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WILD BOAR - THE SOURCE OF HUMAN CONTAMINATION WITH *TRICHINELLA SPP*. IN ROMANIA

MISTREȚUL – SURSA DE CONTAMINARE UMANĂ CU *TRICHINELLA SPP.* ÎN ROMÂNIA

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ABSTRACT

Trichinellosis remains a cosmopolitan parasitosis, affecting mammals, birds, and humans, and in whose etiology species of the genus *Trichinella* are involved. In Romania, culinary traditions and customs favor the transmission of the disease, through the inadequate consumption of insufficiently prepared meat from the domestic pig or from the animals of the game (wild boar). In domestic and game animals, after slaughter, the diagnosis is established following examination of the meat, carried out by trichinoscopy and/or artificial digestion. The investigation of a number of 1364 wild boars hunted on 14 hunting grounds of Vâlcea County (Romania),in the period 2017-2019, revealed the presence of the larvae of *Trichinella spp*. obtained by artificial digestion in three (0.21%) of the total number of monitored animals. The human consumer and hunters should be warned of the risk of infestation, especially in situations where the meat of the hunted animals escapes veterinary control.

Keywords: wild boar, Romania, *Trichinella spp*., prevalence

REZUMAT

Trichineloza rămâne o parazitoză cosmopolită, care afectează mamiferele, păsările și omul și în a cărei etiologie sunt implicate specii ale genului *Trichinella*. În România, tradițiile și obiceiurile culinare favorizează transmiterea bolii prin consumul neadecvat de carne insuficient preparată termic provenită de la porcul domestic sau de la animalele de vânat (mistreț). La animalele domestice și de vânat, după sacrificare, diagnosticul se stabilește în urma examinării cărnii, realizat prin trichineloscopie și / sau digestie artificială. Investigarea unui număr de 1364 mistreți vânați pe 14 fonduri de vânătoare ale județului Vâlcea (România), în perioada 2017-2019, a relevat prezența larvelor de *Trichinella spp*. obținute prin digestie artificială la trei (0,21%) din numărul total de animale monitorizate. Consumatorul uman și vânătorii în mod deosebit trebuie atenționați asupra riscului infestării, mai ales în situațiile în care carnea animalelor vânate scapă controlului veterinar.

Cuvinte cheie: mistreț, Romania, *Trichinella spp*., prevalență

Trichinellosis remains a cosmopolitan parasitosis, diagnosed in mammals, birds, and humans, produced by nematodes belonging to the family *Trichinellidae.* The most important source of human infection worldwide is the domestic pig, but in Europe, horse and wild boar meat have a significant role in transmission (14, 32). Studies conducted in Romania claim that trichinellosis is one of the most serious zoonotic diseases, with a high percentage of prevalence compared to other countries (7, 26).

The importance of trichinoscopy and artificial digestion, the evaluation of epidemiological relations and the knowledge of the trophic links in the sylvatic cycle that relates to the domestic cycle are the main coordinates of the surveillance of this zoonosis (5, 12). The two domestic and sylvatic cycles are not separated. Between them there are connecting links and variables from one area to another, wildlife has an important role in the transmission of trichinellosis to domestic fauna (3, 8, 20, 28).

A review on the ecology, epidemiology, and parasitological control of trichinellosis brings, once again, to the attention of specialists the warning of increasing the risk of infestation in humans through the inappropriate consumption of game meat (wild boar, bear, deer, walrus), as well as the importance of hygiene and temperature conditions that undoubtedly influence the appearance of the disease (10, 18, 24, 31, 35).

In Romania, culinary traditions and customs favor the transmission of the disease, through the inadequate consumption of insufficiently prepared meat from the domestic pig or the game animals (wild boar) (6, 22, 23, 25, 27).

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Besides diseases with parasitic etiology, (6, 15, 23) both domestic pigs and wild boars are receptive to a number of infectious diseases, with viral etiology (1, 16,) and bacterial etiology, (15, 17, 19, 29, 30), which influences the health status of the population of domestic pigs and wild boars.

In domestic and game animals, after slaughter, the diagnosis is established following examination of the meat, carried out by trichinoscopy and/or artificial digestion. The method of artificial digestion is considered the standard golden method used for the direct identification of the larvae of *Trichinella spp*. (2, 11, 13) and is mandatory by European legislation (36).

Considering that, after the domestic pig, the wild boar meat represents the second most important source of human infestation, the purpose of the study was (i) to identify by the artificial digestion the cases of trichinellosis in the wild boars from the hunting grounds in Vâlcea County (Romania) and monitored for a period of three years and (ii) to assess the risk of human contamination.

MATERIAL AND METHOD

The study was carried out over a period of three years (2017-2019) when 1364 wild boars hunted on 14 hunting grounds of Vâlcea County (Romania) were investigated (Fig. 2; Table 1). The biological material collected consisted of flexor and extensor muscles of the anterior limb as places of predilection for *Trichinella* larvae. From these muscle groups, 30 grams were collected from each animal. Portions of musculature in the amount of 5 g from each animal were digested according to the method of artificial digestion (9).

The method of artificial digestion was used in the County Sanitary-Veterinary Laboratory (CSVL) Vâlcea, according to the EU Regulation 1375/2015, for the examination of samples of striated muscle tissue coming from wild boars.

The main stages of artificial digestion comprised the following stages (Fig. 1):

1. the actual digestion of the muscle tissue and the release of the larvae, which consists in the immersion of the sample in the digestive fluid, the continuous homogenization of the meat sample under continuous stirring (with the magnetic stirrer) at the temperature of 44-46° C, for 30-60 minutes, until the disappearance of the muscle fragments.

2. The isolation of the larvae by sedimentation involved filtering the digestion fluid, after which it was left at rest for 30 minutes. The filtrate was then placed in a graduated test tube (40 ml), which was left to rest for 10 minutes. For examination, 10 ml of the filtrate was retained.

3. The highlighting and counting of the larvae were done at the trichineloscope with a horizontal mass. At each positive sample, several 5 to 10 individual *Trichinella* larvae were selected, washed with cold water several times, and stored in Ethanol of 70 volumes for further analysis of molecular biology.

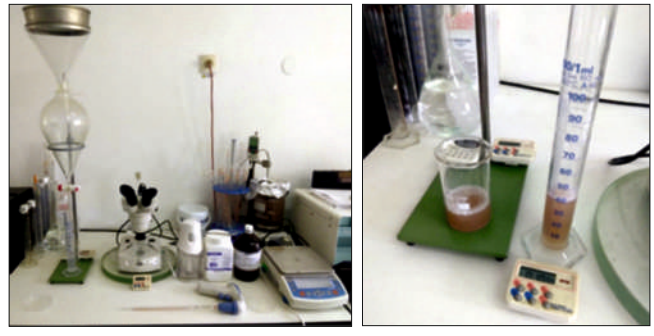

Fig. 1. Method of artificial digestion

Table 1

Hunting grounds and number of samples collected (wild boars) in the period 2017-2019

Name of the hunting stock	Number of samples
Băbuiești	216
Călimanești	23
Goranu	17
Bistrița	65
Romani	43
Colţeşti	15
Nicolae Bălcescu	41
Dănice1	28
Malaia	25
Dobruşa	432
Manăileasa	26
Racoasa	211
Latoriţa	17
Padina	205
Total	**1364**

RESULTS AND DISCUSSION

Muscle samples collected from a number of 1364 wild boars from 14 hunting grounds from Vâlcea County (Romania) and examined by the method of artificial digestion, within CSVL Vâlcea, were positive to the infestation with *Trichinella spp*. in three wild boars (Fig. 3). These positive animals (3) belong to 2 hunting grounds: Padina and Malaia. The data of the samples examined and the distribution by year and months are given in Table 2. It can be noted that the prevalence of trichinellosis in wild boar herds in Vâlcea County, in the period 2017-2019, was 0.21% (3/1364).

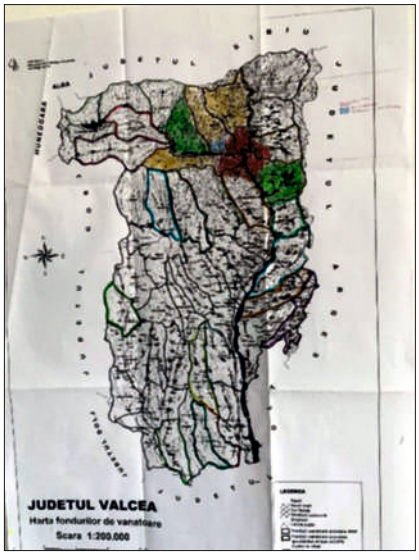

Fig. 2. Hunting grounds from Vâlcea County

Fig. 3. Artificial digestion – larvae of *Trichinella spp.*

The three animals infested with the nematode *Trichinella spp*. were diagnosed in the same year, 2018, in consecutive months, November and December, during the peak season of hunting allowed in the wild boar and around the winter holidays when the consumption of domestic pig/wild boar meat is at its peak.

Comparing our results with those of the authors in the country and abroad, we notice that, in Romania, Blaga et al. (2009) have identified a prevalence of trichinellosis in wild boars, of nine cases / 10^3 wild boars tested (4). If we report the results obtained by Blaga et al. (2009) on the data from the Eastern European area, we notice that the infestation rate of wild boars is higher in our country than in Poland and Latvia (14, 21, 34). In Latvia, the evaluation of the trichinellosis prevalence over a period of 37 years was 2.5%, *Trichinella britovi* being the species involved in the etiology. During the study period, the population of wild boars in Latvia increased 4.9 times and hunting 9.7 times (21).

The samples collected from wild boar populations (*Sus scrofa*) from Bihor County, Romania, were examined by the method of artificial digestion, ELISA, and Western Blot. The results of serological examinations revealed the existence of the nematode *Trichinella spp.* in the wild boar in this area of our country. Moreover, the authors warn people who prefer to consume wild boar meat as a source of infestation with larvae of *Trichinella spp.* even when they are found in low numbers in infested meat (5).

In Portugal, infestations of hunters who consumed uncontrolled wild boar meat through trichinoscopy and artificial digestion and which was infested with the species *T. britovi* have been reported (33).

Table 2

The distribution by years and months, respectively the results obtained from the artificial digestion performed at CSVL Vâlcea, Romania

Month	Year			Result						Total samples
				Negative			*Positive*			
	2017	2018	2019	2017	2018	2019	2017	2018	2019	
January	41	47	58	41	47	58	0	0	0	146
February	12	7	19	12	7	19	0	0	0	38
March	11	14	14	11	14	14	0	0	0	39
April	4	6	17	4	6	17	0	0	0	27
May	3	6	5	3	6	5	0	0	0	14
June	11	12	27	11	12	27	0	0	0	50
July	16	24	46	16	24	46	0	0	0	86
August	13	14	77	13	14	77	0	0	0	104
September	51	37	73	51	37	73	0	0	0	161
October	23	31	84	23	31	84	0	0	0	138
November	98	124	87	98	123	87	0	1	0	309
December	51	56	145	51	54	145	0	2	0	252
TOTAL	334	378	652	334	375	652	0	3	0	**1364**

Although the results of the study highlight the presence of the nematode as just one "episode" from November to December of 2018, in three of the 1364 wild boars examined by digestion artificial for a period of three years, measures are required to monitor the cases of trichinellosis in Vâlcea County and careful surveillance of the controls performed on wild boar meat and, not only.

CONCLUSIONS

The artificial digestion allowed highlighting of the larvae of *Trichinella spp.* in the muscle of wild boars in Vâlcea County, Romania. The prevalence of trichinellosis in wild boars monitored over a period of three years and examined by artificial digestion was 0.21%. In Romania, trichinellosis remains a zoonosis with a high level of infestation, as a result, the assessment of risk factors and the monitoring of the nematode's presence in wild boar and equally in other wild animals, a reservoir of *Trichinella spp.,* could significantly reduce the prevalence of the parasite.

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