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A review of *Trichinella* species infection in wild animals in Romania

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ABSTRACT

Nematodes of the genus *Trichinella* are important zoonotic parasites present throughout Romania. This study aimed to assess the status of *Trichinella species* in wild animals in Romania over the past 30 years. A literature review of original studies concerning the only two species (out of the four in Europe) of *Trichinella* (*T. spiralis* and *T. britovi*) confirmed in wildlife from Romania was conducted and corroborated with the results of our original research concerning the topic. This review article has shown that, in Romania, European minks were infected with *T. spiralis*, while wolves, European wild cats, Eurasian lynx, golden jackals, stone marten, and European badgers were infected with *T. britovi*, respectively. Both *Trichinella* species have been identified in foxes, bears, wild boars, and ermines, but mixed infections have been found only in European polecats. *Trichinella* infection is still significantly present in Romania, infecting several wild omnivorous and carnivorous species in an equal manner, with different prevalence rates over the years. Regarding the spatial distribution of *T. spiralis* and *T. britovi* in Romania, both species can be found all over the country, but in wild animals, *T. britovi* is the most prevalent.

1. Introduction

Romania is a southeastern European country located in the north of the Balkan Peninsula. The Country is characterized by a temperate-continental climate of transitional type, with four clearly defined seasons (Trușcă and Alecu, 2005). Romania's Carpathic-Danubian-Pontic geography is defined by the Carpathian Mountains, the Black Sea, the Danube river, and its Delta. These units are in a nearly balanced combination with the hills and plains, determined by the step-like arrangement of the relief (Ilieş et al., 2017). Due to the forested mountains, wild animals are found in large numbers and show high diversity (Tănase et al., 2019). Many wild omnivorous and carnivorous species can host *Trichinella* species in Romania, thus maintaining the parasite's sylvatic life cycle (Boros et al., 2020).

Nematodes of the genus *Trichinella* are zoonotic parasites, being among the most widespread parasites in domestic and wild omnivores and predatory animals (Campbell, 1988; Pozio et al., 2009; Șuteu and Cozma, 2012). Rodents can act as a source of infection

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with *Trichinella* spp. for domestic and wild animals (Pozio and Zarlenga, 2005). The infection develops after the ingestion of raw meat, harboring the infective larvae (Pozio, 2007).

In Romania, the first information regarding trichinellosis dates back to 1866, when the supreme medical authority introduced the control of all slaughtered pigs in the country, but without any infections identified. In 1868, Schreiber had diagnosed the first case of human trichinellosis in Colţea hospital in Bucharest. In the same year, the first case of swine trichinellosis was confirmed in the southeastern part of the Country (Lupu and Cironeanu, 1960). In 1913, the use of trichinelloscopy was officially introduced in all slaughterhouses from Bucharest. Afterwards, new laws and regulations have been implemented to help reduce the number of human infections (Cironeanu, 1961).

In Romania, a priority epidemiological study on *Trichinella* spp. in domestic and wildlife hosts was conducted in the year 1960 with the use of trichinoscopy (Lupaşcu et al., 1970). Since then, the knowledge regarding *Trichinella* spp. infections has significantly improved, due to the introduction of the artificial digestion method in the 1990s. This method was used initially in parallel with trichinoscopy, whereas later studies focused on the use of artificial digestion. The risk of *Trichinella* infection still remains a concern in Romania, because of local eating habits and customs (Blaga et al., 2007). Most human cases are caused by consuming undercooked meat of pigs infected with *T. spiralis* (Blaga et al., 2007). Additionally, wild boar meat consumed in several local dishes, sometimes infected with *T. britovi*, might represent another source of infestation for the local human population (Blaga et al., 2009a; Blaga et al., 2009b). According to the International Commission on Trichinellosis, Romania accounted for most cases of human trichinellosis reported worldwide in 2004 (Neghină et al., 2010a). Furthermore, an increase in the incidence of trichinellosis in Romania has been observed since the beginning of the 21st century. After the fall of communism in 1989, the annual incidence increased from 0.1 to 4.1 cases per 100,000 inhabitants (until 1989) to 6.2 cases per 100,000 inhabitants, with a range of 2–15.9 per 100,000 inhabitants between 1990 and 2007 (Neghină et al., 2009; Neghină et al., 2010b). In a more recent study from 2018, among 1347 blood donors from Timiş county, aged 18–63 years, *T. spiralis* IgG antibodies were detected only in 2.0%. However, with further development and implementation of sanitary education programs for pig farmers and meat consumers, the number of human infections is expected to further decrease in the future (Pavel et al., 2022).

The present review of studies conducted between 1991 and 2021 aimed to assess the presence of *T. spiralis* and *T. britovi* (the only two species currently present in Romania) in Romania over the past 30 years.

2. Prevalence of *Trichinella* spp. infections in wild animals in Romania

One of the earliest studies aiming to broaden the epidemiological knowledge on *Trichinella* spp. in Romania, was conducted in 1991 in bears (*Ursus arctos*), wolves (*Canis lupus*), foxes (*Vulpes vulpes*), wild cats (*Felis silvestris*), badgers (*Meles meles*), wild boars (*Sus scrofa*), and polecats (*Mustela putorius*) (Tables 1, 2, 3, 4; Figs. 1, 2, 3) (Nesterov et al., 1991). A further study was conducted between 1991 and 1994 in a restricted area of the Carpathian Mountains (Jiu valley), in red foxes and wild boars (Table 2, Table 3, Fig. 1, Fig. 2) (Cristea and Şuteu, 1996), indicating that these animal species play a limited role in the sylvatic cycle in that area. Afterwards, several studies focused on the detection of *Trichinella* spp. infection in wild animals from different regions of Romania. Between 1992 and 1997, wild boars and bears from Transylvania were subjected to larvae detection methods and the results are provided in Tables 1 and 2, and in Fig. 1, respectively (Gherman, 1998). The low prevalence rates detected in wild boars compared to bears show that, in the

Table 1
Trichinella spp. infections in bears (*Ursus arctos*) from Romania between 1991 and 2021.

Year	Location (areas or counties)	Number of animals	Methods	Prevalence	*Trichinella* species (PCR)	Reference
1991	Central Romania	50	Trichinelloscopy	18.5%		Nesterov et al., 1991
1992–1997	Transylvania	503	Trichinelloscopy Artificial digestion	12.1%		Gherman, 1998
1997–2004	Transylvania Other counties	1062	Trichinelloscopy	12.4%		Blaga et al., 2009b
2000–2005	Cluj county Mureş county	2	Artificial digestion PCR	100.0%	*T. spiralis*	Blaga et al., 2009a
2000	Covasna county	6	Trichinelloscopy	66.6%		Oprescu et al., 2007
1997–2007	Covasna county	60	Trichinelloscopy Artificial digestion	38.3%		Oprescu et al., 2007
2010–2015	Eastern Romania	49	Artificial digestion PCR	6.5% 15.6%	*T. spiralis* *T. britovi*	Iacob, 2017
2011-2015	Eastern Transylvania	37	Trichinelloscopy	5.4%		Borka-Vitális et al., 2017
2015	North-Eastern, North-Western, Central regions, Western, South, and South-Eastern regions of Romania	147	Artificial digestion PCR	6.1% 4.7%	*T. spiralis* *T. britovi*	Nicorescu et al., 2015

Table 2
Trichinella spp. infections in wild boars (*Sus scrofa*) from Romania between 1991 and 2021.

Year	Location (areas or counties)	Number of animals	Methods	Prevalence	*Trichinella* species (PCR)	Reference
1991	Central Romania	38,908	Trichinelloscopy	0.1%		Nesterov et al., 1991
1991–1994	Jiu Valley	1210	Trichinelloscopy Artificial digestion	23.5%		Cristea and Șuteu, 1996
1992–1997	Transylvania	17,053	Trichinelloscopy Artificial digestion	0.3%		Gherman, 1998
1997–2007	Covasna county	210	Trichinelloscopy Artificial digestion	9.5%		Oprescu et al., 2007
1990–1999	Constanța county	340	Trichinelloscopy	0.1%		Olteanu, 2001
1997–2004	Transylvania Other counties	29,825	Trichinelloscopy	8.7%		Blaga et al., 2009b
1998–2011	Timiș county	823	Trichinelloscopy	0.5%		Borza et al., 2012
2010–2014	Hunedoara county	973	Trichinelloscopy Artificial digestion	1.3%		Ciobotă et al., 2015
2000–2005	Cluj county Mureș county	5	Artificial digestion PCR	30.0% 70.0%	*T. spiralis* *T. britovi*	Blaga et al., 2009a
2015	North-Eastern, North-Western, Central, Western, South-Western, Southern, and South-Eastern regions of Romania	5596	Artificial digestion PCR	0.8% 0.6% *T. spiralis* + *T. britovi* / 0.0%	*T. spiralis* *T. britovi* *T. spiralis* + *T. britovi*	Nicorescu et al., 2015
2010–2015	Eastern Romania	8024	Artificial digestion PCR	6.5% 0.4%	*T. spiralis* *T. britovi*	Iacob, 2017

mentioned region, bears have a more important role in the maintenance of the sylvatic cycle than wild boars. Furthermore, of two ten-year studies, the first one (1990–1999) focused on wild boars (Olteanu, 2001), while the other study took into consideration the prevalence of *Trichinella* infection in bears and wild boars from Covasna county in central Romania (Table 1, Table 2, Fig. 1) (Oprescu et al., 2007), highlighting the importance of these animal species for trichinellosis in Romania. The presence of *Trichinella* spp. infection in three wild carnivore species from Romania (fox, wolf, and wild cat) was assessed between October 1999 and March 2002, which brought updates regarding the epidemiology of trichinellosis in these wild carnivore species (Table 3, Fig. 2) (Gherman et al., 2002). Based on the results obtained, wild carnivores represent the most important hosts in the sylvatic cycle of *Trichinella* spp. in Romania. Routine *Trichinella* test (trichinelloscopy) was conducted with game species (wild boars, bears), between 1997 and 2004, to investigate the extent of the infection in hunted animals in Romania (Table 1, Table 2, Fig. 1). Apart from their role in the sylvatic cycle, they could represent a source of inter-foci transmission of *Trichinella* spp. due to different feeding habits compared to domestic species (Blaga et al., 2009b). An epidemiological study of *Trichinella* infection in wild boars in Timiș county was done between 1998 and 2011 (Table 2). The data were collected from the Veterinary Public Health Department of Timiș County and show a low prevalence rate in wild boars, meaning this species of animal exhibit a minor role in the local sylvatic life cycle of *Trichinella* (Fig. 1) (Borza et al., 2012).

Another study was conducted between 2010 and 2014 on the epidemiology of *Trichinella* infection in wild boars from Hunedoara county in western Romania. The highest prevalence of infection was established in 2012 (1.3%), followed by 2013 (1.1%), 2010 (0.8%), and then 2014 (0.7%), whereas all animals examined in 2011 were negative (Table 2, Fig. 1). The results indicated that wild boars from this county had a low infection rate with *Trichinella* spp. (Ciobotă et al., 2015) and that, over the years, infected animals became less and less common. Brown bears in eastern Transylvania were also tested for infection between 2011 and 2015 and the results confirmed that bears from this area contribute to the maintenance of the sylvatic life cycle of parasites (Table 1, Fig. 1) (Borka-Vitális et al., 2017). Marian et al. (2015) assessed the prevalence of *Trichinella* spp. infection in large wild carnivores from Romania between 2014 and 2015. The highest prevalence was identified in Eurasian lynx, followed by wolves, golden jackals, and wildcats, as seen in Table 3. The methods used in the detection of *Trichinella* spp. in these studies are presented in Tables 1, 2, and 3.

The present review, which included research conducted over the last 30 years, performed an analysis of the presence of *Trichinella* infection in more than 80% of Romania's territory (33 out of 41 counties), as it can be observed in the figures. Infections of wild animals were found in all studied areas during this period. As the number of examined animals and the detection methods varied drastically among the different studies, the comparative contribution of different host species to the parasite's maintenance is difficult to assess. Looking at the studies with large sample sizes, prevalences of wild canids and felids are at the higher end of the range, while bears and mustelids are less frequently affected. *Trichinella* infection in wild boars seems to be least frequent and may reflect a low proportion of mammalian carcasses in the diet (Tabels 1–4 and Oltean et al., 2014; Boros et al., 2020; Boros et al., 2021b).

Table 3
Trichinella spp. infections in wild carnivores from Romania between 1991 and 2021.

Animal species	Year	Location (areas or counties)	Number of animals	Methods	Prevalence	*Trichinella* species (PCR)	Reference
Wolves (*Canis lupus*)	1991	Central Romania	399	Trichinelloscopy	30.5%		Nesterov et al., 1991
	1999–2002	Transylvania	7	Artificial digestion	71.4%		Gherman et al., 2002
	2014–2015	Transylvania	3	Artificial digestion	66.7%		Marian et al., 2015
Foxes (*Vulpes vulpes*)	1991	Central Romania	972	Trichinelloscopy	15.8%		Nesterov et al., 1991
	1991–1994	Jiu Valley	163	Trichinelloscopy Artificial digestion	23.5%		Cristea and Șuteu, 1996
	1999–2002	Transylvania	50	Artificial digestion	16.0%		Gherman et al., 2002
	2000–2005	Cluj, Covasna and Harghita counties	71	Artificial digestion PCR	14.0% 57.1%	*T. spiralis* *T. britovi*	Blaga et al., 2009a
	2015	Arad, Hunedoara, and Timiş counties	121	Artificial digestion PCR	96.0% 4.0%	*T. britovi* *T. spiralis*	Imre et al., 2015
Wild cats (*Felis silvestris*)	1991	Central Romania	158	Trichinelloscopy	31.5%		Nesterov et al., 1991
	1999–2002	Transylvania	6	Artificial digestion	16.6%		Gherman et al., 2002
	2014–2015	Buzău, Tulcea, and Maramureș counties	3	Artificial digestion	66.7%		Marian et al., 2015
Eurasian lynx (*Lynx lynx*)	2014–2015	Transylvania	3	Artificial digestion	66.7%		Marian et al., 2015
Golden jackals (*Canis aureus*)	2006	Tulcea county	1	Artificial digestion PCR	100.0%	*T. britovi*	Blaga et al., 2008
	2014–2015	Botoșani, Buzău, Brăila, Tulcea, Ialomița, Ilfov, Giurgiu, Teleorman, Olt, Vâlcea, Dolj, Gorj, and Timiș counties	54	Artificial digestion	53.7%		Marian et al., 2015

3. Species of *Trichinella* circulating in wild animals

Identifying the species of *Trichinella* in Romania is important due to the fact that *Trichinella spiralis* is more often found in domestic animals. However, this species can also appear in wild animals. Infection in humans is most often caused by *T. spiralis*. Therefore, identifying the exact species of *Trichinella* present in wild animals in Romania represented an important step in this field (Cozma et al., 2013; Cozma et al., 2016). Several studies have been conducted over the last 15 years and PCR-based methods confirmed the presence of *T. spiralis* and *T. britovi* in wild species in Romania.

3.1. *Trichinella spiralis infections in wild animals in Romania*

Trichinella spiralis infections were found in bears, wild boars, red foxes (Blaga et al., 2009a; Nicorescu et al., 2015; Imre et al., 2015), European minks (*Mustela lutreola*) (Oltean et al., 2014), and European polecats (*Mustela putorius*) (Boros et al., 2021b), as seen in Tables 1, 2, 3, and 4.

The results show that this parasite species was identified more frequently in bears and less frequently in foxes. However, there are few studies using PCR to determine the parasite species in wild animals, so final conclusions regarding this topic still remain to be drawn.

3.2. *Trichinella britovi infections in wild animals in Romania*

Trichinella britovi infections were found in golden jackals (Blaga et al., 2008;), wild cats, wolves, Eurasian lynx (Blaga et al., 2009a), beech martens (*Martes foina*), short-tailed weasels (*Mustela erminea*) (Oltean et al., 2014), foxes (Imre et al., 2015), wild boars, bears (Blaga et al., 2009a; Nicorescu et al., 2015; Iacob, 2017), European badgers (*Meles meles*) (Boros et al., 2021a), as seen in Tables 1, 2, 3, and 4. Mixed infections with *T. britovi* and *T. spiralis* were found in wild boars (Nicorescu et al., 2015) and polecats (Boros et al., 2021b).

The results show that *T. britovi* was identified more frequently in wild boars (Table 2) and mustelids (Table 4) but less frequently in

Table 4
Trichinella spp. infections in mustelids from Romania between 1991 and 2021.

Animal species	Year	Location (areas or counties)	Number of animals	Methods	Prevalence	*Trichinella* species (PCR)	Reference
European badgers (*Meles meles*)	1991	Central Romania	166	Trichinelloscopy	6.0%		Nesterov et al., 1991
	2015–2019	Timiș, Bihor, Sălaj, Maramureș, Cluj, Alba, Mureș, Sibiu, Brașov, Harghita, Ilfov, Giurgiu, Constanța, and Tulcea counties	61	Trichinelloscopy Artificial digestion PCR	1.6%	*T. britovi*	Boros et al., 2021a
Polecats (*Mustela putorius*)	1991	Central Romania	157	Trichinelloscopy	5.2%		Nesterov et al., 1991
	2016–2020	Arad, Brașov, Constanța, Brăila, Călărași, Ialomița, Giurgiu, Teleorman, and Olt counties	75	Trichinelloscopy Artificial digestion PCR	1.3%	*T. spiralis*	Boros et al., 2021b
European mink (*Mustela lutreola*)	2009–2013	Danube Delta	3	Artificial digestion PCR	33.3%	*T. spiralis*	Oltean et al., 2014
Beech martens (*Martes foina*)	2009–2013	Danube Delta	4	Artificial digestion PCR	50.0%	*T. britovi*	Oltean et al., 2014
Short-tailed weasels (*Mustela erminea*)	2009–2013	Danube Delta	4	Artificial digestion PCR	50.0%	*T. britovi*	Oltean et al., 2014

Fig. 1. The map of Romania showing the collection sites of bears and wild boars. Black circles: bear samples; Black stars: wild boar samples; Big circles and stars: general areas; Small circles and stars: counties.

golden jackals (Table 3). Regarding the mixed infection with *T. britovi* and *T. spiralis*, it was identified only two times (Table 2, Table 3), indicating that its occurrence is a rare phenomenon.

3.3. Serology in wild animals with Trichinella spp. infections

The serology approach regarding *Trichinella* infection in wild and domestic animals has been used less frequently in Romania. Nevertheless, a study from 2018 reported the seroprevalence of *Trichinella* spp. in wild boars (84 plasma samples) from Bihor county, located in western Romania. These animal samples were tested by ELISA and Western blot, although the artificial digestions of the tissue samples ($n = 84$) were negative. At analysis by indirect ELISA, 65.4% ($n = 55$) were positive, 7.1% ($n = 6$) were doubtful, and 27.38% ($n = 23$) were negative. On analysis by Western blot, from 26 samples, only 23.7% (n = 6) were positive, whereas 76.9% ($n = 20$) were negative, thus indicating the presence of anti-*Trichinella* antibodies in these animals (Boros et al., 2020). This study is important because it shows that antibodies can be found in animals that are negative in the golden standard method, thus indicating these animals probably had a very small infection or the samples (tissue) weren't taken correctly. The same situation might occur in other similar contexts and by this exposing the local population to this parasitic infection.

Fig. 2. The map of Romania showing the collection sites of wolves, foxes, wild cats, lynxes and golden jackals. Black circles: wolf samples; Black rectangles: fox samples; Black stars: wild cat samples; Black triangle: lynx samples; Black diamonds: golden jackal samples; Big circles, rectangles, stars, triangles, and diamonds: general areas; Small circles stars, rectangles, triangles and diamonds: counties.

Fig. 3. The map of Romania showing the collection sites of badgers, polecats, European minks, beech materns, and short-tailed weasels. Black circles: badger samples; Black stars: polecat samples; Black rectangles: beech matern samples; Black triangle: European minks samples; Black diamonds: short-tailed weasel samples; Big circles, rectangles, stars, triangles, and diamonds: general areas; Small circles and stars: counties; The rectangle, triangle and diamond: Danube Delta.

The results referenced above regarding *Trichinella* infection in wild species from Romania seem to reconfirm the combined statements of Campbell (1983) and Neghină et al. (2012) according to which „the saga of the helminth, destined to remain with us, both in nature and in the laboratory, will still haunt and fascinate scientists at the same time!" from both developing and developed countries, as they try to answer new questions regarding the parasite's evil nature.

4. Conclusions

Trichinella infection is still significantly present in Romania, infecting several wild omnivorous and carnivorous species in an equal manner, with different prevalence rates over the years, thus maintaining the sylvatic focus of the parasites. Two species of *Trichinella*, namely *T. spiralis* and *T. britovi*, were identified in wild animals. Although the relative frequency of the two parasite species and the contribution of different host species are difficult to assess given the heterogenous data available, it is clear that dietary habits of the carnivores and omnivores play a major role, which needs to be addressed in future studies.

Declaration of Competing Interest

All authors declare no conflicts of interest regarding the content in this article.

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