## **Review article**

# **Biodiversity of lung helminths in terrestrial mammals** from Eastern Europe

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**ABSTRACT.** This review provides the results of many years of original studies of the authors published in different journals and compilations taking into account an analysis of literature data. The studies on biological diversity of lung helminths of terrestrial mammals were performed by the authors in Armenia, Bulgaria and Poland. Literature data on European Russia were also taken into account. Sixteen animal species from the families Cervidae (six species), Bovidae (nine species) and Leporidae (one species) were investigated. In these animal taxons 27 helminth species from the families Dictyocaulidae (five species), Protostrongylidae (20 species), and Taeniidae (two species) have been found. Lung nematodes *Muellerius capillaris* and *Protostrongylus hobmaieri*, as well as zoonotic parasite *E. granulosus* were found to be common for all the countries studied. Also, relatively many species appeared common between several studied regions. Taking into account morphology, ecology and life cycles, we support a validity of genera *Echinococcus* and *Alveococcus* in the structure of the subfamily Echinococcinae of family Taeniidae.

Keywords: lung helminths, terrestrial mammals, Armenia, European Russia, Bulgaria, Poland

#### Introduction

This article's goal is to summarize results of many years of studies of its authors performed in the framework of international cooperation between Academy Institutes of Armenia, Bulgaria, Poland and Russia. It was of a significant interest to explore a host character of formation of biodiversity of helminth fauna in these divers regions. In this respect Armenia is characterized by presence of sharply pronounced vertical landscape zones and continental climate. The relief of Bulgaria is strongly vertically divided, with lowlands, plains and hilly lands predominating. The southern border parts of the country belong to the subtropical climate zone, and the remaining larger part is in the temperate one. Poland is characterized by lowland landscape and temperate, warm, intermediate climate. It is well known that Russia is characterized by broad diversity of both landscape and climate, with predominately plain landscape throughout most part of Eastern European Plain giving way to mountains at its South and East borders, with climate being predominately temperate/boreal in the parts studied.

#### **Biodiversity of lung helminths in Armenia, European Russia, Bulgaria and Poland**

The helminth material was collected from 16 terrestrial mammal species in Armenia, European

Russia, Bulgaria and Poland (Tab. 1, 2). Table 1 shows there were six host species studied in Armenia, 13 - in Russia, seven host species - in Bulgaria and 10 - in Poland. Numbers of host species studied was six for Cervidae, nine - for Bovidae and one - for Leporidae.

species of family Dictyocaulidae Five nematodes have been found in the regions studied: Dictyocaulus filaria, D. viviparus, D. capreolus, D. eckerti and D. cervi [1-16]. Two of these species were reported from Armenia: D. filaria and D. viviparus [1,2]. In conditions of pronounced vertical belts (800-2000 m above sea level) and the pronounced continental climate of Armenia Dictyocaulus nematodes were found everywhere. The prevalence of Dictyocaulus spp. in conditions of mountain and foothill climate belts (height above sea level 1400-2000 m, yearly rainfall 400-560 mm, median yearly temperature 4-10°C) was 42.0-57.0% in lambs and 35.0-45.0% in sheep above 2 years old, while in highlands climate and landscape belts (height above sea level 800-860 m, yearly rainfall 244 mm, median temperature 11.3°C) the extensity of infection level was significantly lower - at 23.0-37.0% in lambs and 18.0-24.0% in adult sheep. The average parasite load was 64.3 (37–90) nematodes per animal in all studied climate conditions [1,2]. Previously D. filaria was also registered in mouflons here [3].

In Russia, D. filaria and D. viviparus are also broadly distributed throughout its European territories. Here a nematode D. capreolus was also registered from wild ruminants Alces alces, Cervus dama and C. nippon. According to [4-6], wild ruminants from European part of Russia carry D. viviparus, D. filaria and D. eckerti. Excepting Bison bonasus and Bos taurus with a single nematode species D. viviparus, all other hosts were found to be infected with mixes of several nematode species. Samojlovskaya [4] shows some preference between host and lung nematode species: in Alces alces -D. filaria, in Cervus nippon – D. viviparus. Interesting data on biodiversity of lung nematodes in Russian Federation were given by Gadaev [5,6] for Chechnya Republic and Krotenkov for Western Region of Russia [7]. They note a broad distribution of D. filaria and D. eckerti in domestic animals and wild fauna throughout all latitude zones of Russian Federation. E.g., the following parasite species were registered: in West Caucasian tur -D. filaria with extensity of infection (prevalence)  $(E_i) = 5.9\%$  and intensity of infection  $(I_i) = 3-41$  specimens, D.

*eckerti* ( $E_i = 5.9\%$ ,  $I_i = 1-17$ ); in Bezoar goats – *D. filaria* ( $E_i = 14.9\%$ ,  $I_i = 1.32$ ), in roe deer – *D. filaria* ( $E_i = 9.5\%$ ,  $I_i = 3-31$ ), *D. eckerti* ( $E_i = 4.8\%$ ,  $I_i = 1-14$ ), *D. railieti* ( $E_i = 19.0\%$ ,  $I_i = 9-34$ ) [5,6]. The recent study [8] from nearby Terek-Sulak lowland (Dagestan Republic, Russian Federation) also shows a presence of *D. filaria* in goats with higher prevalence (12.9%) at semi-desert ecosystems, though generally considering this species to be of lower importance than some other parasites of goats there.

Distribution of the dictyocaulids in wild ruminants from 7 mountain and coastal regions of Bulgaria was studied by Panayotova-Pencheva et al. [9,10]. *Dictyocaulus* sp. was diagnosed in mouflons from the Rodope Mountains with prevalence 41.4% and *D. eckerti* were found by necropsy of a red deer from the region of Svoge in West Balkan Mountains. In general, however, decreased distribution of dictiocaulids was noted compared to the data on the country available for the previous twenty-five years. In a subsequent study *D. eckerti* was reported in red deer from the Rila Mountain and from the Balkan Mountains [10].

In Poland, *D. viviparus* has been found in 94.4% of European bisons, and *D. eckerti* in 21% of red deer studied in Białowieża Forest Biosphere Reserve [11,12]. In addition, *D. cervi* was found in deer and elks of Poland, *D. capreolus* – in roe deer and elks and *D. filaria* – in sheep [13]. A new species from deer and elks – *D. cervi* have been recently described with molecular evidence in Poland [13,14].

The lung helminths from family Protostrongylidae have been found in all the regions studied. There were 20 Protostrongylidae species altogether. In Armenia 8 Protostrongylidae species have been found, in Russia – 19, in Bulgaria – 12 and in Poland – 14. There were only two species common for all the regions: *Muellerius capillaris* and *Protostrongylus hobmaieri*, while eight species were found to be common for European Russia, Bulgaria and Poland (Tab. 1, 2).

Data on protostrongylids of ruminants in Armenia were summarized by Movsesyan et al. [15]. There were six species (*Cystocaulus ocreatus*, *M. capillaris*, *Protostrongylus davtiani*, *P. hobmaieri*, *P. muraschkinzewi and P. rufescens*) reported in this region. Studies by Gadaev [5] on Chechnya Republic and Krotenkov [7] on Western Region of Russia note that domestic animals and wild fauna in European Russia are commonly

Host species	Armenia	Russia* +	Bulgaria +	Poland	Nematode species D. eckerti, D. filaria, D. viviparus, V. sagittatus			
Dama dama	_			+				
Cervus nippon	_	+	_	_	D. filaria, D. viviparus, E. cervi, M. capillaris, V. sagittatus			
C. elaphus	-	+	+	+	D. cervi, D eckerti, D. filaria, D. viviparus, E. cervi, V. sagittatus			
C. elaphus sibiricus	_	+	_	_	E. cervi, V. capreoli, V. sagittatus			
Capreolus capreolus Alces alces	_	+ +	_	+ +	C. ocreatus, C. vsevolodovi, D. capreolus, D. eckerti, D. filaria, D. railieti, D. viviparus, M. capillaris, P. davtiani, P. hobmaieri, P. rufescens, P. skrjabini, V. capreoli D. capreolus, D. cervi, D. filaria, D. viviparus, E. alces, E. cervi, M. capillaris, V. alces, V. capreoli			
Bison bonasus	_	_	_	+	D. viviparus			
Bos taurus	+	+	_	+	C. ocreatus, C. vsevolodovi, D. eckerti, D. filaria, D. viviparus, M. capillaris, N. linearis, P. davtiani, P. hobmaieri, P. raileiti			
Rupicapra rupicapra Capra aegagrus	- +	+ +	+ _	+	C. ocreatus, M. capillaris, M. tenuispiculatus, N. linearis, P. hobmaieri, P. rupicaprae C. ocreatus, D. filaria, M. capillaris, N. linearis, P. davtiani, P. muraschkinzewi, P. hobmaieri, P. rufescens			
Ovis ammon	+	+	_	_	C. ocreatus, P. davtiani, P. hobmaieri, P. raillieti			
Ovis musimon/ Mouflon musimon	_	_	+	+	M. capillaris, P. davtiani, P. hobmaieri, P. raillieti, P. rufescens, V. capreoli			
Ovis ophion armeniana/ Ovis orientalis gmelini	+	_	_	_	C. ocreatus, P. davtiani, P. muraschkinzewi			
Ovis aries/Ovis ammon. dom.	+	+	+	+	C. ocreatus, D. filaria, M. capillaris, N. linearis, P. brevispiculum, P. hobmaieri, P. rufescens			
Capra hircus	+	+	+	+	D. filaria, C. ocreatus, M. capillaris, N. linearis, P. davtiani, P. hobmaieri, P. muraschkinzewi, P. rufescens			
Lepus europeus	+	+	+	-	P. tauricus			
Total animal species 16	7	13	7	10				

Table 1. A list of definitive host species of lung nematodes

\*Russia - here means south and central regions of European Russia

infected with Protostrongylidae nematodes (*C. ocreatus*, *C. vsevolodovi*, *M. capillaris*, *Neostron*gylus linearis, *P. davtiani*, *P. hobmaieri*, *P. raillieti*, *P. rufescens* and *P. skrjabini*). It is established that in cases of mixed infections the mean prevalence was 24% while for monoinfections it was significantly higher – at 70.9%. Differences in prevalence were also found depending on vertical zones of the grazing lands. E.g., in Chechnya Republic the mean prevalence in sheep was 42.3% (38.2–46.4%) in a mountain zone, 39.7% (37.0–42.4%) in lowlands and 57.5% (44.1–70.9%) in foothills. The subsequent study by Gadaev [6,16] mainly support these data, but also detect previously absent

*Varestrongylus capreoli* in roe deers and *P. rufescens* in West Caucasian tur. The recent study of goats in Terek-Sulak lowland [8] has detected the following Protostrongylidae: *C. ocreatus* ( $E_i = 7.8\%$ ,  $I_i = 3-26$ ), *M. capillaris* ( $E_i = 3.5\%$ ,  $I_i = 3-23$ ), *P. hobmaieri* ( $E_i = 7.4\%$ ,  $I_i = 5-23$ ) and *P. rufescens* ( $E_i = 8.1\%$ ,  $I_i = 3-22$ ), noting higher infection in bush and woodland ecosystems.

In Bulgaria, it has been found that the common prevalence of protostrongylids infections for red deer, fallow deer, mouflons and chamois living in regions with different ecological and geographical characteristics was 46.5% [17]. Twelve protostrongylid species were found in different Table 2. Species of lung nematodes found in the countries studied

Nematode species	Armenia	Russia*	Bulgaria	Poland
Dictyocaulidae Skrjabin,1933				
Dictyocaulus filaria (Rudolphi, 1809)	+	+	+	+
D. viviparus (Bloch, 1782)	+	+	_	+
D. capreolus Gibbons, Höglund, 2002	-	+	_	+
D. eckerti Skrjabin, 1931	-	+	+	+
D. cervi sp. nov. Pyziel, Laskowski, Demiaszkiewicz, Hoglund, 2017	-	_	-	+
Total Dictyocaulidae 5 species	2	4	2	5
Protostrongylidae (Leiper, 1926) Boev et Schulz, 1950				
Cystocaulus ocreatus (Railliet et Henry, 1907)	+	+	+	+
C. vsevolodovi Boev, 1946	-	+	-	-
Elaphostrongylus alces Steen, Chabaud, Rehbinder, 1989	-	+	-	+
E. cervi Cameron, 1931	-	+	+	+
Muellerius capillaris (Mueller, 1889)	+	+	+	+
M. tenuispiculatus Gebauer, 1932	_	+	+	+
Neostrongylus linearis (Marotel, 1913)	-	+	+	+
Protostrongylus brevispiculatum Mikačič, 1940	-	+	+	-
P. cuniculorum (Joyeux & Gaud, 1946)	-	_	+	-
P. davtiani (Savina, 1940)	+	+	_	+
P. hobmaieri (Schulz, Orlow et Kutass, 1933)	+	+	+	+
P. muraschkinzewi (Davtian, 1940)	+	+	_	_
P. raillieti (Schulz, Orlow et Kutass, 1933)	+	+	-	+
P. rufescens (Leuckart, 1865)	+	+	+	+
P. rupicaprae Gebauer, 1932	_	+	+	+
P. tauricus Schulz et Kadenazii, 1949	+	+	+	-
P. skrjabini Boev, 1936	_	+	_	_
Varestrongylus capreoli (Stroh et Schmid, 1938)	_	+	_	+
V. sagittatus (Mueller, 1890)	_	+	+	+
V. alces Demidova et Naumitscheva, 1953	_	+	_	+
Total Protostrongylidae 20 species	8	19	12	14

\*Russia - here means south and central regions of European Russia

animals: in sheep – C. ocreatus, M. capillaris, N. linearis, P. brevispiculum, P. hobmaieri, P rufescens, in goats – M. capillaris, N. linearis, P. hobmaieri, P. rufescens, in mouflons – C. ocreatus, M. capillaris, N. linearis, P. hobmaieri, P. rufescens, in red deer – E. cervi, V. sagittatus, in fallow deer – Protostrongylus spp., in chamois – M. capillaris, M. tenuispiculatus, N. linearis and P. rupicaprae, in European hares – P. cuniculorum and P. tauricus [17–22]. Morphometric descriptions of these species were performed on the materials from the country. P. cuniculorum was reported for the first time as a part of the helminth-fauna of the European brown hare from Southeast Europe and Bulgaria in particular [22].

Data on Protostrongylidae of ruminants from some locations of Poland are presented in several studies [23–26]. Among other Protostrongylidae species the recently substantiated *Varestrongylus alces* was also discovered in elks from Poland [26].

The Echinococcinae lung helminths were found in all the regions studied, with both *E. granulosus* and *E. multilocularis* (*=Alveococcus multilocularis*) present. It should be noted that while historically there were a number of *Echinococcus* species proposed which were later considered synonymous with only *E. granulosus* and *E. multilocularis* being recognized as valid species, recent studies using molecular approach and taking into account host specificity and other factors often show levels of difference between various strains which are characteristic of species level [27–30]. This can often be important due to noticeable differences in pathogenicity to humans between even close species of these cestodes [27,30]. Unfortunately, literature and even recent data from our regions often didn't take these factors into account, so recognition of species here requires further studies.

While genus level for *Alveococcus* was mainly rejected it should be noted that some molecular methods such as DNA sequencing of nuclear protein-coding genes actually demonstrate it showing in a group significantly differing from other echinococcids [29] and in some cases different methods provide different results. The main differences between the genus-level taxons *Echinococcus* and *Alveococcus* are as following:

- morphology - *Alveococcus* has a globe-shaped uterus in mature sections, its genital opening is in the front part of a section, while *Echinococcus* uterus is generally sack-shaped, going throughout whole section's length with branches to the sides, i.e. tree-shaped. Its genital opening lies in the rear part of a section.

- biology - definitive hosts of *Alveococcus* cestodes are mainly Red and Polar foxes, adult forms may also parasitize dogs, wolves and cats. Their intermediate hosts are rodents and humans. For *Echinococcus* there are many species of definitive hosts: dogs, wolves, jackals, with foxes being rare. Intermediate hosts here are many ungulate species and humans.

- geography - *Echinococcus granulosus* is a cosmopolite species while *Alveococcus* is mainly distributed in the North regions.

Such differences inside this cestode group are mainly characteristic of genus level, as was proposed by Abuladze [31]. At the same time, geographical distribution of all these species is still not adequately studied, with up to 10 species being recognized currently [30].

Data on distribution of *E. granulosus* in Armenia are given in [32,33]. Cattle of Armenia appeared to be infected by echinococci at 49%, sheep and goats at 22%, pigs at 15%. Studies of human population in Armenia have shown the infection in all age groups with the highest in people of 30-35 age. *Echinococcus* cysts were found in all organs, but lungs and liver were the most commonly infected (42-43%) [33]. Alveolar echinococcosis remains almost lacking studies in Armenia. The most complete data on distribution of both the species in Russia are given in [34] and show broad distribution of *E. granulosus*. The data from Azizova [8] confirm presence of *E. granulosus* in goats of Caucasus region (20%, mainly in bush and salt desert areas). Alveolar echinococcosis had also been noted in various regions of Russia [34] but this question requires further more intensive studies.

In Bulgaria, *E. granulosus* was reported with prevalence in dogs of 7%, in sheep of 32%, in cattle – 19%, in swine – 1.5%, and human annual load (per 100 000 population) was 3.3 [35,36]. In Poland, study of 2 951 red foxes showed, that 76 (2.6%) appeared to be infected with *E. multilocularis* [37]. Recent studies in Poland showed, that *E. granulosus* has been found in 0.3% of swine studied, with prevalence being higher in central Poland, from 0.4 to 1.2% of animals studied [38].

In summary, sixteen species of terrestrial mammals were studied for infection with lung helminths, including seven species in Armenia and Bulgaria each, 10 in Poland and 13 in Russia. There were studied six species from Cervidae, nine species from Bovidae and one species from Leporidae. There were 27 helminth species found in these animals, of the families: Dictyocaulidae (5 species), Protostrongylidae (20), Taeniidae (2).

The species of lung helminths found to be common for all the regions were *Muellerius capillaris* and *Protostrongylus hobmaieri*. *E. granulosus* was also noted in all of the countries analyzed. Also, relatively many species are common between several regions.

Taking into account morphology, ecology and life cycles, we support a validity of genera *Echinococcus* and *Alveococcus* in the structure of the subfamily Echinococcinae of family Taeniidae.

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