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GASTROINTESTINAL PARASITES AND LUNG WORMS OF WILD RUMINANTS FROM SOUTHWESTERN BULGARIA. I. CERVIDAE: RED DEER (*CERVUS ELAPHUS* L. 1758) AND FALLOW DEER (*DAMA DAMA* L. 1758)

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ABSTRACT

Both the fallow deer and the red deer are valuable hunting objects in Europe, including in our country. In order to obtain current data on the parasitological status, 46 fallow deer (*Dama dama* (Linnaeus, 1758) and 15 red deer (*Cervus elaphus* Linnaeus, 1758) were studied. The aim was to use conventional methods in parasitological research to obtain useful data for the implementation of effective preventive measures in animal health protection. The investigated red deer (*Cervus elaphus)* were from the hunting farms as follow: DLS „Iskar" (n = 5), DLS "Vitoshko-Studena" (n = 6), DGS "Blagoevgrad" (n = 1), and DGS "Krichim" (n = 3). The investigated fallow deer (*Dama dama*) were from DLS "Dikchan" (n = 29), DLS "Iskar" (n = 13), DGS "Mesta" (n = 2), and DLS "Vitoshko-Studena" (n = 2). Invasions with representatives of 3 classes of helminths (Trematoda, Cestoda, and Nematoda) as well as with protozoa of the Coccidia subclass after parasitological examinations were found. Some of them are known as the causes of economically important parasitic diseases. Trematodes of the genera *Dicrocoelium* and *Paramphistomum,* and cestodes of the genus *Moniezia* were also determined. The gastrointestinal parasites were identified as belonging to the families Trichostrongylidae Leiper, 1912 (*Trichostrongyus*), Molineidae Durette-Desset and Chabaud, 1977 (*Nematodirus*), Strongylidae Baird, 1853 (*Oesophagostomum*), Ascarididae Baird, 1853 (*Toxocara*), Trichuridae Railliet, 1915 (*Trichuris*), Eimeriidae Minchin, 1903 (*Eimeria*) and lung worms as members of the families Dictyocaulidae Skrjabin, 1941 (*Dictyocaulus*) and Protostrongylidae Leiper, 1926 (*Protostrongylus*).

Key words: *Cervus elaphus*, *Dama dama*, gastrointestinal parasites, lung worms, *Eimeria*.

Introduction

Studies on parasites of red deer (*Cervus elaphus* Linnaeus, 1758) in Europe have been observed for a long time. Invasions by *Dictyocaulus viviparus* and *Setaria labiatopapillosa* were among the first to be reported in Switzerland (Schweizer, 1949). Cases of invaded *C. elaphus* with helminth species in the former Czechoslovakia (Kotrly, 1958) and Poland (Drozdz, 1961) were later reported. The authors found the presence of *D. viviparus*, *Bicaulus sagittatus,* now known as *Varestrongylus sagittatus*, *Apteragia quadrispiculata* n. sp., *Grosspiculagia lasensis* n. comb., *Haemonchus contortus*, *Ostertagia leptospicularis, Ostertagia mossi*, *Rinadia mathevossiani* n. comb., *Spiculopteragia asymmetrica*, *S. spiculoptera*, and *Trichostrongylus axei*. The knowledge of parasites of *C. elaphus* in Europe has been expanded by a large number of researchers through findings in Scotland (Dunn, 1964) with the description of a new species of *Trichostrongylus askivili* n. sp., in Poland (Drozdz, 1965) with the identification of 19 species of helminths, in Germany with the identification of 14 species (Deckelmann, 1968), and later 17 species (Schweisgut, 1975), and by the detection of helminth infestations of 10 species in Slovakia (Páv & Zajcek, 1971) and others. Helminths that are not typical of the local fauna have also been found in Europe, such as *Ashworthius sidemi* and *A. gagarini* in Poland (Drozdz, 1966).

The first data on the helminth fauna of red deer in Bulgaria has been the result of a study of 17 animals (Yanchev, 1976), in which 15 species of nematodes were identified as *Capillaria bovis*, *Cooperia pectinata*, *C. punctata*, *Dictyocaulus eckerti*, *Gongylonema pulchrum*, *Nematodirus roscidus*, *Oesophagostomum cervi*, *O. sikae*, *O. venulosum*, *Ostertagia leptospicularis*, *Rinadia mathevossiani*, *Skrjabinagia kolchida*, *Spiculopteragia asymmetrica*, *S. spiculoptera*, and *Trichostrongylus axei*. Later, the information about parasites of *C. elaphus* in Bulgaria was enlarged by the establishment of lung worms from the genera *Muellerius*, *Cystocaulus*, *Varestrongylus*, and representatives of the subfamily Elaphostrongylinae (Panayotova-Pencheva et al., 2004). Some gastrointestinal nematodes were also found (Radev et al., 2011).

As a result of consistent and in-depth studies on fallow deer's parasites, a significant number of gastrointestinal and lung nematodes of *Dama dama* have been found in the Netherlands (Jansen, 1958) and registered as: *Apteragia quadrispiculata* n. sp., *Haemonchus contortus*, *Ostertagia circumcincta*, *O. leptospicularis*, *O. mossi*, *O. ostertagi*, *O. trifurcate*, *Rinadia mathevossiani* n. comb. *Spiculopteragia asymmetrica*, *S. spiculoptera*, *Teladorsaigia davtiani*, and *Trichostrongylus axei*. The species *A. quadrispiculata*, *G. lasensis*, and *R. mathevossiani* are classified as *Spiculopteragia quadrispiculata* (Jansen, 1958), *Ostertagia kolchida* Popova, 1937, *and Spiculopteragia mathevossiani* Ruchliadev, 1948, and are now known, respectively. Invasions of *Dictyocaulus filaria* and *Dictyocaulus viviparus* have been reported in Germany (Hildebrandt, 1962). Similar studies were continued later. For example, in Poland (Drozdz, 1966) and in Great Britain (Batty & Chapman, 1970), 17 and 6 species of helminths were reported, respectively; in Austria (Kutzer, 1971) and Italy (Goffredo & Sobrero, 1972; Sobrero & Goffredo, 1972), 5 species in each were noted.

The first data on fallow deer helminths in Bulgaria was obtained from a study of 12 animals from two hunting farms. The findings of them were identified as representatives of 14 species of nematodes (Yanchev, 1979): *Apteragia quadrispiculata*, *Capillaria bovis*, *Gongylonema pulchrum*, *Nematodirus filicolis*, *Oesophagostomum cervi*, *O. sikae*, *O. venulosum*, *Ostertagia drozdzi*, *O. leptospicularis*, *S. asymmetrica*, *Skrjabinagia ryjikovi*, *Spiculopteragia spiculoptera*, *Trichocephalus ovis*, and *Trichostrongylus axei*.

Later pulmonary worms, members of 3 genera-*Cystocaulus*, *Muellerius*, and *Varestrongylus*, were identified in fallow deer from the DGS "Devin", "Kardzhali", "Palamara", "Ropotamo", and "Sherba" (Panayotova-Pencheva et al., 2004). In addition, in the study of *D. dama* from 2 hunting farms ("Ropotamo" and "Zhenda"), infestations with helminths belonging to 9 genera were also found: *Chabertia*, *Dicrocoelium*, *Gongylonema*, *Haemonchus*, *Nematodirus*, *Oesophagostomum*, *Ostertagia*, *Protostrongylus*, and *Trichostrongylus* (Nanev et al., 2010).

After research on parasites of wild ruminants, including red deer and fallow deer from different climatic regions in Bulgaria, the invasions with parasites of the genera *Cystocaulus*, *Dicrocoelium*, *Gongylonema*, *Haemonchus*, *Muellerius*, and *Oesophagostomum* as dominant have been noted (Todev et al., 2004). The authors found that in the same regions, less common invasions are those with representatives of the genera *Capillaria*, *Cooperia*, *Dictyocaulus*, *Nematodirus*, *Neostrongylus*, *Ostertagia*, *Paramphistomum*, *Protostrongylus*, and *Trichostrongylus*.

Cases of invasion with lung worms of the genera *Elaphostrongylus* and *Varestrongylus* in red deer from DLS "Vitinia" have also been found (Panayotova-Pencheva, 2006). Lung nematodes obtained in *C. elaphus* from the Rhodope Mountains region (DLS "Izvora") as *Protostrongylus rupicaprae* (Panayotova-Pencheva, 2008), and in the DLS "Vitinya" area as *Elaphostrongylus cervi* and *Varestrongylus sagittatus* have been identified (Panayotova-Pencheva & Alexandrov, 2011).

Based on the analysis of 140 fecal samples (Dakova et al., 2017), invasions by lung worms and gastrointestinal parasites of 16 genera in red deer and 15 genera in fallow deer have been discovered. In studying parasitic diseases on wild animals in Bulgaria, invasions with representatives of the genera *Bunostomum*, *Capillaria*, *Cooperia*, *Dictyocaulus*, *Elaphostrongylus*, *Moniezia*, *Nematodirus*, *Oesophagostomum*, *Ostertagia*, *Paramphistomum*, *Protostrongylus*, *Strongyloides*, *Trichostrongylus*, *Varestrongylus*, and *Eimeria* in red deer have been found (Dakova, 2020). Two species of coccidian (*E. robusta* and *E. asymmetrica*), two species of gastrointestinal nematodes (*Oesophagostomum sikae* and *O. venulosum)*, and a lung worm, *Varestrongylus sagittatus*, have been identified. In addition, gastrointestinal helminths as members of the genera *Chabertia*, *Dicrocoelium*, and *Haemonchus*, and protostrongylids as members of *Muellerius* and *Neostrongylus* were detected.

The above data show the presence of a rich variety of gastrointestinal parasites and lungworms in *C. elaphus* and *D. dama* in Europe, including Bulgaria, and were obtained after regular non-abatement parasitological studies.

Materials and methods

Materials from 61 wild ruminants (Cervidae) from five hunting ranches in Southwestern Bulgaria between September 1, 2020, and January 31, 2021 were studied. There were 46 fallow deer (*Dama dama* Linnaeus, 1758) and 15 red deer (*Cervus elaphus* Linnaeus, 1758). Samplings were from the lungs, liver, small and large intestines, rumen, and individual fecal samples were also studied. Samples from male animals up to 1 year of age or between the ages of 8 and 11 years old were examined.

The studied fallow deer (*Dama dama*) were from DLS* "Dikchan" (n = 29), DLS "Iskar" (n = 13), DGS "Mesta" (n = 2), and DLS "Vitoshko-Studena" (n = 2). The red deer (*Cervus elaphus)*, were from DLS "Iskar" (n = 5), DLS "Vitoshko-Studena" (n = 6), DGS “Blagoevgrad” (n = 1), and DGS "Krichim" (n = 3).

A partial helminthological autopsy according to a procedure proposed by Skrjabin and accepted by a number of authors (Kamenov & Radev, 2002; Koinarski et al., 2014, Kanchev et al., 2016; Soulsby, 1982) and others was performed. Flotation and sedimentation methods were used to test for the presence of helminth eggs in fecal samples. A simplified Berman method was used to detect parasitic larvae. For morphological characteristics, larvae were obtained by culturing in a manner described in detail in the same cited sources. The third-stage larvae were obtained based on their morphological features and biological characteristics recommended by a number of authors (Anderson, 2000; van Wyk & Mayhew, 2013). By the sedimentation method, eggs of parasites of the classes Trematoda and Cestoda by the sedimentation method were established and those of Nematoda by the flotation method for ovoscopical examination of fecal samples after using well-known laboratory-diagnostic methods (Kamenov & Radev, 2002; Koinarski et al., 2014; Kanchev et al., 2016; Soulsby, 1982).

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

Results and discussion

Of the 46 fallow deer (*D. dama*) examined, 35 (76%) were positive for invasion, and the remaining 11 (24%) were free from invasion. The predominant findings in animals from DLS "Dikchan" were for the presence of parasite members of the family Trichostrongylidae Leiper, 1912 (Figures 1b and 3). After the larvae culturing, they were found to be nematodes of the genera *Ostertagia* Ransom, 1907 (Figure 4), *Trichostrongylus* Looss, 1905 and *Oesophagostomum* Molin, 1861 (Figure 2). Next in frequency are the findings of nematode infestations of the family Chabertiidae (Lichtenfels, 1980). Less commonly, eggs of trematodes of the genus *Dicrocoelium* Dujardin, 1845 (Figure 7) and larvae of lung parasites of the family Protostrongylidae Leiper, 1926 (Figures 5 and 9) were found, followed by those from representatives of the genera *Paramphistomum* Fischoeder, 1901 and *Eimeria* Schneider, 1875 (Figure 10). In isolated cases, infestation with nematodes of the genera *Trichuris* Roederer, 1761 (Figure 1a), *Nematodirus* Ransom, 1907 (Figure 6), *Toxocara* Stiles, 1905 (Figure 8) and cestodes of the genus *Moniezia* Blanchard, 1891 were found. Invasions by nematodes of the genus *Trichostrongyus* and trematodes of the genus *Paramphistomum* were found more often in the materials from DLS "Iskar". Less frequently, the presence of representatives of the genera *Ostertagia* and *Oesophagostomum*, cestodes of the genus *Moniezia*, and protozoa of the genus *Eimeria* were found. Twelve of the studied *D. dama* were free from invasion-five from DLS "Dikchan", four from DLS "Iskar", two from "Vitoshko-Studena" and one from DGS "Mesta". The negative results for the DLS "Vitoshko-Studena" materials, as well as those for the detected presence of parasites of the genera *Oesophagostomum* and *Eimeria* in fallow deer from DGS "Mesta", are not significant enough for reliable analysis to be imposed.

Mixed invasions with two or more species of parasites were found more frequently in the studied animals. This explains why the number of positive results exceeds that of positive samples. Invasions with gastrointestinal nematodes (*Oesophagostomum*, *Ostertagia*, *Trichostrongyus*, *Nematodirus*, *Toxocara*, and *Trichuris*), pulmonary nematodes (Protostrongylidae, Dictyocaulidae), trematodes (*Dicrocoelium*, *Paramphistomum*), and protozoa (*Eimeria*) were detected as positive (Figure 11).

Of the gastrointestinal nematodes, the presence of representatives of the genus *Trichostrongyus* was predominant, followed by those of the genera *Ostertagia* and *Oesophagostomum*. Invasions with *Nematodirus* sp. and, in some cases, with *Toxocara* sp. and *Trichuris* sp. (Figure 12 b) were also found. The trend for the relative share of the found findings in relation to the total number of positive results is preserved, as is the extent of the invasion (Table 1). In fallow deer from Southwestern Bulgaria, the highest extent of invasion (IE) is shown by *Trichostrongyus* sp. (26.08%), followed by that for *Ostertagia* sp. and *Oesophagostomum* sp. (15.21%) and 13.04%, respectively (Table 1). The extent of invasion for *Trichuris* sp. was 4.34%, and the lowest was for *Nematodirus* sp. and *Toxocara* sp. was 2.17%.

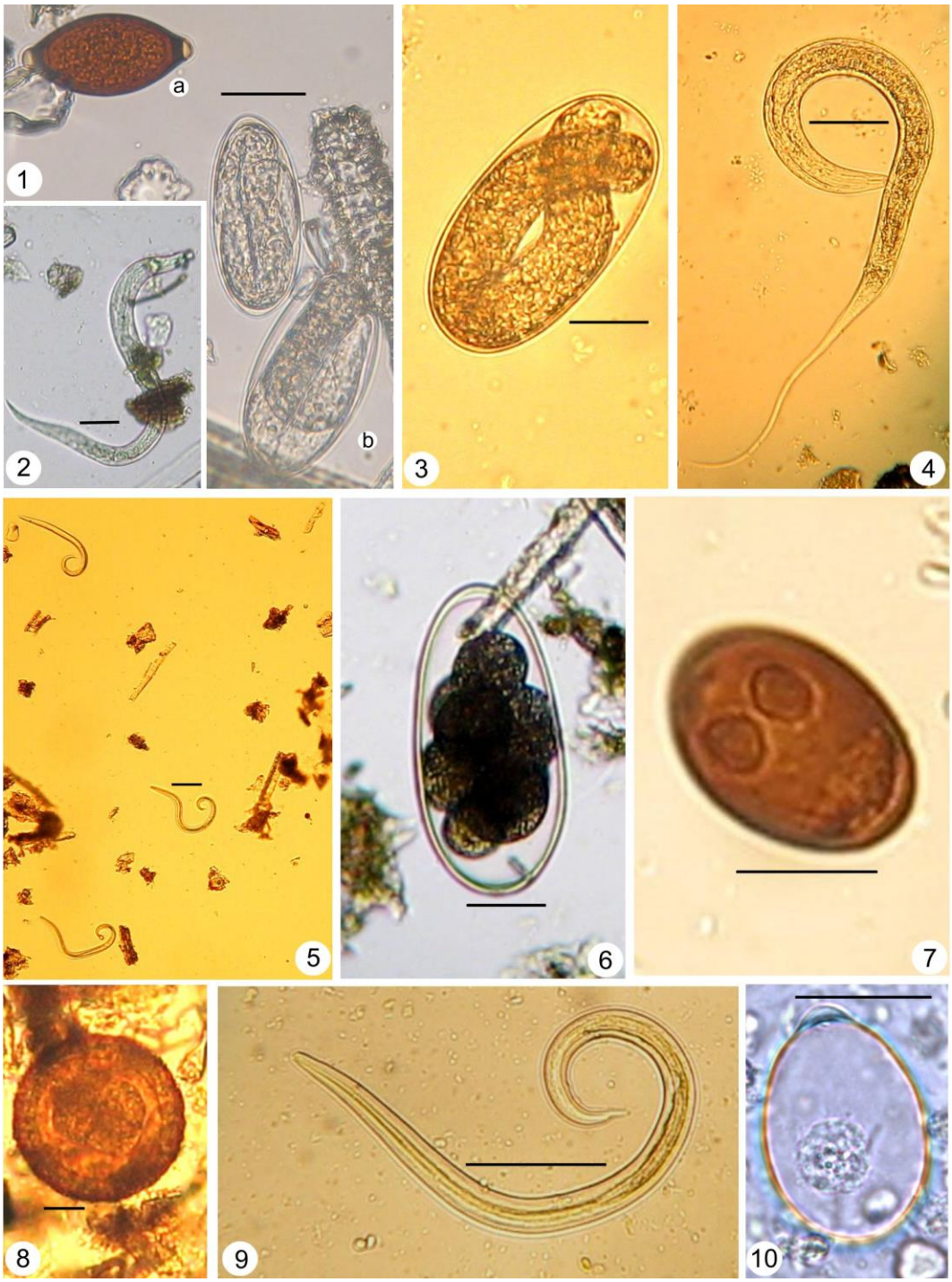

Figure 1 – 10: Results of ovoscopic and larvoscopic examinations of cervid's fecal samples: eggs and oocysts: 1a - *Trichuris* (egg), 1b; 2 - *Oesophagostomum* (larvae); 3 - Trichostrongylidae (eggs); 4- *Ostertagia* (egg); 5 - Protostrongylidae (larvae); 6 - *Nematodirus* (egg), 7 - *Dicrocoelium* (egg); 8 - *Toxocara* (egg); 9 – Protostrongylidae (larva); 10 - *Eimeria* (oocyst). Scale bars: Figs. 1, 3, 7, 8 and 10 = 20 µm; Fig. 2 and 6= 50 µm; Figs. 4, 5 and 9 = 100 µm.

Table 1: Percentage of positive samples and invasion extent.

Kind of infestation	Fallow deer (Dama dama)			Red deer (Cervus elaphus)		
	Positive samples - number	Percentage of positive samples	Invasion extent (%)	Positive samples - number	Percentage of positive samples	Invasion extent (%)
Gastrointestinal nematodes						
Nematodirus	1	2.85	2.17			
Oesophagostomum	6	17.14	13.04	2	20	13.33
Ostertagia	7	20	15.21	3	30	20
Toxocara	1	2.85	2.17			
Trichostrongyus	12	34.29	26.08	3	30	20
Trichuris	2	5.71	2.17			
Trematoda						
Dicrocoelium	11	31.42	23.91			
Paramphistomum	7	20	15.21			
Cestoda						
Moniezia	2	5.71	4.34			
Lung worms						
Dictyocaulidae	3	8.57	6.52	2	20	13.33
Protostrongylidae	11	31.42	23.91			
Protozoa						
Eimeria	6	17.14	13.04	1	10	2.85

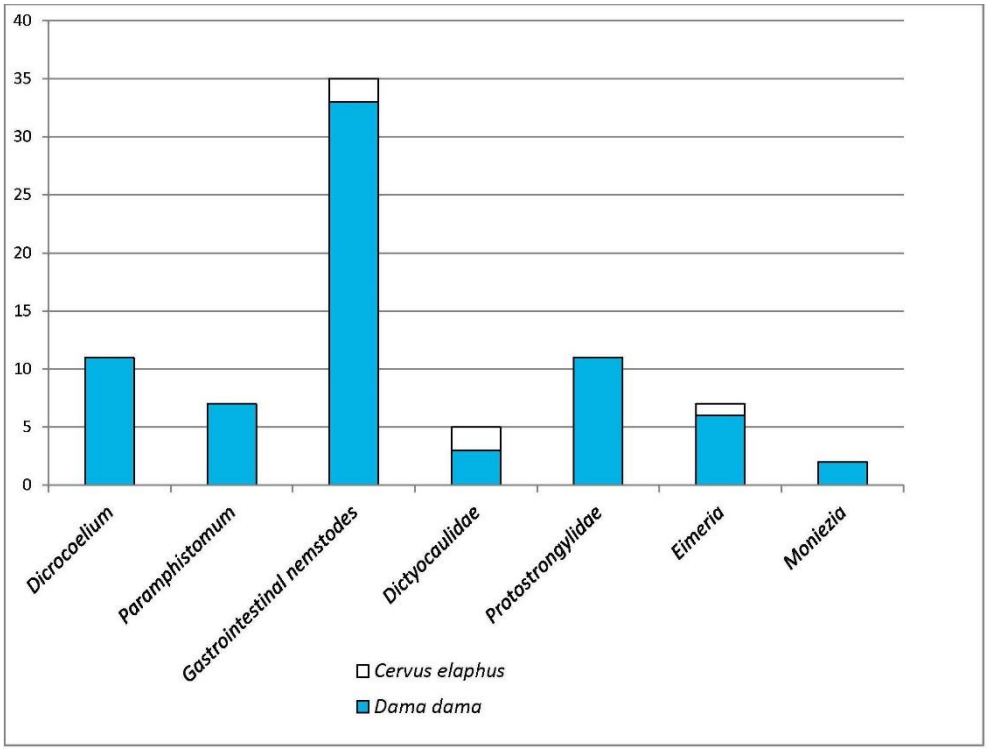

Figure 11: Parasites of red deer and fallow deer from the region of Southwestern Bulgaria. Identity and commonness.

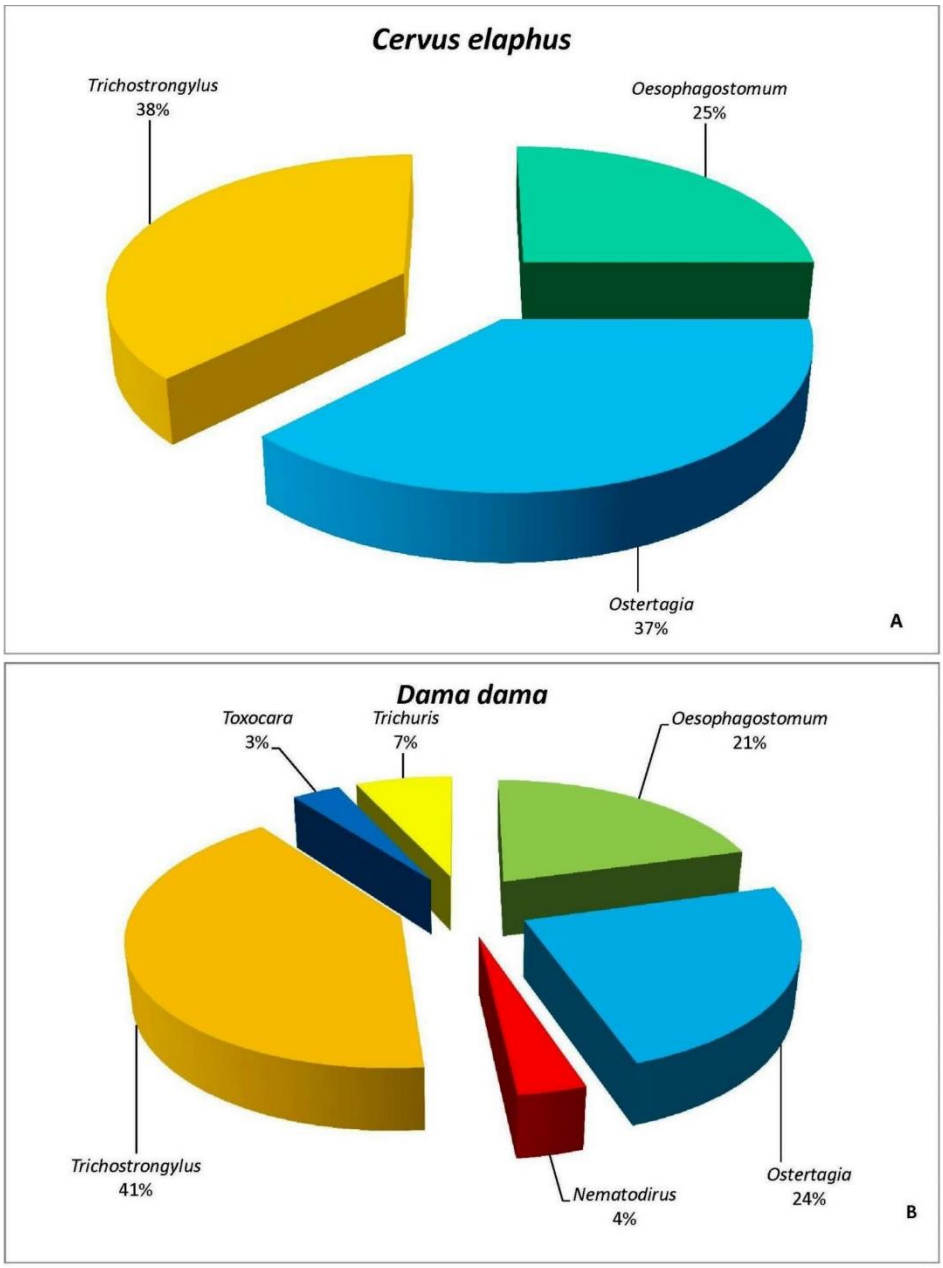

Figure 12: Percentage between gastrointestinal nematodes in *Cervus elaphus* (A) and *Dama dama* (B).

In pulmonary nematode cases, the representatives of the Potostrongylidae family show a larger share of the positive results (Table 1). In fallow deer, IE with them was 23.91%, and with those of the family Dictyocaulidae, it was 6.52%. The results of the studies show that *Dicrocoelium* sp. occupies a larger share of the positive results (Table 1), with an IE of 23.91%, than the representatives of the genus *Paramphistomum*, with an IE of 15.21%. When studies were performed at this time of year, the representatives of the genus *Moniezia* (Cestoda) were isolated sporadically with an IE of

4.34%. Such cases allow the invasion to overwinter in the final host and to intensify under favorable conditions.

In fallow deer, representatives of the genus *Eimeria* (Protozoa) as single and mixed invasons were found. They account for 17.14% of the positive results with a 13.04% extent of invasion (Table 1). Taking into account the low degree of invasion and the age of the animals tested, we believe that in these cases the deer are carriers at 13.04% EI.

Positive for invasion with internal parasites were 10 (67%) of the 15 investigated red deer. In *C. elaphus* from DGS Blagoevgrad (Figure 11), infestation with gastrointestinal nematodes of the genera *Oesophagostomum*, *Ostertagia,* and *Trichostrongyus* in mixed and single invasions was found. In the materials from DLS "Vitoshko-Studena", larvae of lung worms (Dictyocaulidae) and oocysts of *Eimeria* were found, in these ones from DGS "Krichim"-eggs of *Ostertagia* and *Trichostrongyus,* and in those from DLS "Iskar", eggs and larvae of *Ostertagia*, *Trichostrongyus,* and Protostrongylidae.

The presence of invasions with parasites of the genera *Ostertagia* and *Trichostrongyus* was found in 3 of the four studied farms-DGS "Blagoevgrad", DLS "Iskar" and DGS "Krichim". Infections with representatives of Dictyocaulidae and *Eimeria* were found in *C. elaphus* from DLS "Vitoshko-Studena", and in the materials from DLS "Iskar"-evidence of invasion with parasites from the family Protostrongylidae. Five of the studied *C. elaphus*-two from DLS "Iskar" and one each from DGS "Blagoevgrad", DLS "Vitoshko-Studena" and DGS "Krichim"-were free from parasite presence.

In the period September 2020–February 2021, in red deer (*C. elaphus*) from Southwestern Bulgaria were found infestations with parasites, representatives of 5 taxonomic groups: genera *Oesophagostomum*, *Ostertagia,* and *Trichostrongyus*, from the family Dictyocaulidae, all of the type Nematoda, and from the genus *Eimeria* (Protozoa) (Figure 11).

The largest share of gastrointestinal nematodes have representatives of the genus *Trichostrongylus* (38%), followed by those of the genera *Ostertagia* (37%) and *Oesophagostomum* (25%) (Figure 12a).

Infestations with gastrointestinal nematodes are most often found in positive samples of fallow deer. Invasions with members of the genera *Trichostrongyus* and *Ostertagia* have the highest share of positive results (30% each), followed by those with members of the genus *Trichostrongyus* (20%) (Table 1). The extent of invasion with nematodes from the genera *Trichostrongyus* and *Ostertagia* is 20%, and 13.33% with nematodes from the genus *Oesophagostomum*. Concerning the lung nematodes, infestations with members of the Dictyocaulidae family with an IE of 13.33% were found (Table 1). Invasions with protozoa (genus *Eimeria*) account for 10% of positive results, with an IE of 2.85%.

The current results are partly consistent with previous studies by other authors over the last decade (Dakova, 2020; Dakova et al., 2017; Todev et al., 2004). In our studies, infestations of fallow deer were found more often, which is due to the significant difference in the number of studied animals of the two deer species. Both in the fallow deer and the red deer, mixed invasions were more often found than single invasions. During the autumn-winter study period, gastro-intestinal parasites from four genera (*Oesophagostomum*, *Ostertagia*, *Trichostrongyus*, and *Eimeria*) and lung worms from two families (Dictyocaulidae and Protostrongylidae) were found in red deer from four farms in South-western Bulgaria. In addition to them, infestations with representatives of 6 other genera-*Dicrocoelium*, *Paramphistomum*, *Nematodirus*, *Trichuris*, *Toxocara,* and *Moniezia* in fallow deer were established (Figure 11).

The frequency of encounters with infestations of members of the Cervidae family in southwestern Bulgaria raises the question of whether effective prevention and control measures can be tested. Anthelmintics used in feed have been used in Poland and other countries (Cisek et al., 2003). Good results in the fight against lung and gastrointestinal parasites were obtained after the addition of Eprinex and Ivomec premix. With long-term use, Cermix has shown lower efficacy against lung nematodes (*E. cervi*) but has given good results in the fight against gastrointestinal nematodes. Good results have been obtained after the seasonal application of Panacur against *V. sagittatus* and *D. noerneri*.

According to literature data for the treatment of parasitic diseases in wild animals (Panayotova-Pencheva et al., 2015), ivermectin is the most widely used and most effective in controlling parasites in Cervidae. Treatment of trematode infections with albendazole has shown an effectiveness of 20–80% when administered orally, and in moniesiosis, use of Niclosamide gives good results.

Conclusion

Invasions of gastrointestinal parasites, including members of the families Trichostrongylidae Leiper, 1912, Molineidae Durette-Desset and Chabaud, 1977 (Nematodirus), Ascarididae Baird, 1853 (Toxocara), Trichuridae Railliet, 1915, and lung nematodes, including members of the families Dictyocaulidae Skrjabin, cestodes belonging to the Anoplocephalidae Cholodkovsky, 1902 (Moniezia) and trematodes in Dicrocoeliidae Odhner, 1911 and Paramphistomidae Fishoeder, 1901 families were found as well.

Dama dama (fallow deer) has been infected with parasites from the genera *Dicrocoelium*, *Paramphistomum*, *Moniezia*, *Nematodirus*, *Oesophagostomum*, *Ostertagia*, *Toxocara*, *Trichostrongylus*, *Trichuris*, *Eimeria*, and the families Dictyocaulidae and Protostrongylidae. Invasions with gastrointestinal helminths, such as *Trichostrongyus* and *Ostertagia*, are more common, while those with parasites from the genera *Oesophagostomum* and *Eimeria* are less common. *Trichuris*, *Nematodirus*, *Toxocara*, and *Moniezia* representatives are rarely found in invasion cases.

Gastrointestinal parasites from the genera Oesophagostomum, Ostertagia, Trichostrongylus, Eimeria, and pulmonary parasites from the family Dictyocaulidae are found in C. elaphus (red deer), much as they are in fallow deer invaders. Representatives of the family Protostrongylidae are the most common lung nematode invaders in fallow deer, but only members of the family Dictyocaulidae have been found in red deer.

The obtained results show the necessity of constant control over the parasitological status of cervidae and the periodic deworming carried out.

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