



## SPECIES COMPOSITION OF *TRICHINELLA* IN DOMESTIC AND WILD ANIMALS IN BULGARIA

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### Summary

Lalkovski, N., 2019. Species composition of *Trichinella* in domestic and wild animals in Bulgaria. *Bulg. J. Vet. Med.*, 22, No 1, 99–104.

Four *Trichinella* species cause trichinellosis in Europe: *Trichinella spiralis*, *Trichinella britovi*, *Trichinella nativa* and *Trichinella pseudospiralis*. The aim of our study was to determine the preponderance of *Trichinella* species in Bulgaria. The research covered the period 2010–2016. Molecular analysis was performed with 120 *Trichinella* isolates. Two species were discovered: *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. It was found in samples from domestic and wild animals from all over the country, while *T. spiralis* has only been found in several areas.

**Key words:** bear, badger, domestic pig, fox, jackal, *Trichinella britovi*, *Trichinella spiralis*, wild boar, wolf

### INTRODUCTION

Trichinellosis is a foodborne disease that affects skeletal muscle tissue of wild and domestic carnivores and omnivores. The causative agents of this disease are nematodes belonging to the genus *Trichinella*, family *Trichinellidae*. By the middle of last century it was thought 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 (Pozio & Zarlenga, 2005). According to FAO/WHO/OIE Guidelines 11 genotypes are identified in the genus *Trichinella* (Anonymous, 2007). Two main clades are recognised in the genus: the first that encapsulates in host muscle tissue and a second that does not. The representatives of eight genotypes have species names. The encapsulated clade includes 5 species: *T. spiralis* – genotype T1, *T. nativa* – genotype T2, *T. britovi* – genotype T3, *T. murrelli* – genotype T5 and *T. nelsoni* – genotype T7 and three genotypes (*Trichinella* T6, T8 and T9),

which invades mammals. The non-encapsulated (acapsular) clade includes three taxonomically distinct species: *T. pseudospiralis* – genotype T4, *T. papuae* – genotype T10 and *T. zimbabwensis* – genotype T11. The first one invades mammals and birds and the other two species – mammals and reptiles. The existence of genotype T12 isolated from a cougar in Argentina is supposed (Krivo-kapich *et al.*, 2008).

From epidemiological point of view there are two cycles of trichinellosis: synantropic (domestic) cycle where *T. spiralis* is usually involved and in rare cases *T. britovi*, as well as a sylvatic cycle specific for all *Trichinella* species and genotypes.

The most commonly spread etiological agents of trichinellosis in European wild and domestic animals are *T. spiralis* and *T. britovi*. Two other species – *T. nativa* and *T. pseudospiralis* are of minor importance as pathogens in domestic animals (Pozio, 2007). According to Pozio *et al.* (2009) *T. britovi* is more widespread in the Member States of the European Union and it can be found in 62.5% to 100% of the samples. *T. spiralis* is widespread in Finland, Germany, Poland and Spain – 56.3% to 92.8% of the samples. In Romania, the ratio is 49.2% to 50.8% in favour of *T. britovi*. *T. britovi* is more widespread than *T. spiralis* in wild carnivores – 89% vs. 11% respectively. According to this research *T. spiralis* is more commonly found in wild boars (62% vs. 38%), domestic pigs (82% vs. 18%) and rodents (75% vs. 25% respectively).

In Bulgaria, Kurdova *et al.* (2004) investigated parasite isolates obtained upon 15 epidemic outbreaks. Obtained results showed the predominance of *T. britovi* (etiological agent in 10 outbreaks) while *T. spiralis* was found in 5 outbreaks. The

authors identified *T. britovi* in 4 wild boars and *T. spiralis* in only one sample from wild boar. Between 1995 and 2002, 18 human outbreaks have been recorded (Petkova *et al.*, 2008). In seven of them the etiological agent was identified as *T. spiralis* and in 11 outbreaks as *T. britovi*. In wild boars the authors determined only *T. britovi* whereas in domestic pigs – both species.

The purpose of this research was to conduct a more extensive survey on species composition and distribution of agents of trichinellosis in domestic and in wild animals in our country.

## MATERIALS AND METHODS

The research covered the period 2010–2016. The isolated larvae of *Trichinella* were detected during the routine official control of samples obtained from meat products, slaughtered domestic pigs, wild pigs and shot wild carnivores. The studies were carried out following the reference method of digestion using a magnetic stirrer according to Regulation (EU) 2015/1375. A total number of 120 isolates were typified and sorted as followed: 8 from domestic pigs, 92 from wild boars, 5 from foxes, 3 from jackals, 3 from wolves, 3 from badgers, 1 from a bear, 3 from raw dried sausage made of wild boar meat and 2 from minced meat (made of mixture of domestic pig meat, beef/horse meat). *Trichinella* spp. larvae were identified at the species level by multiplex PCR, which allowed differentiating the eight species of *Trichinella* and one of the genotypes – T6.

Ninety one *Trichinella* spp. isolates were tested in the European Union Reference Laboratory for Parasites (EURLP) in Italy according to the laboratory protocol ([http://www.iss.it/binary/crlp/cont/MI\\_02\\_WEB\\_SITE\\_REV\\_6.pdf](http://www.iss.it/binary/crlp/cont/MI_02_WEB_SITE_REV_6.pdf)).

**Table 1.** Species composition and distribution of causes of trichinosis in domestic and wild animals in Bulgaria in the period 2010–2016

District	Hosts and sources	Isolates number	Species
Blagoevgrad	Wild boar	32	<i>Trichinella britovi</i>
Burgas	Wild boar	2	<i>Trichinella britovi</i>
	Jackal	1	<i>Trichinella britovi</i>
Varna	Wild boar	1	<i>Trichinella britovi</i>
	Minced meat (pig+beef)	1	<i>Trichinella spiralis</i>
Veliko Tarnovo	Wild boar	3	<i>Trichinella britovi</i>
Vidin	Wild boar	2	<i>Trichinella britovi</i>
	Fox	1	<i>Trichinella britovi</i>
Vratsa	Wild boar	1	<i>Trichinella britovi</i>
Gabrovo	Wild boar	8	<i>Trichinella britovi</i>
	Wild boar	1	<i>Trichinella spiralis</i>
Dobrich		No isolates	
Kardzhaly	Wild boar	6	<i>Trichinella britovi</i>
Kyustendil	Wild boar	3	<i>Trichinella britovi</i>
Lovech	Wild boar	1	<i>Trichinella britovi</i>
	Wild boar	1	<i>Trichinella spiralis</i>
Montana	Fox	1	<i>Trichinella britovi</i>
Pazardzhik	Badger	1	<i>Trichinella britovi</i>
	Fox	1	<i>Trichinella britovi</i>
Pernik	Wild boar	1	<i>Trichinella britovi</i>
Pleven		No isolates	
Plovdiv	Wild boar	3	<i>Trichinella britovi</i>
	Jackal	1	<i>Trichinella britovi</i>
Razgrad		No isolates	
Rousse	Wild boar	1	<i>Trichinella britovi</i>
Sliven	Wild boar	7	<i>Trichinella britovi</i>
Smolyan	Wild boar	2	<i>Trichinella britovi</i>
	Wolf	2	<i>Trichinella britovi</i>
	Fox	1	<i>Trichinella britovi</i>
Silistra	Wild boar	2	<i>Trichinella britovi</i>
	Fox	1	<i>Trichinella britovi</i>
Sofia-town	Wild boar	7	<i>Trichinella britovi</i>
	Domestic pig	2	<i>Trichinella spiralis</i>
	Domestic pig	2	<i>Trichinella britovi</i>
	Raw dried sausage	2	<i>Trichinella britovi</i>
Sofia-district	Wild boar	3	<i>Trichinella britovi</i>
	Domestic pig	1	<i>Trichinella britovi</i>
	Domestic pig	1	<i>Trichinella spiralis</i>
	Wolf	1	<i>Trichinella britovi</i>
	Minced meat (pig+ horse)	1	<i>Trichinella spiralis</i>
	Raw dried sausage	1	<i>Trichinella britovi</i>
Stara Zagora	Wild boar	2	<i>Trichinella britovi</i>
	Badger	1	<i>Trichinella britovi</i>
	Bear	1	<i>Trichinella britovi</i>
Targovishte	Wild boar	1	<i>Trichinella britovi</i>
Haskovo	Wild boar	2	<i>Trichinella britovi</i>
Shumen	Domestic pig (free ranging)	2	<i>Trichinella britovi</i>
	Jackal	1	<i>Trichinella britovi</i>
	Badger	1	<i>Trichinella britovi</i>
Yambol		No isolates	

Twenty-nine samples were tested in the National Reference Laboratory of Parasitic Zoonoses, Sofia (Lalkovski *et al.*, 2011).

## RESULTS

The results of the multiplex PCR for *Trichinella* spp. isolates identification are shown in Table 1.

Molecular analysis was performed with 120 larval isolates from 24 districts of the country. Two species were discovered: *Trichinella britovi* and *Trichinella spiralis*. *T. britovi* was found in 113 (94.17%) and *T. spiralis* in 7 (5.83%) samples. Both types were found in domestic pigs and wild boars. Concerning species prevalence, there was a predominance of *T. britovi* (97.83%) in wild boars compared to *T. spiralis* (2.17%). In domestic pigs the ratio *T. britovi*:*T. spiralis* was 1:1. In wild carnivores only *T. britovi* was found. *T. britovi* is the geographically mo-

re widespread *Trichinella* species (Fig. 1) found in samples from domestic pigs, wild boars and wild carnivores from 24 districts of the country, while *T. spiralis* has been found in only 5 districts.

## DISCUSSION

The above mentioned results showed that trichinellosis in domestic pigs, wild boars and wild carnivores in the country was caused by two *Trichinella* species – *T. spiralis* and *T. britovi*. According to Kurdova *et al.* (2004) the same two species caused human trichinellosis outbreaks. Our results confirm the opinion of Pozio *et al.* (2009), that in most countries of the European Union, including Bulgaria, *T. britovi* was the more prevalent species. *T. spiralis* was detected in meat and meat products in five backyard domestic pigs. Given that four of them originated from two adjacent districts (Sofia-town and

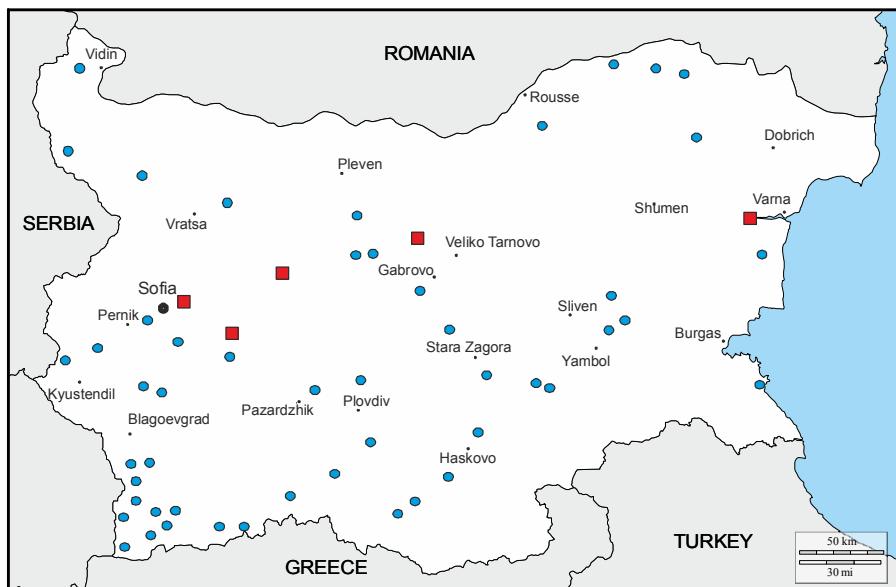


Fig. 1. Geographical distribution of *Trichinella britovi* and *Trichinella spiralis* in Bulgaria (2010–2016); ● *Trichinella britovi*; ■ *Trichinella spiralis*

Sofia-district) this region can be considered as a risk area. In the same districts Petkova *et al.* (2008) identified *T. spiralis* as etiological agent in 6 outbreaks during 2000–2001 and Rainova *et al.* (2016) reported 10 human outbreaks from 2008–2014. Unlike Petkova *et al.* (2008) we found *T. spiralis* in a wild boar which suggests that this species is not restricted only to domestic cycle of trichinellosis in our country. Two *T. spiralis*-infected wild boars were shot in the central north region of Bulgaria, where *T. britovi*-infected wild boars were also detected, but we didn't find a mixed *Trichinella* spp. infection as reported in Spain. However, most of *T. britovi*-infected wild boars were from southwestern region of Bulgaria, where Rainova *et al.* (2016) recorded most human outbreaks in the country. Our studies did not detect the non-encapsulated species *Trichinella pseudospiralis* reported by Michov *et al.* (2006).

The results of our research give us the reason to make the following important conclusions:

- A widespread species in the country was *Trichinella britovi*, which was the only etiologic agent of trichinellosis in wild carnivores in Bulgaria;
- The high extensity of invasion in foxes – 42.3% (Georgieva *et al.*, 2000) plays an important role in a stable trend towards increase in the number of wild boars invaded by *Trichinella* spp.;
- The wild boars were the only reservoir of *Trichinella spiralis* in the sylvatic cycle of trichinellosis in Bulgaria;
- The high incidence and wide spread of *Trichinella britovi* in wild boars in Bulgaria posed a high risk for transmission into the domestic cycle, especially where free ranging pigs are in contact with wildlife reservoirs.

## ACKNOWLEDGEMENTS

The author thanks Dr. Edoardo Pozio and Dr. Gianluca Marucci from EURLP, Italy for their help in *Trichinella* species identification by multiplex PCR.

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Paper received 24.03.2017; accepted for publication 07.04.2017

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