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 Carpathian-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 (Ilieș 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...
with *Trichinella* spp. for domestic and wild animals (Pozio and Zarlenka, 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 Colțea 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 trichinelloscopy (Lupascu 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 trichinelloscopy, 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 Timiș 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 Șuteu, 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

| 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, Gherman, 1998 |
| 1992-1997     | Transylvania                 | 503               | Trichinelloscopy         | 12.1%                                  | Gherman, 1998 |
| 1997-2004     | Transylvania                 | 1062              | Artificial digestion     | 12.4%                                  | Blaga et al., 2009b |
| 2000-2005     | Cluj county                  | 2                 | Artificial digestion     | 100.0%                                 | Blaga et al., 2009a |
| 2000          | Covasna county               | 6                 | Trichinelloscopy         | 66.6%                                  | Oprescu et al., 2007 |
| 1997-2007     | Covasna county               | 60                | Artificial digestion     | 38.3%                                  | Oprescu et al., 2007 |
| 2010-2015     | Eastern Romania              | 49                | Artificial digestion     | 6.5% 15.6%    *T. spiralis* *T. britovi* | Iacob, 2017 |
| 2011-2015     | Eastern Transylvania         | 37                | Trichinelloscopy         | 5.4%                                   | Borka-Vitáliás et al., 2017 |
| 2015          | North-Eastern, North-Western, Central regions, Western, South, and South-Eastern regions of Romania | 147 | Artificial digestion | 6.1% 4.7%    *T. spiralis* *T. britovi* | Nicorescu et al., 2015 |
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 Şuteu, 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       |
| 1999-1994| Constanţa county                           | 340               | Trichinelloscopy               | 0.1%       |                             | Olteanu, 2001              |
| 1997-2004| Transylvania                               | 29,825            | Trichinelloscopy               | 8.7%       |                             | Blaga et al., 2009         |
| 1998-2011| Timiș county                               | 823               | Trichinelloscopy               | 0.5%       |                             | Borza et al., 2012         |
| 2010-2014| Hunedoara county                           | 973               | Trichinelloscopy               | 1.3%       |                             | Ciobotă et al., 2015       |
| 2000-2005| Cluj county                                | 5                 | Artificial digestion PCR        | 30.0%      | *T. spiralis*               | Blaga et al., 2009         |
|          | Mureș county                               |                   |                                | 70.0%      | *T. britovi*                |                            |
| 2015     | North-Eastern, North-Western, Central,    | 5596              | Artificial digestion PCR        | 0.8%       | *T. spiralis*               | Nicorescu et al., 2015     |
|          | Western, South-Western, Southern, and South-|                   |                                | 0.6%       | *T. britovi*                |                            |
|          | Eastern regions of Romania                |                   |                                | *T. spiralis + *T. britovi / 0.0% | *T. spiralis + *T. britovi |                            |
| 2010-2015| Eastern Romania                            | 8024              | Artificial digestion PCR        | 6.5%       | *T. spiralis*               | Iacob, 2017                |
|          |                                             |                   |                                | 0.4%       | *T. britovi*                |                            |

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., 2015). 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-Vitáliš 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.
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 T. spiralis was identified more frequently in wild boars (Table 2) and mustelids (Table 4) but less frequently in...
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.

### 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        | 1991       | Central Romania                                                                            | 166               | Trichinelloscopy                 | 6.0%       |                             | Nesterov et al., 1991 |
| (Meles meles)           | 2015–2019  | Timiș, Bihor, Sălaj, Maramureș, Cluj, Alba, Mureș, Sibiu, Brașov, Harghita, Ițî, Giurgiu, Constanța, and Tulcea counties | 61                | Trichinelloscopy Artificial digestion PCR | 1.6%       | *T. britovi*               | Boros et al., 2021a |
| Polecats                | 1991       | Central Romania                                                                            | 157               | Trichinelloscopy                 | 5.2%       |                             | Nesterov et al., 1991 |
| (Mustela putorius)      | 2016–2020  | Arad, Brașov, Constanța, Braila, Câlărași, Ialomița, Giurgiu, Teleorman, and Olt counties    | 75                | Trichinelloscopy Artificial digestion PCR | 1.3%       | *T. spiralis*              | Boros et al., 2021b |
| European mink           | 2009–2013  | Danube Delta                                                                                | 3                 | Artificial digestion PCR          | 33.3%      | *T. spiralis*              | Oltean et al., 2014 |
| (Mustela lutreola)      |            |                                                                                             |                   |                                   |            |                             |             |
| Beech martens           | 2009–2013  | Danube Delta                                                                                | 4                 | Artificial digestion PCR          | 50.0%      | *T. britovi*               | Oltean et al., 2014 |
| (Martes foina)          |            |                                                                                             |                   |                                   |            |                             |             |
| Short-tailed weasels    | 2009–2013  | Danube Delta                                                                                | 4                 | Artificial digestion PCR          | 50.0%      | *T. britovi*               | Oltean et al., 2014 |
| (Mustela erminea)       |            |                                                                                             |                   |                                   |            |                             |             |

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.
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 heterogeneous 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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