (drawing)

and abroad, it is obvious that the lungworm species *Cystocaulus ocreatus, Neostrongylus linearis, Protostrongylus rufescens, P. rupicaprae, P. hobmaieri* and *Varestrongylus sagittatus* have been recorded here for the first time as part of the helminth fauna of Bulgarian wild ruminants, and *P. rupicaprae, P. hobmaieri* and *V. sagittatus* have been recorded for the first time for the Bulgarian fauna.

The morphological characteristics of the male specimens of *P. hobmaieri* are identical with those indicated by Boev (1975) in the materials from domestic sheep (*Ovis ammon f. domestica* L.) and argali mouflon (*Ovis ammon* L.) from Kazakhstan. There are no significant differences (Table 4) when our metric data are compared with those indicated by Boev either. The same seems true about the morphometric features of L1, after comparison of our data with those obtained in materials from domestic sheep in Armenia (Table 5). The maximum width of the body is the only exception. Our average value was one micrometer higher than the maximum value indicated by Davtian (cited by Boev, 1975).

These facts indicate that regardless of the remote geographic habitats and different hosts of the populations, the lungworm *P. hobmaieri* does not undergo population variability.

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