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# Associative Helminthiasis and Protozoasis in Wild ARTIODactyl Animals of the Boreal Forest Subzone in Belarus

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**Abstract:** The studies were conducted to evaluate a comparative efficacy of broad – spectrum anthelmintics with an immunomodulatory effect used for animal dehelminthization on the basis of an epizootic situation in helminthiasis and protozoasis of wild artiodactyl animals of the northern forest subzone of Belarus. Helminthological and protozoological investigations were based on the methods generally accepted in parasitology. The data obtained show infestation of a wild boar with 14 species of helminths belonging to four classes (Trematoda, Cestoda, Nematoda, Acanthocephala); moose – with 13 helminth species from three classes (Trematoda, Cestoda, Nematoda) and one species of Eimeria from Sporozoa class; European roe deer – with 11 helminth species from three classes (Trematoda, Cestoda, Nematoda) and 1 species of Eimeria from Sporozoa class. In terms of species the helminths belonging to Nematoda class predominate in helminthocenosis. The most widespread helminthosis in wild boar is Metastrongylosis, in moose is Mecistociriosis, in European roe deer is Trichostrongylidosis. Four preparations for dehelminthization of wild animals under laboratory and field conditions were tested. They were «22% fenbendazole granulate», «20% tetramizole granulate», «Polyparacid», «Pentavet». It was established that Polyparacid and Pentavet having and immunostimulating effect are more effective than «22% fenbendazole granulate» and «20% tetramizole granulate» having an immunosuppressive.

**Keywords:** Helminthiasis, Protozoasis, Infestation, Dehelminthization, Antihelmintic Drugs

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## 1. Introduction

In Belarus, the boreal forest subzone stretches across the Belorussian Land District and the uplands of Belarus' central watershed divide: Orsha Upland, Minsk Upland, Oshma Ranges, Orsha-Mogilev Plateau and the northern, most elevated part of Central Berezina Plain. The latter, located between Orsha and Minsk Uplands, is characterized by a harsher climate compared to the southern part of the plain adjacent to the Belorussian Forest District and has a characteristic landscape of the plains bordering the Forest District.

The study area is characterized by a high population density of wild artiodactyl animals [1-4] and a high degree of infestation by parasites [5-11].

## 2. Method

Ecological and parasitological studies of wild artiodactyl animals were conducted in 2012-2018 at 3 veterinary clinics (Braslavsky Lakes, Berezinsky Biosphere Reserve, Begomlsky Leskhov) of the game ranches in 13 districts of the

boreal forest subzone of Belarus and using a transect sampling method [1-2, 4].

498 samples of excrements, organs, and tissues collected from 119 killed animals were studied by applying recognized parasitology methods of study. In the statistical processing of the collected material in order to assess the parasitosis index, following indicators were used – infestation prevalence (IP) and infestation intensity (II).

**Table 1.** Prevalence and intensity indices of helminth invasion of a wild boar in fall-winter period in the subzone of oak-dark coniferous forests (N=297).

	Helminth species	Number of infested animals	Prevalence of invasion (%)	Intensity of invasion, min-max
	Class Trematoda Rudolphi, 1808			
1	<i>Dicrocoelium lanceatum</i>	7	10,9	1–6
	Class Cestoda Rudolphi, 1808			
2	<i>Spirometra erinacei-europeaei</i> , larvae	34	26,8	1–27
3	<i>Taenia hydatigena</i> , larvae	6	4,7	1–4
4	<i>Echinococcus granulosus</i> , larvae	8	12,5	1–8
	Class Nematoda Rudolphi, 1808			
5	<i>Trichocephalus suis</i>	102	34,4	2–25
6	<i>Oesophagostomum dentatum</i>	38	12,9	1–49
7	<i>Globocephalus urosululatus</i>	34	26,8	2–298
8	<i>Metastrongylus elongatus</i>	248	83,6	1–59
9	<i>Metastrongylus pudendotectus</i>	291	97,9	2–47
10	<i>Metastrongylus salmi</i>	181	61,1	1–40
11	<i>Ascaris suum</i>	53	17,8	1–6
12	<i>Physocephalus sexalatus</i>	15	11,8	3–98
13	<i>Trichinella spiralis</i> , larva	2	3,1	1–23
	Class Acanthocephala Rudolphi, 1801			
14	<i>Macracanthorhynchus hyrudinaceus</i>	5	1,7	1–4

In wild boars, the most widespread helminthiasis form is metastrongylosis with a infestation rate of 97.9% in adult animals and up to 100% in young animals. Metastrongylidae are represented by 3 species: *Metastrongylus pudendotectus* (Wostokow, 1905) (most common, IP 97.9%, II from 2 to 47 worms per animal), *Metastrongylus elongatus* (Dujardin, 1845) (IP 83.6%, II 1-59 worms per animal), and *Metastrongylus salmi* (Geddoelst, 1923) (IP 61.1%, II 1-40 worms per animal). From one to three *Metastrongylidae* species were found in a single wild boar individual. The share of infested animals in the population is the largest in the fall-winter period (up to 97.9%). By spring, the infestation percentage drops due to non-infested young animals and some adult wild boars getting naturally dewormed. In summer, IP rises to 78.2%. In winter, the *Metastrongylidae* infestation level is the highest and constant, as the wild boar no longer feeds on earthworms. By the end of winter, the *Metastrongylidae* infestation figures reduce significantly, and by the spring, infestation prevalence and intensity levels are the lowest. In summer, there is a sharp increase in wild boar infestation due to larval *Metastrongylidae* invasion from earthworms and a minor growth by fall. At the same time, in summer, the wild boar develops immunity, which prevents large numbers of *Metastrongylidae* from entering the lungs, and they stay in the lymphatic system. By winter, resistance decreases, the wild boar's health is weakened, larvae invade the lungs from the lymph nodes, and the infestation curve rises sharply. The intensity of infestation by these species can be regarded as low: 1 to 59 *Metastrongylus elongatus* worms per host animal have been recorded.

### 3. Result and Discussion

Epizootic status of wild artiodactyl animal helminthiasis and protozooasis. Helminthological studies revealed the infestation of wild boars with 14 species of helminths belonging to 4 helminthocenosis (table 1) by the greatest number of species (9), followed by tapeworms (3 species), trematodes, and acanthocephales (one species each).

Of other helminthiasis types, the prevalence of infestation with *Trichocephalus* and *Globocephalus* is high – 34.4% and 26.8%, respectively. The intensity of infestation with *Globocephalus* was an order of magnitude higher than that with other species and was up to 298 worms per host animal. Of the tapeworm species, sparganosis pathogens, larvae of *Spirometra erinacei-europeaei* (Rudolphi, 1819) were quite common with a low infestation intensity (IP 26.8%, II 1-27 worms per animal). Such frequent occurrence of sparganosis pathogens is more common in the south of Belarus, where favorable conditions exist for this helminth [1]. Other helminth species were much less common.

Assessment of the parasitological status of the wild boar habitats revealed that five intermediate *Metastrongylidae* hosts – earthworms – are infested with helminth larvae: *Dendrobaena octaedra*, *D. rubidus*, *Allolobophora caliginosa*, *Lumbricus terrestris*, *L. rubellus* (table 2). The overall average infestation rate was 17.3% in all collected worms, with an average infestation intensity of 25.8 larvae per earthworm. The maximum infestation intensity was 178 larvae. Earthworm infestation with *Metastrongylidae* larvae was the highest in mixed forests of coniferous and small-leaved deciduous tree species, where infestation prevalence ranged from 20.1% to 37.5% with an average of 28.8%, and in floodplain meadows (IP 19.4-28.0% with an average of 23.7%). Same biotopes had the highest infestation intensities of *Lumbricidae* – 40.1 and 37.4 larvae per worm, respectively. A lower degree of infestation was recorded in worms collected in alder forests (IP 17.2-22.4%, with an average of 19.8%) and spruce forests (IP 7.5-13.3%, with an

average of 10.4%), the lowest was observed in pine forests (IP 2.9-5.1%, with an average of 4.0%).

**Table 2.** Specific composition of earthworms and their infestation with *Metastrongylidae* in the biotopes of the boar habitat.

Biotope	Earthworm species	Mean density of lumbricids in biotope, specimen/m <sup>2</sup>	Mean prevalence of invasion, %	Mean intensity of invasion, specimen/species
Pine forests	<i>Dendrobaena octaedra</i> , <i>Lumbricus rubellus</i> ,	0,7	4,0	8,2
Spruce forests	<i>Dendrobaena octaedra</i> , <i>Octolasion lacteum</i> .	5,4	10,4	11,2
Mixed coniferous - small leaved forests	<i>Allolobophora caliginosa</i> , <i>Dendrodrilus rubidus</i> <i>Dendrobaena octaedra</i> <i>Allolobophora caliginosa</i> , <i>Octolasion lacteum</i> ,	17,1	28,8	40,1
Alder forests	<i>Lumbricus terrestris</i> , <i>Lumbricus rubellus</i> , <i>Octolasion lacteum</i> .	20,3	19,8	32,1
Floodplain meadows	<i>Dendrobaena octaedra</i> <i>Allolobophora caliginosa</i> , <i>Octolasion lacteum</i> , <i>Lumbricus rubellus</i> ,	11,2	23,7	37,4
On average foe subzone	<i>Lumbricus terrestris</i> ,	10,9	17,3	25,8

13 helminth species belonging to 3 classes (Trematoda, Nematoda, Cestoda) and 1 eimeria species belonging to the Sporozoa class were observed in moose (table 3). Nematodes are represented in the helminthocenosis by the greatest number of species (11), followed by tapeworms and

trematodes (one species each). In moose, the most common helminthiasis types are mecostocirrosis, with an infestation rate of up to 86.1% in adult animals and up to 97% in young animals, and strongyloidosis (IP 75.6%, II 9-91 worms per animal).

**Table 3.** Indices of helminth and Protozoa infestation prevalence and intensity in moose in the subzone of oak-dark coniferous forests (N= 115).

Helminth species	Number of infested animals	Prevalence of invasion (%)	Intensity of invasion, min-max
Class Trematoda, Rudolphi, 1808			
1 <i>Fasciola hepatica</i>	24	20,8	3-24
Class Nematoda, Rudolphi, 1808			
2 <i>Trichocephalus skrjabini</i>	18	15,6	4-15
3 <i>Cooperia oncophora</i>	9	7,8	1-3
4 <i>Oesophagostomum venulosum</i>	32	27,8	7-46
5 <i>Strongyloides papillosus</i>	87	75,6	9-91
6 <i>Haemonchus contortus</i>	11	9,6	2-14
7 <i>Nematodirus spathiger</i>	5	4,3	1-2
8 <i>Nematodirus filicollis</i>	17	14,7	3-19
9 <i>Bunostomum trigonocephalum</i>	4	3,4	1-2
10 <i>Gongylonema pulcarum</i>	20	17,4	4-15
11 <i>Mecistocirus digitatus</i>	99	86,1	7-94
12 <i>Ostertagia orlaffi</i>	6	5,2	1-2
Class Cestoda, Rudolphi, 1808			
13 <i>Echinococcus granulosus larvae</i>	3	2,6	1-2
Class Sporozoa, Leuckart, 1879			
14 <i>Eimeria zuernii</i>	4	3,5	21-29

Of the other helminthiasis, high prevalence of esophagostomosis, fascioliasis, gongylonemosis, and trichocephalic infestations was observed – 27.8%, 20.8%, 17.4%, and 15.6%, respectively. The intensity of infestation with *Oesophagostomum* was an order of magnitude higher than that with other species and was up to 46 worms per host animal. Less frequent were *Ostertagia* (IP 5.2%, II 1-2 worms), *Nematodirus* (IP 4.3%, II 1-2 worms), and *Bunostomum* (IP 3.4%, II 1-2 worms).

The cestode class is represented by the causative agent of echinococcosis – *Echinococcus granulosus* larvae (Batsch, 1786) with a low infestation intensity (IP 2.6%, II 1-2 worms).

Such frequent occurrence of echinococcosis pathogens is more common in the south of Belarus, where favorable conditions exist for this helminth. A single moose had simultaneously from 1 to 3 helminth species. A low infestation intensity was observed with the causative agent of eimeriosis – *Eimeria zuernii* (Nenez, 1989) (IP 3.5%, II 21-29 worms).

The highest species diversity (table 4) was found in young pine stands (6 to 8 species), the lowest – in aspen (3 species) and common alder stands (3) and abandoned farmland (1). In the examined biotopes, *Mecistocirus digitatus* (Linstow, 1906; Raillet et Henry, 1912), *Strongyloides papillosus* (Weld, 1856), and *Trichocephalus skrjabini* (Baskakow, 1924) dominated.

Echinococci and Eimeria were less common. Infestation prevalence was the highest in young pine stands (7-13.1%) and mixed spruce stands (4.7-7.9%) and the lowest on abandoned farmland (1.1-1.9%) and in aspen stands (2.1-3.2%).

**Table 4.** Indices of helminth and Protozoa infestation in moose in the territory GPU NP «Braslavsky Lakes», GPU «Berezinsky Biosphere Reserve» and GLKHU «Begomlsky Leskhoz».

	<b>Biotope</b>	<b>Number of species tested</b>	<b>Prevalence of invasion, %</b>	<b>Species of helminthes and Protozoa</b>
GPU NP «Braslavsky Lakes»				
1	Aspen forest (willow brushwood)	4	25,0	<i>Strongyloides papillosus</i> , <i>Mecistocirus digitatus</i> , <i>Trichocephalus skrajbini</i>
2	Young pine trees	9	11,1	<i>Strongyloides papillosus</i> , <i>Haemonchus contortus</i> , <i>Trichocephalus skrajbini</i> , <i>Mecistocirus digitatus</i> , <i>Gongylonema pulcarum</i> , <i>Fasciola hepatica</i>
3	Complex spruce forest	9	22,2	<i>Strongyloides papillosus</i> , <i>Gongylonema pulcarum</i> , <i>Mecistocirus digitatus</i> , <i>Fasciola hepatica</i>
4	Black alder forest	6	16,7	<i>Strongyloides papillosus</i> , <i>Gongylonema pulcarum</i> , <i>Mecistocirus digitatus</i>
GPU «Berezinsky Biosphere Reserve»				
5	Young pine trees	3	33,3	<i>Trichocephalus skrajbini</i> , <i>Mecistocirus digitatus</i> , <i>Gongylonema pulcarum</i> , <i>Fasciola hepatica</i> , <i>Cooperia oncophora</i> , <i>Ostertagia orlaffi</i>
6	Complex spruce forest	3	33,3	<i>Strongyloides papillosus</i> , <i>Mecistocirus digitatus</i> , <i>Ostertagia orlaffi</i> , <i>Echinococcus granulosus larvae</i>
7	Enclosure	7	28,6	<i>Trichocephalus skrajbini</i> , <i>Nematodirus filicollis</i> , <i>Ostertagia orlaffi</i> , <i>Haemonchus contortus</i> , <i>Oesophagostomum venulosum</i> , <i>Eimeria zuernii</i>
GLKHU «Begomlsky Leskhoz»				
8	Young pine trees	6	33,3	<i>Strongyloides papillosus</i> , <i>Haemonchus contortus</i> , <i>Nematodirus spathiger</i> , <i>Bunostomum trigonocephalum</i> , <i>Gongylonema pulcarum</i> , <i>Mecistocirus digitatus</i> , <i>Trichocephalus skrajbini</i> , <i>Fasciola hepatica</i>
9	Complex spruce forest	6	16,6	<i>Mecistocirus digitatus</i> , <i>Strongyloides papillosus</i> , <i>Haemonchus contortus</i>
10	Renewed deforested areas	3	33,3	<i>Nematodirus spathiger</i> , <i>Gongylonema pulcarum</i> , <i>Trichocephalus skrajbini</i>
11	Brushwood (abandoned farmland)	3	33,3	<i>Mecistocirus digitatus</i>
Total:		59		

In European roe deer, the most often observed species is *Chabertia* (73.1%); in 60.9% of the animals *Trichocephalus*, *Oesophagostomum*, and *Bunostomum* (26.6%) have been observed simultaneously. A health species in 74.2% of the population.

Parasitosis prevention in wild artiodactyl animals. Based on a study of the properties of existing anthelmintics, timbendazole (22% fenbendazole granulate) and the new preparation «Polyparacid» – a complex broad-spectrum anthelmintic with an immunomodulatory effect – were chosen

for trials on wild boar feeding sites.

Trials on experimental sites demonstrated that 22% timbendazole at 45 mg/kg and the new preparation «Polyparacid» at 50 mg/kg, when administered with food to groups of animals for 2 consecutive days as a treatment of metastrongylosis and associative helminthiasis of the gastrointestinal tract in wild boars, had a therapeutic efficacy of 97-100%.

The efficacy of «Polyparacid» when administered to wild boars as a single dose with feed in a real-life setting was 96.2% in ascaridosis, 88.6% in trichocephalosis, 100% in oesophagostomy and metastrongylosis.

The new preparation «Pentavet» – a complex broad-spectrum anthelmintic with an immunomodulatory effect – was chosen for trials on feeding sites and in enclosures to compare it with 20% tetramizole granulate and thymbendazole (22% fenbendazole granulate).

Trials of the selected drugs on experimental sites demonstrated that 22% thymbendazole at 50 mg/kg and the new preparation «Pentavet» at 50 mg/kg, when administered as a single to moose with fodder, bread (in enclosures), and salt bricks with aspen wood (Figure 1) as a treatment of mecistocirosis, nematodirosis, trichocephalosis, and monisiosis, had a therapeutic efficacy of 90-100%.



**Figure 1.** Feeding site for mooses in Begomlsky Leskhoz.

Parasitological studies of «Pentavet» in a real-life setting showed that its efficacy when administered to moose as a single dose with feed was 100% in mecistocirosis and nematodirosis, 98.5% in trichocephalosis, and 100% in mecistocirosis and monisiosis at Berezinsky Biosphere Reserve.

## 4. Conclusions

Helminthological and protozoological investigation revealed wild boar infestation with 14 helminth species belonging to four classes; moose – with 13 helminth species from three classes and one species of Eimeria; European roe deer – with 11 helminth species from three classes and 1 species of Eimeria. In terms of species the helminths belonging to Nematoda class predominate in helminthocenosis. The most widespread helminthosis in wild boar is metastrongylosis, in moose is Mecistocirosis, in European roe deer is Trichostrongylosis.

It was established that the anthelmintics «Polyparacid»

and «Pentavet» administered with food one time to groups of wild artiodactyl animals at the dose of 50 mg/kg in parasitoses showed 90-100% therapeutic efficacy. Besides these new complex preparations have an immunostimulating effect. As to «22% fenbendazole granulate», «20% tetramizole granulate». They have an immunosuppressive effect.

We think that in future anti-parasitic measures should be taken with application of anthelmintics with an immunomodulating effect.

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