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Original Scientific Article

DETERMINATION OF ENDOPARASITES BY FAECAL EXAMINATION IN THE WILD BOAR POPULATION IN VOJVODINA (SERBIA)

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ABSTRACT

The aim of our study was to determine whether and to what extent certain species of helminths and protozoa are present in the wild boar population living in hunting grounds in Vojvodina. For this purpose, 52 faecal samples of hunted wild boars (aged 6 months to 2 years) were examined. Examination of the faeces was performed using classical coproscopic laboratory methods. The following parasite species were identified: *Metastrongylus* spp. *Ascaris suum, Trichuris suis, Physocephalus sexalatus, Strongyloides ransomi, Oesophagostomum* sp. */ Globocephalus* sp., *Hyostrongylus rubidus, Gnathostoma hispidum, Eimeria deblecki* and *Eimeria suis*. The obtained results from this study indicated that wild boars are a potential reservoir of a variety of endoparasites, thus endangering the surrounding ecosystem.

Key words: wild boar, endoparasites, Vojvodina, faecal examination

INTRODUCTION

Wild animals are an integral part of the natural world. Continuous monitoring and control of wild animals is necessary in order to assess the extent of human impact on nature and, on the other hand, the impact of wild animals on the natural environment and humans. Fluctuations in population size of particular wild animal species often indicate an excessive interference by men in nature, which may be manifested by changes in the susceptibility to certain diseases (15, 19).

Within the European free-living wild animal population, the wild boar (*Sus scrofa*) deserves special attention as both an important part of hunting husbandry and as a distant ancestor of the domestic pig (6, 36). Wild boars are a potential reservoir of a variety of bacterial, viral and parasitic diseases endangering the health of domestic swine, especially those grown in an extensive system, that is, on pasture (22, 26).

Presence of parasites and their impact on the health of wild boars in Serbia hunting areas has not yet been comprehensively investigated. We have only a limited number of papers published on this topic (4, 26, 27, 34, 35). Because of the scarcity of such data in the literature, the present study was undertaken to evaluate helminths and protozoa species and their prevalence in hunted wild boars from nine hunting grounds in Vojvodina. Besides that, we would also like to find out if there is a difference in the presence and prevalence of types of parasites in relation to sex and age of hunting animals, as well as to animals hunting on open and closed hunting grounds.

MATERIAL AND METHODS

Study area

Vojvodina is situated in the northern part of Serbia.The region is divided by the Danube and Tisa rivers into: Bačka in the northwest, Banat

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Table 1. Examined hunting grounds and number of samples

Name	Characteristic	Surface Area	No of sample
Titel	Encompassing hunting grounds nearby Tisza, Danube and Begej rivers	39637 ha	12
Plavna (Bač)	Lowland type hunting-ground with height between 80 and 87 metres above sea level, hunting ground bordering the Danube fen	2619 ha	8
Koviljski rit	Lowland type hunting-ground with height between 78 and 84 metres above sea level. Greater part of it is under forests	1955 ha	6
Bosutske forest	Hunting ground bordering Bosutski rit	12274 ha	6
Kućine Sid	Lowland type hunting-ground with height between 79 and 80 metres above sea level. It is almost completely under forests	1955 ha	4
Vranjak – Višnjićevo, Sid	Lowland type hunting-ground with height between 79 and 80 metres above sea level	12274 ha	4
Lower Danube "Pancevo"	Includes a large water surface of the Danube and marshy area around Danube aits.	3526.59 ha	4
Deliblato Sandpit, Pancevo	This is a lowland type hunting-ground with height between 70 and 85 metres above sea level and lies close to the Danube (Dragićev hat are enclosed on 1,850 ha)	31036.55 ha	4
Hrastovaca forests, Subotica	This is a lowland type hunting-ground with height ranging between 114 and 143 metres above sea level. Enclosed part comprises 500 ha of forests, 35 ha of pastures and meadows and 8 ha of other vegetation	4760 ha	4

in the east and Srem in the southwest. For testing purposes, we used wild boar faeces collected from 9 hunting grounds in Vojvodina during the autumn hunting season 2015. Samples originated from the following hunting grounds: from Bačka hunting grounds Titel, Hrastovača forest (Subotica) and Plavna (Bač), from Srem: Koviljski rit, Bosutske forest, Kućine-Šid, and Vranjak-Višnjićevo (Šid) and from Banat: Lower Danube "Pančevo" and Deliblato Sandpit, Pančevo. The data concerning the hunting grounds and the number of samples taken from them are shown in Table 1.

Faecal sample collection and examination

During the research period, we collected a total of 52 faeces samples from hunted wild boar. The animals that the samples were collected from were categorized according to the animals' age as a young (6 - 8 months) or an older group (over 8 months), while according to sex as female or male animals. After the evacuation, the samples from the various segments of the hose were collected and packaged in PVC bags that were marked on the outside. The following data were specified: sex and age of animals, hunting ground and date of hunting. All faecal samples were examined using a sedimentation and flotation method with saturated solution of $ZnSO_4$ as previously described (10, 16, 31, 32).

We performed the determination of helminth eggs and protozoon oocysts based on morphometric characteristic using keys by Beugnet et al. (5), Euzeby (10), Kaufman (16), Kozlov (17) and Soulsby (32). Correct identification of *Oesophagostomum* sp. and *Globocephalus urosubulatus* with the coproscopic methods and without rearing larvae can be burdened with the possibility of too much error. For this reason we used eggs, whose morphology and biometrics allowed for classifying them as one of the two mentioned genera *Oesophagostomum* sp. / *Globocephalus* sp. In infected animals helminth eggs in per gram faecal sample (EPG) were determined by using the McMaster technique. Degrees of infection were assessed according to EPG and 50-200 eggs were was considered to be a low rate of infection, up to 300 was moderate and more than 300 was considered high.

The data obtained was analyzed using Chi-squere test (χ^2) to determine if the prevalence and intensity of infection depended on age, sex of examined wild boar and types of hunting grounds. In all analyses, the confidence level was held at 95%.

RESULTS

A total of 52 faecal samples were examined. The results of the coproscopy revealed that 46 (88.46 %) wild boars harboured parasitic infections. According to the faecal examination, the following parasite species were identified: *Metastrongylus* sp., *Strongyloides* sp., *Oesophagostomum* sp./ *Globocephalus* sp., *Hyostrongylus rubidus, Gnathostoma hispidum, Ascaris suum, Trichuris suis, Physocephalus sexalatus, Eimeria deblecki* and *Eimeria suis*. The prevalence rates and EPG values of related parasite species were presented in Table 2.

Table 2. Prevalence and EPG value of parasites in the investigated samples from 52 wild boars

Parasites species	Examined animals		
	infected	%	EPG
Metastrongylus spp.	46	88.46	498
Ascaris suum	16	30.76	3290
Trichuris suis	11	21.15	165
Physocephalus sexalatus	3	05.76	57
Strongyloides ransomi	11	21.15	121
Oesophagostomum sp. / *Globocephalus* sp.	16	30.76	479
Hyostrongylus rubidus	7	13.46	110
Gnathostoma hispidum	6	11.53	198
Eimeria deblecki	11	21.15	192
Eimeria suis	10	19.23	217

In relation to the age of animals, our results suggested a higher prevalence of various parasite species in adult wild boars as compared to the younger ones. On the other hand, the extent of the infection was greater in younger than in adult wild boars, which is explained by the development of the immune response to certain parasitic species. In both age categories, *Metastrongyloidea* were the dominant parasites. The results of the coproscopic examination of wild boars in relation to age are presented in Table 3.

There was no significant age-related difference in the prevalence of parasite species. It was found that in younger animals the intensity of infection was lower than in older animals. However, statistically the intensity of infection did not significantly differbetween younger and older wild boars. Out of the total 46 infected wild boars, 16 (84.21%) were young and 33 (90.90%) were adult animals (Table 4).

Table 3. Intensity of infections in relation to the age of the wild boars

Examined group	Total	Presence of parasites		
		Parasites species	infected	%
Younger (6-8 month)	19	*Metastrongylus* spp.	16	84.21
		Ascaris suum	12	63.15
		Trichuris suis	4	21.05
		Strongyloides ransomi	6	31.57
		Oesophagostomum sp. / *Globocephalus* sp.	3	15.78
		Hyostrongylus rubidus	1	05.26
		Gnathostoma hispidum	2	10.52
		Eimeria deblecki	9	47.36
		Eimeria suis	7	36.84
Older (> 8 month)	33	*Metastrongylus* spp.	30	90.90
		Ascaris suum	4	12.12
		Trichuris suis	7	21.21
		Physocephalus sexalatus	3	09.09
		Strongyloides ransomi	5	15.15
		Oesophagostomum sp. / *Globocephalus* sp.	7	21.21
		Hyostrongylus rubidus	6	18.18
		Gnathostoma hispidum	4	12.12
		Eimeria deblecki	2	06.06
		Eimeria suis	3	09.09

Table 4. Intensity of infections in relation to the age of the wild boars

Age	examined			Intensity of infection					
				Low		Moderate		High	
	total	infected	%	no	%	no	%	no	%
Young	19	16	84.21	3	15.78	9	47.36	4	21.05
Adult	33	30	90.90	7	21.21	20	60.60	3	09.09

Table 5. Mixed infection in relation to the age of the wild boars

Age	examined	Number of nematode species						Total
		two	%	three	%	four	%	
Young	19	6	31.57	7	36.84	3	15.78	16
Adult	33	4	12.12	14	42.42	2	06.06	20

The results of the faecal examination revealed a greater number of parasite eggs in young wild boars than in adult animals.

Infection with only one nematode genus was found in 10 wild boars – 4 young and 6 adult animals. Mixed infection was observed in 36 animals. In young animals, mixed infection occured in 84.21% (16/19) and in 90.90 % (20/33) of adult wild boars. Infection with two species was determined in 10 (19.23%) faecal samples, with three species were 21 (40.38) faecal samples and with four species were 5 (09.61%) faecal samples (Table 5). Adult animals were infected with more parasite species than the young and as such presented a potential source of infection and contaminants of hunting grounds.

Chi-square test analysis revealed that mixed infections did not significantly differ between younger and older wild boars.

The examination encompassed 30 male and 22 female samples originating from hunted animals. The results revealed no differences in the types of parasites with respect to the sex of the animals. Prevalence of infection was 95.45% in male animals (21/22) and 83.33% (25/30) in female animals (Table 6).

Table 6. Prevalence of parasites according to sex of animals

Examined group	Presence of parasites			
	Parasites species	**examined**	**infected**	**%**
Female	*Metastrongylus* spp.	22	21	95.45
	Ascaris suum	22	6	27.27
	Trichuris suis	22	5	22.72
	Physocephalus sexalatus	22	2	09.09
	Strongyloides ransomi	22	6	27.27
	Oesophagostomum sp. / *Globocephalus* sp.	22	7	31.81
	Hyostrongylus rubidus	22	2	09.09
	Gnathostoma hispidum	22	2	09.09
	Eimeria deblecki	22	4	18.18
	Eimeria suis	22	7	31.81
Male	*Metastrongylus* spp.	30	25	83.33
	Ascaris suum	30	10	33.33
	Trichuris suis	30	7	23.33
	Physocephalus sexalatus	30	3	10.00
	Strongyloides ransomi	30	5	16.66
	Oesophagostomum sp. / *Globocephalus* sp.	30	10	23.33
	Hyostrongylus rubidus	30	5	16.66
	Gnathostoma hispidum	30	4	13.33
	Echinococcus granulosus (larvae)	30	6	20.00
	Eimeria deblecki	30	7	23.33
	Eimeria suis	30	3	10.00

Table 7. Intensity of infection with parasites in relation to the sex of the wild boars

Sex	examined			Intensity of infection					
				Low		Moderate		High	
	total	infected	%	no	%	no	%	no	%
Male	22	21	95.45	4	18.18	15	63.63	2	09.09
Female	30	25	83.33	4	01.33	14	46.66	7	23.33

Table 8. Mixed infections in relation to the sex of the wild boars

Sex	examined	number of nematode genus						Total
		two	%	three	%	four	%	
Male	22	4	18.18	4	18.18	7	31.88	15
Female	30	9	30.00	11	36.66	1	03.33	21

Intensity of infection according to the sex of animals is presented in Table 7.

There was no evidence for sex-related differences in the prevalence and intensity of parasite species.

Infection with only one nematode genus was found in 4 female and 6 male animals. Meanwhile, mixed infections were observed in 36 animals. In female animals mixed infections occured in 68/61% (15/22) and in male wild boars the number was 70.00 % (21/30) (Table 8).

The statistical analysis showed that the sex of wild boars had an influence on infection with four parasite species $P<0.05$. Intensity of mixed infections did not significantly differ with regards to infection with two and three parasites species.

DISCUSION

During our examination, the most abundant parasite species was *Metastongylus* sp. A high percentage of *Metastongylus* species found in wild boars was reported in France (13), Germany (18), Spain (8) and Poland (28, 29). Lungworms also usually occurred outside the European continent, such as in Turkey (30), Iran (9, 31) and China (37). These findings indicate a wide global distribution of transient hosts for these parasites - Earthworm („rain worm“) genera *Eisenia, Allolobophora, Dendrobaena, Lumbricus, Octolasium* or *Heledrillus*, which are commonly part of the diet of wild pigs (23). Research performed in several regions of Serbia - Belgrade, Branicevski and Severnobacki (4, 26, 34, 35) demonstrated a high prevalence of *Metastrongylus* infection in wild boars, though the prevalence of particular species varied from area to area. These findings concerning the *Metastrongylus* species is similar to our result (88.46% of processed material). *Metastrongylus pudendotectus* was the dominant species in the northern areas of Serbia, whereas *M.apri* dominated in the south. Similar findings were recorded in domestic pigs in organic production (23).

The European research on wild boar helminths resulted in a list of 30 species, encompassing flukes, tapeworms, nematodes, and acanthocephalans, as well as their larvae. Examinations performed in Germany (2, 3, 18), France (13), Hungary (35) and in Czech Republic and Slovakia (1, 21) indicated that the helminth and protozoa species which we found usually occurred in wild boars in Europe. The importance of testing wild boars for the presence of endoparasites has been gaining increasing attention taking into account the importance of parasites for domestic pigs and the losses and damage they can cause, as well as the problems related to management and its effects on pig production. According to the reports of several authors (19, 22, 30, 37), it is apparent that the parasitic fauna of domestic pigs is identical with the corresponding fauna of wild boars. These findings suggest that there is a possibility of mutual transmission of parasites between the two groups of animals, which largely depends on the production and husbandry system. Some authors (1, 19, 23, 25) reported that the presence of parasites affects the production of pigs and that pigs raised in an open system or an extensive production system are potential reservoirs of various parasitic pathogens that continuously circulate in the particular area of natural environment of pigs. This statement is supported by the global presence of some parasitic

species such as *Ascaris suum*, *Trichuris suis*, *Oesophagostomum* spp, *Metastromngylus* spp. or *Macracanthorhynchus hirudinaceus* (23, 24, 25). Similar problems occur in organic pig production, which does not implicate the application of preventive anthelmintic drugs (26).

Problems related to the presence of parasites in wild pigs are very similar with problems related to parasitosis in swine production. Consequently, continuous monitoring and control of wild boar population is highly important for this sector of animal husbandry.

The problem with the parasitic fauna of wild pigs in relation to their habitats has been present in several studies. Jarvis et al. (14) reported on testing of wild boar for the presence of parasites in an isolated territory in Estonia, i.e. on an island. Popiołek et al. (29) examined the influence of natural and breeding conditions of helminths of wild boar (*Sus scrofa L.*) in Lubin Forest (Poland). The presence of seven species of helminths was established, including several *Metastrongylus* species, *Ascaris suum*, *Trichuris suis*, *Dicrocelium dendricum* and larvae of *Tenia hydatigena*. The most prevalent finding was the presence of lung nematodes (82% of examined animals). Similar results were obtained by comparing the findings of parasites in pigs held in free hold and wild boar in the same areas (26, 34, 35).

Jarvis et al. (14) noticed that natural barriers, that is, sufficient distance of the island from the mainland, prevented the transmission of other types of parasites, thus the spectrum of parasites on the island was lesser than the parasitic fauna of wild pigs on the mainland. This natural barrier particularly affects biohelminths, whose intermediate hosts are not globally distributed, yet depend on the type of habitat and its ecological valence (prevalence of dry or moist habitats, microclimate, flora and fauna of the habitat, the impact of biotic factors, etc.). During our examination at various hunting grounds in Vojvodina, we concluded that there was no fundamental difference in the fauna of the parasite, though there were natural barriers like rivers in some hunting grounds.

The importance of territorial isolation of the wild boars and its relation to the parasitic fauna was addressed in the study of Fernandez-de-Mera et al. (11). The authors investigated the presence of parasites in the wild boar population in central Spain and compared it with findings in the same animal species in France, which were intended for import. The obtained results confirmed that the movement of animals would pose an increased risk because of significant differences in the number and species of identified parasites in animals from different regions. In imported wild boars, different parasitic species (*Oesophagostomum dentatum*, *Ascaris suum* and *Trichuris suis*) were identified, which had not existed in central Spain. Similar observations were reported in Poland, where the types and prevalence of helminths in wild boars in the northwestern region of Poland (21) were different from the findings in central Poland (7). Such findings correspond with the results reported in Serbia, where helminth fauna in wild boars significantly differed between the hunting grounds in North Backa, Braničevo (South Serbia) and Belgrade area (Central Serbia) (4, 26, 27, 34).

Examination of the presence of parasites with regard to animals' gender did not reveal any differences in parasitic fauna between female and male animals (Table 3). Our results are thus consistent with the reports of other authors (2, 3, 12, 14, 18).

CONCLUSION

Our research revealed the presence of parasitic fauna in wild boar (*Sus scrofa*) population in the nine investigated hunting grounds. The detection of the same parasites species in different hunting grounds, which are separated with rivers, canals and hills, is very important. Because of that, we may conclude that natural barriers do not prevent the spread of parasitic fauna in the examined regions (Vojvodina). Our results indicate that parasites are uniformly expanded in all examined groups.

Damages associated with the presence of parasites in wild animals are difficult to estimate. This problem requires a systematic and continuous approach in the future. Damages resulting from reduced weight, lower fertility rates, burdening of the immune system and increased susceptibility to other infectious diseases can be assessed directly or indirectly. In that respect, development of an appropriate methodology adjusted to our conditions is necessary for determining the actual damage in this segment of the ecosystem and improving the wildlife welfare.

CONFLICT OF INTEREST

The authors declared that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

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