

## Trichinella britovi, Etiologic Agent of Trichinellosis in Wild Carnivores in Bulgaria

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Trichinellosis is one of the most common helminthozoonoses in Bulgaria. While in previous years the main source of infection were wild boars, today there is a shift in the tendency towards the domestic breed of swine as well as to the consumption of meat from bears, badgers, etc. which constitute a major link in the epidemiological chain of Trichinellosis. The objective of the present study was to better characterize the *Trichinella* genotypes found in wild boars and wildlife carnivores from different regions in Bulgaria. A molecular characterization by PCR of 24 *Trichinella* isolates from a variety of game has been performed. The prevalence of *T. britovi* among the wild animals in the country was confirmed.

*Key words: Trichinella britovi, species determination, polymerase chain reaction (PCR)*

### Introduction

Trichinellosis is a food-borne parasitic disease caused by nematodes of the genus *Trichinella* which are zoonotic parasites with cosmopolitan distribution and major socio-economic importance. Human infection is acquired through consumption of undercooked meat from domestic or wild animal. Penetration of trichinella larvae into striated skeletal muscle cells results in ultrastructural and metabolic changes [2]. Migration of larvae causes the typical symptoms and signs of the disease. The severity of the symptoms depends on the number of ingested trichinella larvae and the immune response of the host [1, 2]. Based on epidemiological observation, [3] it is assumed that approximately 100 and 300 larvae of *Trichinella spiralis* cause disease and an intake of 1,000 to 3,000 or more larvae can cause severe disease. The incubation period for development of the disease after consumption of contaminated meat or meat products ranges from 1 to 51 days [5]. The main reason for global distribution of this parasite is the fact that trichinella has a wide range of hosts and can infect more than 150 species of animals, including humans. It is evaluated that around 11 million people may be infected by trichinella [4]. Until recently it has been accepted that the cause of trichinellosis in all animal species was the same – *Trichinella spiralis*. However, the implementation of molecular biology methods proved the existence of a significant diversity of species and differences in the taxonomy, the geographical dissemination and the epidemiology of trichinellosis [10]. Today, nine species and three genotypes are recognized in this genus [6, 9].

In Bulgaria, Kurdova et al. [7] investigated parasite isolates obtained upon 15 epidemic outbreaks. Received results showed the predominance of *T. britovi* (etioloical agent in 10 outbreaks) while *T. spiralis* was found in 5 outbreaks. For the period from 2008 to 2014, 29 outbreaks were recorded in Bulgaria. Of 1 670 people who consumed meat or meat products contaminated with trichinella larvae, 710 were infected. The annual incidence of human trichinellosis for the period varied from 0.22 to 5.82 per 100,000 population [12]. An in-depth analysis based on molecular research, performed with 120 trichinella isolates was made by Lalkovski [8]. He found the appearance of two species: *Trichinella britovi* and *Trichinella spiralis*. *T. britovi* predominated over *T. spiralis* – 113 isolates (94.17%) and 7 (5.83%) respectively. Both species were identified in domestic pigs and wild boars, with *T. britovi*:*T. spiralis* ratios in 45:1 in wild boars and 1:1 in domestic pigs. *T. britovi* was the geographically more widespread species. (**Fig. 1**).



**Fig.1.** *Trichinella britovi* larvae within the diaphragm muscle of mouse.

## Materials and Methods

The studies were carried out following the reference method of digestion using a magnetic stirrer according to Regulation (EU) 2015/1375. Muscle larvae were preserved in ethanol. *Trichinella spp.* larvae were identified at the species level by multiplex PCR, which has been developed for the simple and unequivocal differentiation of trichinella species and genotypes. Partial DNA sequence data were generated from the internal transcribed spacers ITS1 and ITS2 and from the expansion segment V region of the rRNA repeat from different trichinella species and genotypes.

Twenty four *Trichinella spp.* isolates were tested, typified and sorted according to the laboratory protocol of The European Union Reference Laboratory for Parasites (Rome, Italy).

## Results

A total number of 24 isolates typified and sorted as followed:

**Table 1.** The Data obtained from the multiplex PCR.

N	Source of infection	Type of muscle in the original host	Species identification PCR	Number of larvae/g diaphragm	Number of passages <i>in vivo</i>	Place of origin locality	Latitude longitude
1	w. boar	masseter diaphragm	Tb	4	2	Elhovo	42° 31'N 26° 71' E
2	w. boar	masseter diaphragm	Tb	8	2	Grudovo	42° 41'N 27° 17' E
3	w. boar	masseter diaphragm	Tb	9	2	Chiprovci	43° 45'N 22° 79' E
4	w. boar	masseter diaphragm	Tb	5	2	Bankia	42° 78'N 22° 63' E
5	w. boar	masseter diaphragm	Tb	6	2	Melnik	41° 32'N 23° 45' E
6	fox	masseter diaphragm	Tb	2	2	Rudarci	42° 33'N 23° 19' E
7	jackal	masseter diaphragm	Tb	3	2	Peshtera	42° 05'N 23° 90' E
8	jackal	masseter diaphragm	Tb	2	2	Elena	43° 00'N 25° 86' E
9	Jackal	masseter diaphragm	Tb	7	2	Kula	43° 89'N 22° 51' E
10	jackal	masseter diaphragm	Tb	4	2	Razlog	41° 82'N 23° 46' E
11	jackal	masseter diaphragm	Tb	3	2	Govedarci	42° 36'N 23° 53' E
12	wild cat	masseter diaphragm	Tb	4	2	Anton	42° 69'N 24° 38' E
13	m. foina	masseter diaphragm	Tb	6	2	Belchin	42° 44'N 23° 41' E
14	m. foina	masseter diaphragm	Tb	4	2	Kostenec	42° 56'N 23° 98' E
15	badger	masseter diaphragm	Tb	14	1	Svoqe	42° 91'N 23° 32' E
16	badger	masseter diaphragm	Tb	7	2	Iskretc	42° 94'N 23° 26' E
17	rat	masseter diaphragm	Tb	23	1	Sofia	42° 44'N 23° 21' E

N	Source of infection	Type of muscle in the original host	Species identification PCR	Number of larvae/g diaphragm	Number of passages <i>in vivo</i>	Place of origin locality	Latitude longitude
18	rat	masseter diaphragm	Tb	14	2	Elin Pelin	42° 43'N 23° 29' E
19	rat	masseter diaphragm	Tb	25	1	Slivnitca	42° 51'N 23° 15' E
20	wolf	masseter diaphragm	Tb	11	2	Grudovo	42° 21'N 27° 26' E
21	wolf	masseter diaphragm	Tb	9	2	Mramor	42° 75'N 22° 60' E
22	bear	masseter diaphragm	Tb	17	1	Madan	41° 42'N 24° 91' E
23	bear	masseter diaphragm	Tb	6	2	Batak	41° 88'N 24° 20' E
24	otter	masseter diaphragm	Tb	12	2	Samokov	42° 19'N 23° 34' E

## Discussion

The results obtained in the study of the trichinella species from wild animals: wild boars - 5 specimens, fox-1, waiting-5, wild cat - 1, m. foia-2, badger-2, rat-3, wolf-2, bear-2 and 1 otter showed that parasites cause of infection belong to *T. britovi*.

The detection of only *T. britovi* in wild animals shows that this species is prevalent in wildlife. Our results confirm the opinion of Pozio et al. [11] that in most countries of the European Union, including Bulgaria, *T. britovi* was the more prevalent species. These results confirm the assumption that wild animals (wild boar, fox, jackal, etc.) represent wildlife reservoir for *T. britovi* in Bulgaria. Previous investigation of trichinella isolates obtained upon 15 epidemic outbreaks revealed the predominance of *T. britovi*, as a cause of infection. Therefore, when people fail to properly breed domestic pigs this parasite reaches the domestic environment as it is registered in three outbreaks caused by the consumption of pork from backyard pigs [7]. As data indicate a steady increase in the number of outbreaks and of sporadically occurred individual cases, as well as due to public health importance of the disease, many questions remain open. We think that the main efforts should have been focused on the control or elimination of trichinella from the food chain and hope that our discoveries may have an impact on the transmission and control of trichinellosis in Bulgaria.

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