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Original paper

Endoparasites of the European brown hare (*Lepus europaeus* Pallas, 1778 L.) (Lagomorpha: Leporidae) from Bulgaria

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ABSTRACT. In recent decades in Europe there has been a decline in the population of European brown hare (*Lepus europaeus* L.). Diseases, including parasitoses, are one of the factors considered to be the cause for the phenomenon. In this connection the aim of the present work was to update the data on the composition and distribution of endoparasites of this small mammal in Bulgaria against the background of the situation in Europe. Thirty sets of abdominal organs and forty seven faecal samples of hares from different regions of the country were investigated for presence of parasites. The faeces were analyzed with the coproscopical methods of Fulleborn, serial sedimentations, and the modified Baermann technique. Helminthological necropsies of the abdominal organs were carried out through the common technique. Parasites of the following taxa were established: Trematoda (*Dicrocoelium dendriticum*), Cestoda (*Cysticercus pisiformis*, *Mosgovoyia pectinata*), Nematoda (*Trichuris sylvilagi*, Strongylidae, *Strongyloides* sp., *Protostrongylus* spp.), Protozoa (*Eimeria* spp.) and Arthropoda (*Pentastomum dentatum*). *Strongyloides* sp. is reported for the first time as a part of the parasite fauna of the European brown hare in Bulgaria. *Trichuris sylvilagi* was the most prevalent parasite (63.3%), followed by gastrointestinal strongylids (57.43%), *Eimeria* spp. (55.3%), *Protostrongylus* spp. and *Strongyloides* sp. (31.9%), *D. dendriticum* (14.3%), *C. pisiformis* (10%), and *M. pectinata* (6.67%). The studies showed that eimeriid coccidia, gastrointestinal strongylids, and trichurids are the most common endoparasites among the population of the European brown hare both in Bulgaria and the European continent.

Keywords: European brown hare, *Lepus europeus*, parasites, Bulgaria

Introduction

The European brown hare (*Lepus europaeus* L.) is a species native to Europe and parts of Asia. It is considered probably the most important game animal in Europe throughout its historical distribution [1]. In Bulgaria, the brown hare is a major representative of small game and is found throughout the country – from the level of the Black Sea, all the way to the high parts of the mountains. Despite the wide range of habitat, its population in our country has decreased significantly in recent decades [2]. This negative trend has been reported in a number European countries such as France [3], Denmark [4], Slovakia [5], Poland [6], Serbia [7], and Germany [8].

The decline in hare populations have been attributed to factors such as reproductive rate and the ability for adaptation, climate, feed availability, predators, anthropogenic factors and diseases [1]. Apart from common diseases of hares with a high impact on their mortality such as European brown hare syndrome, the parasite diseases are also considered to be a regulative factor of hare population [9]. The negative influence of parasite infections on the abundance of European brown hare was increasingly discussed in recent years [5,6,10–12]. It was found for example that parasite factors were responsible for 25% of deaths among the brown hares in the Czech Republic [12].

Studies on the etiological agents of parasitoses lead to accumulation of fundamental knowledge about biodiversity in the different ecosystems. They could be also of practical importance. According to

Table 1. Parameters of parasite infections in abdominal organs of brown hares *(Lepus europeus* L.) from Bulgaria

Parasite species	Site of infection	Prevalence %	Infection intensity Min–max (mean)	Districts of distribution
Dicrocoelium dendriticum	Liver	6.67	13–70 (41.5)	Burgas
Cysticercus pisiformis	Liver	10	12–50 (32.33)	Burgas, Silistra, Sliven
Mosgovoyia pectinata	Small intestines	6.67	1–5 (3)	Burgas
Trichuris sylvilagi	Large intestines	63.33	2–36 (12.53)	Burgas, Silistra, Sliven, Vratsa
Pentastomum dentatum	Liver	3.33	1	Burgas

Bordes et al. [13] for example, the detailed knowledge of the game parasitofauna from different geographic regions may be used to protect from introduction of new parasites via transfer of animals for repopulation purposes.

The aim of the present work was to update the data on the composition and distribution of endoparasites of European brown hare in Bulgaria against the background of the situation in Europe.

Materials and Methods

The studies were carried out on 30 sets of hares' abdominal organs and 47 faecal samples collected independently of each other from 9 districts of Bulgaria (Fig. 1). The faecal samples were collected from Blagoevgrad, Kyustendil, Pazardzhik, Sofia Province, and Veliko Tarnovo districts. The abdominal organs were obtained mainly by plan shot of the animals during the hunting seasons 2009–2017 (according to the Law of Hunting and Game Conservation, 2000) and at a small extent from hares found dead. These animals came from Burgas, Silistra, Sliven, and Vratsa districts. The coproscopical methods of Fulleborn, serial sedimentations, and the modified Baermann technique were used to analyze the faecal samples and the helminthological necropsies of the abdominal organs were carried out through the common technique [14].

The detected mature helminths were collected and cleaned in saline solution and after that were stored in 70% ethanol. Nematodes were studied after clearing in lactophenol. Cestodes were stained in 1% eosin B, dehydrated and cleared in eugenol. The identification of the parasites was performed according to their morphometric features according to Gvozdev et al. [15], Thienpont et al. [16] and Panaiotova et al. [17].

Results

Endoparasites of 5 species were found in the hares' abdominal organs: one trematode – *Dicrocoelium dendriticum* Rudolphi, 1819; two cestodes – the larval form of *Taenia pisiformis* Bloch (1780) – *Cysticercus pisiformis* and *Mosgovoyia pectinata* (Goeze, 1782); one nematode

Table 2. Results from coproscopical analysis of faecal samples of brown hares (*Lepus europeus* L.) from Bulgaria

Parasites	Prevalence %	Districts of distribution
Dicrocoelium sp.	19.1	Blagoevgrad, Sofia Province, Veliko Tarnovo
Cestoda	4.3	Sofia Province, Veliko Tarnovo
Trichuris spp.	12.8	Blagoevgrad, Kyustendil
Gastrointestinal strongylids	57.4	Blagoevgrad, Kyustendil, Pazardzhik, Sofia Province, Veliko Tarnovo
Strongyloides sp.	31.9	Sofia Province, Kyustendil
Protostrongylus spp.	31.9	Blagoevgrad, Kyustendil, Pazardzhik, Sofia Province, Veliko Tarnovo
Eimeria spp.	55.3	Blagoevgrad, Kyustendil, Pazardzhik, Sofia Province

Figure 1. Origin of materials: 1, 3, 4, 7, 8 – districts of Bulgaria of which faecal samples of brown hares were collected; 2, 5, 6, 9 – districts of Bulgaria of which abdominal organs of brown hares were obtained

– *Trichuris sylvilagi* Tiner 1950; and one arthropod – the nymph of *Linguatula serrata* Fröhlich, 1789 – *Pentastomum dentatum*. The parameters of infections are shown in table 1. In the faecal samples parasites of seven taxa were detected: *Dicrocoelium* sp., Cestoda, *Trichuris* spp., gastrointestinal strongylids, *Strongyloides* sp., *Protostrongylus* spp., and *Eimeria* spp. (Tab. 2).

Discussion

The full reference we made to the available literature showed that the composition and distribution of endoparasites in European brown hare on the continent have been investigated in a number of countries: Austria [12,18], the Czech Republic [12,19], Finland [20], France [13], Germany [11,21–24], Great Britain [25], Greece [26], Hungary [27,28], Italy [29,30], Republic of Moldova [31], Poland [7,32–34], Romania [35], Serbia [36], Slovakia [5], Spain [9]. Data on the topic are also found in the studies by Taşan [37], Tacconi et al. [38], Vysotskaya [39], Shimalov and Shimalov [40], Wibbelt and Frölich [10], Da Costa [41], Haukisalmi [42], Aoutil et al. [43], Chaignat et al. [44], Gabrielli et al. [45], Liatis et al. [46], Yevstafieva et al. [47], and Movsesyan et al. [48]. The parasite taxa that have been recorded in these studies are shown in table 3.

In Bulgaria endoparasites found in brown hares until now are *Fasciola hepatica* Linnaeus, 1758 [49–52], *Dicrocoelium dendriticum* (Rudolphi, 1819) [49–54], *Plagiorchis muris* (Tanabe, 1922) [51], larval form of *Taenia pisiformis* (Bloch, 1780) – *Cysticercus pisiformis* [49–51,53–56], *Mosgovoyia pectinata* (Goeze, 1782) [49–51], *Andrya rhopalocephala* (Riehm, 1881) [49–51,53], *Echinococcus granulosus* (Batsch, 1786) [53], *Trichuris sylvilagi* Tiner, 1950 [49–53], *Trichostrongylus retortaeformis* (Zeder, 1800) [49–51,53], *Trichostrongylus colubriformis* (Giles 1892) [49–51,53], *Nematodirus aspinosus* Schulz, 1931 [50,52], *Nematodirus* sp. [51], *Passalurus ambiguus* (Rudolphi, 1819) [49–53], *Gongylonema pulchrum* Molin, 1857) [49–51,53], *Micipsella numidica* (Seurat, 1917) [49,50,53,54], *Protostrongylus terminalis* (Passerini, 1884) Kamensky 1905 [53], *Protostrongylus tauricus* Schulz et

Table 3. Parasites recorded in brown hares *(Lepus europeus* L.) from Bulgaria and other European countries (according to the present study and reference in the available literature)

Parasites	Armenia	Austria	Belarus	Bulgaria	Czech Rep.	Finland	France	Germany	Great Britain	Greece	Hungary	Italy	Moldova	Netherlands	Poland	Romania	Russia	Serbia	Slovakia	Spain	Switzerland	Turkey	Ukraine
Dicrocoelium dendriticum				+		+	+	+		+		+	+						+	+			
Fasciola hepatica		+	+	+			+	+					+		+				+				
Plagiorchis muris				+																			
Andrya rhopalocephala		+		+	+			+															
Andrya spp.								+				+											
Cittotaenia spp.		+			+	+		+															
Ctenotaenia ctenoides		+			+																		
Cysticercus pisiformis		+		+		+		+			+	+		+	+	+							
Echinococcus granulosus				+																			
Echinococcus multilocularis																					+		
Leporidotaenia wimerosa							+																
Mosgovoyia pectinata		+		+	+	+		+											+			+	+
Mosgovoyia spp.							+																
Paranoplocephala wimerosa								+															
Capillaria hepatica		+																					
Dirofilaria scapiceps										+													
Gastrointestinal strongylids												+		+									
Gongylonema pulchrum				+																			
Graphidium strigosum		+			+		+	+	+				+		+				+				
Micipsella numidica				+								+											
Nematodirus aspinosus				+																			
Nematodirus spp.				+									+										
Passalurus ambiguus				+			+	+		+		+	+		+				+				
Protostrongylus commutatus								+											+				
Protostrongylus cuniculorum				+								+											
Protostrongylus pulmonalis		+			+	+		+							+								
Protostrongylus tauricus	+			+													+						
Protostrongylus terminalis				+																			
Protostrongylus spp.					+					+													
Strongyloides sp.				+	+			+					+	+	+								
Trichostrongylus retortaeformis		+		+	+	+	+	+		+	+	+	+		+			+	+				

Table 3. cont.

Parasites	Armenia	Austria	Belarus	Bulgaria	Czech Rep.	Finland	France	Germany	Great Britain	Greece	Hungary	Italy	Moldova	Netherlands	Poland	Romania	Russia	Serbia	Slovakia	Spain	Switzerland	Turkey	Ukraine
Trichostrongylus colubriformis				+																			
Trichostrongylus spp.										+					+	+							
Trichuris leporis		+			+			+		+			+		+	+		+	+		+		
Trichuris sylvilagi				+				+			+												+
Eimeria spp.		+		+	+	+	+	+		+	+	+	+	+	+	+		+	+	+			
Coccidia								+				+			+								
Pentastomum dentatum				+						+													

Kadenazii 1949 [51,56–58], *Protostrongylus cuniculorum* (Joyeux et Gaud, 1946) [59], nymph of the arthropod *Linguatula serrata* Fröhlich, 1789 – *Pentastomum dentatum* [17,56], and *Eimeria* spp. [60]. In the present study, we have identified *D. dendriticum*, *C. pisiformis*, *M. pectinata*, *T. sylvilagi*, gastrointestinal strongylids, *Strongyloides* sp., *Protostrongylus* spp., *Eimeria* spp., and *P. dentatum*. In the present work *Strongyloides* sp. is reported for the first time as a part of the parasite fauna of the European brown hare in Bulgaria.

Dicrocoelium dendriticum was found in 4 out of 9 districts of the country, materials were collected from. The common prevalence (14.3%), calculated on the basis of the total number of tested faecal samples and livers, is much lower than found by Yanchev [49,50,53,61] in different regions of Bulgaria about 40 years ago (43–50%). For this period however, Genov [51] has given results closer to ours – 16% prevalence of infection for the region of coastal Dobrudzha and 10.11% for Danube Dobrudzha. Recent studies have also shown closer to our results – 10.59% prevalence [54]. The intensity of *D. dendriticum* infection found by us (mean 41.5) was significantly lower than indicated by Yanchev [49,50,53,61] – from several to several thousand flukes in one animal. Studies from different regions of Bulgaria showed intensity of dicrocoelid infection 43–80.57 [51] and 1–184 (mean 26.67) [54]. The literature data give us a reason to speculate that in general the parameters of *D. dendriticum* infection in brown hares in Bulgaria are decreased in recent decades. This could be due to various factors, including the declining population of this animal species in the country.

Cysticercus pisiformis was found in 10% of the necropsied livers from 3 districts. This result is relatively close to those previously established in Bulgaria. According to Trifonov and Meshkov [55] the prevalence in Burgas district in three consecutive years was 18.3%, 12.5% and 6.5%. Yanchev [49,50, 53,61] found 0–28% prevalence of *C. pisiformis* infection in different regions and Genov [50] – 2% in coastal Dobrudzha and 10.11% in Danube Dobrudzha. Trifonova et al. [54] found *C. pisiformis* in 10 of 25 studied areas of the country with a total prevalence of 16.8%.

A relatively low was prevalence of *M. pectinata* infection (6.67%). It was only established in hares from Burgas district. In the past, Yanchev [49,50,53,61] found *M. pectinata* in almost all studied areas, and the prevalence of infection was higher (13.79–40%). Genov [51] found 6% prevalence, a result similar to our one.

Among the identified parasites *Trichuris sylvilagi* was the species with the highest prevalence (63.33%), infection intensity ranged from 2 to 36. High parameters of the infection with this parasite in brown hares from our country were reported in the past decades. According to Yanchev [49,50,53,61] the prevalence was 25–66.66% in Southern Bulgaria and 7.46–90.32% in Northern Bulgaria, and infection intensity ranged from 1–9 specimens in Kardzhali region to 3–289 in Sofia region. According to Genov [51], the prevalence for the regions of the Danube Dobrudzha and coastal Dobrudzha was 53.93% and 60%, and the mean infection intensity was 35.79 and 71.87,

respectively. Tsolov and Gechev [52] reported 80% prevalence and 4–67 infection intensity in Vidin and Pleven districts. Thus, the data from the current and previous studies show that *T. sylvilagi* is one of the most common helminths in brown hares from Bulgaria.

Mature gastrointestinal strongylids and strongyloids were not found during the necropsies, but such type of eggs were observed in a large percentage of faecal samples (57.44% strongylid, 31.9% strongyloid ones). The various shape and size of the found strongylid eggs showed that hares were infected with a large number gastrointestinal strongylid species, although three species only were reported for Bulgaria [50]. The variety of detected strongylid eggs and the presence of faeces from ruminants and equids in hares' habitats we observed, are resons to consider that brown hares could be hosts for parasites specific to other herbivore species. This speculation is confirmed by experimental data of Stott et al. [62] according who mature specimens of ruminant-specific strongylids may develop in brown hares, with egg release also has been observed.

Larvae of *Protostrongylus* spp. were found in 31.9% of the faecal samples in 5 districts of the country. Yanchev [49,50,53,61] has found protostrongylids in brown hares from southwestern Bulgaria, but has not detected these helminths in northern Bulgaria and other southern parts of the country such as the regions of Stara Zagora, Nova Zagora, Kardzhali, Haskovo and Asenovgrad. Genov [51] found protostrongylids in 56% of hares in coastal Dobrudzha, but not in the Danube part of this region. Panayotova-Pencheva et al. [56] established *Protostrongylus* spp. in hares in 12 of 24 surveyed districts of the country with a total prevalence of 7.59%, as the northwestern and north-central part of the country were not affected. The above-mentioned data indicate the irregular distribution of protostrongylids in brown hares from different regions. This could be due to the differences in species composition and distribution of mollusks, which are intermediate hosts of the lungworms.

The infection with *Eimeria* spp. was widespread – oocysts were observed in 55.3% of faecal samples. However, the subjectively determined degree of infection intensity was low. The established widespread distribution of *Eimeria* spp. in our study is in agreement with the data of Golemansky [60] according to which these protozoa are distributed among brown hares througout the country.

Pentastomum dentatum finding was accidental. Only one nymph in one of the livers was found. Low parameters of infection with this arthropod were also found in a study on lung parasites of brown hares from Bulgaria – 0.89% prevalence and only a few parasites in a pair of lungs [56].

Among the parasites identified in the present study, those with the highest prevalence were trichurids, gastrointestinal strongylids and *Eimeria* spp. A widespread distribution of these parasites among brown hare populations was also observed in other European countries. *Eimeria* spp., *T. retortaeformis* and *Trichuris leporis* were the most prevalent endoparasites in Austria [12]. In the Czech Republic *Eimeria* spp. were the most commonly occurring parasites (90.5%), followed by *T. retortaeformis* (75.8%) and *T. leporis* (39.8%) [19]. The prevalence with *Eimeria* spp. in Greece was 64.28%, and this with *T. retortaeformis* and *T. leporis* – 50% and 21.42%, respectively [26]. This parameter was 64.9% for coccidia and 87.1% for *T. retortaeformis* in Italy [29]. The prevalence of eimeriid infections in Slovakia was 91.89%, *T. leporis* was the second in distribution (55.41%), and *T. retortaeformis* the third (6.76%) [5]. In Romania the prevalence with *Eimeria* spp. was 80.95%, with *T. leporis* – 61.90% and with *Trichostrongylus* spp. – 33.33% [35]. In Finland European brown hare has been more commonly infected with *Eimeria* spp. and *T. retortaeformis* [20].

The summarized data from the reference in the available literature (Tab. 3) show that generally gastrointestinal strongylids have been recorded in the largest number of European countries. Of these *Trichostrongylus* spp. – in 17 countries, *Graphidium strigosum* – in 8, and *Nematodirus* spp. – in 2. Coccidia, mostly *Eimeria* spp., have been registered in 19 European countries, *Protostrongylus* spp. – in 15, *Trichuris* spp. – in 14, and *P. ambiguus* – in 8. These parasite taxa, with the exception of *Nematodirus* spp., are common to lagomorphs and this explains their more frequent registration. However, *Nematodirus* spp., are parasites affecting ruminants [63]. Their establishment in present study and Republic of Moldova [31] shows that European brown hare can be also an epidemiological factor of nematodirosis. *Micipsella numidica* has been recorded only in Bulgaria and Italy. This may be due to the fact that the life cycle of this filarial nematode requires arthropods, common in the warmer and

humid climate of Southern Europe.

In the present study the parameters of infection with trematodes and cestodes compared to those with nematodes and coccidia were significantly lower. Such a tendency is also observed abroad [5,12]. The two cestode species found by us, *C. pisiformis* and *M. pectinata*, have been registered respectively in 9 and 8 other European countries (Tab. 3). *Dicrocoelium dendriticum* was the only trematode species we found. It is also the most commonly recorded parasite of class Trematoda in brown hares on the continent (Tab. 3). Here we should mention the fact that during this study we were not found *E. granulosus* cysts in the hares' abdominal organs. *Echinococcus granulosus* larvae were also not detected in our previous investigation on the lung parasites of brown hares from Bulgaria [56]. Yanchev [53] has found echinococcal cysts only in one of the total 55 hares from Bulgaria. According to him although *E. granulosus* has been reported in helminthological literature as a parasite of rabbits, its occurrence in brown hare is an extremely rare phenomenon. Our data confirm his opinion. The study of Yanchev [53] is actually the only one we found documenting *E. granulosus* infestation of a brown hare from Europe.

During the present study nine parasite taxa were identified in brown hares from Bulgaria. They were *Dicrocoelium dendriticum*, *Cysticercus pisiformis*, *Mosgovoyia pectinata*, *Trichuris sylvilagi*, Strongylidae, *Strongyloides* sp., *Protostrongylus* spp., *Eimeria* spp., and *Pentastomum dentatum*. *Strongyloides* sp. is reported for the first time as a part of the parasite fauna of the European brown hare in the country. The most prevalent parasites were trichurids, gastrointestinal strongylids, and eimeriid coccidia. According to the available literature data they are also the most common parasites in brown hare populations in Europe. This ranks trichuroses, gastrointestinal strongylidoses and eimerioses among the important diseases of this animal species with a potential impact on its abundance.

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