

**MOLECULAR CHARACTERIZATION OF *TRICHINELLA BRITОВI*
IN LYNX (*LYNX LYNX* L.) FROM BIHOR COUNTY, ROMANIA: A CASE REPORT**
**CARACTERIZAREA MOLECULARĂ A SPECIEI *TRICHINELLA BRITОВI*
LA RÂS (*LYNX LYNX* L.) PROVENIT DIN JUDEȚUL BIHOR, ROMANIA: RAPORT DE CAZ**

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ABSTRACT | REZUMAT

Trichinella spp. are worldwide etiological agents that affect mammals, birds, and reptiles and are involved in the evolution of two cycles: domestic and sylvatic. The Eurasian lynx is a medium-sized mammal whose favourable habitat extends throughout Eurasia, registering the highest densities in the area of the Carpathian Mountains and having apex predator status in the areas where it lives. The top predator in the category of large carnivores, the lynx is the largest representative of the *Felidae* family in Romania. The food used by this species is extremely varied, from various species of mice and small rodents to birds, cervids (e.g., roe deer, fallow deer, and red deer), and wild boar, mostly preferring to actively hunt them, few being the situations in which the lynx feeds on found corpses, necrophagy not being a quality of this species. The aim of this study was to evaluate the possible presence of larvae of *Trichinella* spp. in the muscle tissue of a lynx from Bihor County, in Romania. The muscle samples were examined by artificial digestion, and the obtained larvae were processed by multiplex PCR. *Trichinella britovi*, a species frequently identified in wild carnivores in temperate zones, has been confirmed. *T. britovi* remains a constant in the infection of wild fauna in Romania.

Keywords: *Trichinella* spp., Romania, lynx

Trichinella spp. sunt agenți etiologici răspândiți în întreaga lume care afectează mamiferele, păsările și reptile implicate în evoluția celor două cicluri, domestic și silvatic. Râsul euroasiatic este mamifer de talie mijlocie a cărui habitat favorabil se întinde în întreaga Eurasie, înregistrând cele mai mari densități în zona munților Carpați, având statut de prădător apex (de vârf) în zonele unde acesta trăiește. Prădător de top din categoria marilor carnivore, râsul este cel mai mare reprezentant al familiei *Felidae* din România. Hrana utilizată de această specie este extrem de variată de la diverse specii de șoareci și rozătoare mici până la specii păsări, cervide (căprior, cerb lopătar, cerb comun) și mistreț, de cele mai multe ori preferând să le vâneze în mod activ, puține fiind situațiile în care râsul se hrănește cu cadavre găsite, necrofația nefiind o calitate a acestei specii. Scopul studiului a fost acela de a evalua posibila prezență a larvelor de *Trichinella* spp. din musculatura unui râs provenit din județul Bihor din România. Probele musculare au fost examinate prin digestie artificială, iar larvele obținute au fost procesate prin PCR multiplex. *Trichinella britovi*, specie frecvent identificată la carnivorele sălbatice din zonele temperate, a fost confirmată. Specia *T. britovi* rămâne o constantă în infecția faunei sălbatice din România.

Cuvinte cheie: *Trichinella* spp., România, râs

Trichinella spp. are the etiological agents of a zoonosis that affects humans, caused by the consumption of raw or undercooked meat of animals infected with the larvae of these zoonotic nematodes (28). Trichinellosis is a parasitic disease that affects mammals, birds, and carnivorous and omnivorous reptiles.

The disease is widespread everywhere except Antarctica (25).

Currently, 13 taxa are described in the *Trichinella* genus, namely the encapsulated species *T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, *T. nelsoni*, *T. patagoniensis*, *T. chanchalensis*, and *Trichinella* genotypes T6, T8, and T9, exclusively for mammals. Non-encapsulated species are represented by *T. pseudospiralis*, *T. papuae*, and *T. zimbabwensis*, which infest mammals and birds or mammals and reptiles (28).

In Romania, trichinellosis is a zoonosis with a high level of infestation, and inadequate consumption of pork and game meat (boar, bear) represents the major cause of human infestation (11, 17).

The Eurasian lynx (*Lynx lynx* L.), the largest representative of the *Felidae* family in Europe, is a medium-sized mammal whose favourable habitat extends over

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the entire European and Asian continent, recording the highest densities and implicitly the best living conditions in the area of the Carpathian Mountains, having an indisputable status as an apex predator (8).

A mysterious species that prefers isolated and wild areas, the lynx is directly dependent on the abundance of food in the occupied habitat, an aspect that is also reflected in the density of this species on various hunting grounds in Romania. Its absence entails the migration of individuals from their respective territories (8). A twilight and predominantly nocturnal animal, the lynx is endowed with exceptional physical qualities for climbing, swimming, and running, but it is also an opportunist that alternates between active hunting and ambush technique with the obvious aim of maximising the success of capturing prey (8).

The lynx is a protected species that, in certain situations, generates interactions and conflicts with human society (of course not as frequent as the brown bear or the wolf). This animal causes damage mainly to wildlife species but also to domestic animals. The lynx is a wild animal that remains by its nature a secretive, reclusive species that is difficult to study, possibly due to the extremely small number of study samples existing over time for this species (8).

In Romania, the lynx and other carnivores (foxes, jackals, wolves, wild cats, etc.) represent a source of infection with larvae of *Trichinella* spp. and have an important epidemiological role in the evolution of sylvatic trichinellosis (3).

In Europe, the raccoon dog (*Nyctereutes procyonoides*) and the red fox (*Vulpes vulpes*) are considered to be the most important hosts for infection with *Trichinella* spp. (22). Other carnivores such as lynx, wolf, and bear, as well as several species of the *Mustelidae* family (marten, badger, etc.), can also represent a source of infection with *Trichinella* spp. (22).

Larvae of *T. britovi* have been identified in lynxes in three counties from Romania (Bistrita-Năsăud, Neamț, and Covasna) (3); however, the information about the presence of *T. britovi* species in lynxes in Romania remains limited.

The present study aimed to evaluate the possible presence of *Trichinella* spp. larvae in the muscles of a lynx from a Surducel hunting ground in Bihor County and to characterize the species involved by mPCR.

MATERIALS AND METHODS

Target hosts

The Eurasian lynx is a medium-sized mammal whose favourable habitat extends throughout Eurasia, registering the highest densities in the area of the Carpathian Mountains and having apex predator status in the areas where it lives (8). A top predator in the category of large carnivores, the lynx is the largest repre-

sentative of the *Felidae* family in Romania, with a length of 104-174 cm, the females being 20 cm smaller, and a height at the withers of approximately 45-86 cm, with a variable weight up to 50 kg (8). The food used by this species is extremely varied, from various species of mice and small rodents to species of birds or *Cervidae* (deer, red deer, fallow deer) or wild boar, most of the time preferring to hunt them actively. There are few situations in which the lynx feeds on found corpses; necrophagy is not a quality of this species (8). The habitat and feeding characteristics make it easier to understand the involvement of this wild animal in the maintenance and transmissibility of various pathogens, especially parasites with zoonotic potential, such as the nematode *Trichinella*.

Diagnostic procedures

On March 2, 2022, a male lynx was found dead (road killed) in the Apuseni mountains on the Surducel hunting ground in Bihor County (lat. 46.820240, long. 21.669641). The lynx specimen was collected and transported to the Faculty of Veterinary Medicine / University of Life Sciences "King Mihai I" from Timisoara, Romania under legal conditions. The animal was examined at the Parasitic Diseases Clinic of the Faculty of Veterinary Medicine Timisoara/ULST.

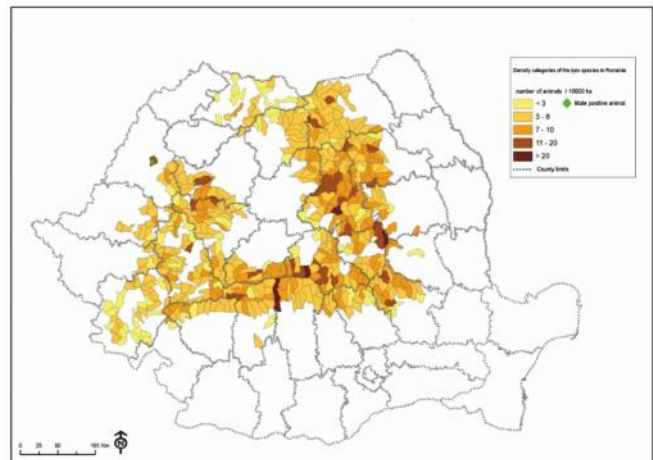


Fig. 1. Density categories of lynx species in Romania and the area where lynx carcasses were collected

About 30 g of muscle from the diaphragm and fore-leg muscles were harvested from the lynx and tested for the presence of *Trichinella* spp. larvae by the artificial digestion method according to Commission Regulation (EC) no. 1375/2015 (7).

After artificial digestion, larvae were collected, counted, stored in 96% ethanol, and sent to the European Reference Laboratory for Parasites (EURLP) (Rome, Italy) for species identification by multiplex PCR (27).

RESULTS AND DISCUSSION

Lynx specimens collected from Bihor County were positive for the presence of *Trichinella* spp. larvae using the artificial digestion method. The larval burden was estimated to be 26 larvae /g (LPG) of muscle tissue. Ten *Trichinella* larvae were individually tested by multiplex PCR and identified as *T. britovi* (Fig. 2).

Identification of species *T. britovi* in the lynx specimen from Bihor County emphasises not only the vector role of this sylvatic carnivore in the transmission of trichinellosis but also the fact that the species *T. britovi* remains a constant presence of wild fauna in Romania.

T. britovi is parasitic on wild mammals of the families *Canidae*, *Felidae*, *Mustelidae*, *Ursidae*, and *Viverridae* living in the temperate regions of Europe, Western and Northern Asia, and West Africa (25).

In Europe, there are four species of the genus *Trichinella*, but the most prevalent species isolated from wildlife are *T. britovi* and *T. spiralis*. *T. britovi* is more widespread than *T. spiralis*, with a different prevalence depending on the carnivore family (26).

Analysing the results from the literature, it could note the study carried out by Kołodziej-Sobocińska et al., 2018, in Poland, signals, for the first time, the presence of *T. britovi* species in lynx (14). *T. nativa*, *T. spiralis*, *T. britovi*, and *T. pseudospiralis* larvae were identified in the lynx muscle from Finland (1, 21).

In Estonia, the raccoon dog, fox, lynx, and wolf are the most important reservoirs of *Trichinella*, in the sylvatic cycle. In 2001, Järvis et al. identified *T. spiralis*, *T. britovi*, and *T. nativa* in muscle samples collected from the same wild carnivores (12). For the first time, Kärssin et al. isolated *T. spiralis* larvae from lynx muscles in 2021 (13). Pozio et al. (2004) isolated the spe-

cies *T. pseudospiralis* from a lynx in Froso, Sweden (24). Frey et al. (2009) initiated a study to reassess the epidemiological status of *Trichinella* infection in wildlife in Switzerland. Muscle samples from foxes and lynxes have been examined, and *T. britovi* has been identified (10). On the American continent, the lynx appears for the first time as the host of the species *T. pseudospiralis* (29).

Epidemiological investigations conducted in seven areas of Alaska revealed a 21% prevalence of infection with *Trichinella* spp. in the lynx, which indicates that they are good indicators for assessing the risk of infection with this nematode. The species isolated from the lynx muscle was *T. nativa* (30).

T. britovi, but also in a smaller percentage, *T. nativa*, and *T. spiralis* were identified in the muscle tissue of wild animals (badger, marten, jackal, raccoon dog, fox, wolf, and lynx) from Latvia (9). *T. britovi* is the most widespread species of wild carnivore in Europe, Asia, and North and West Africa. *T. britovi* can also affect domestic pig populations and is the second species of *Trichinella* to affect human health (2, 23).

In Romania, there are only two species of the genus *Trichinella*, namely *T. spiralis* and *T. britovi*, which significantly affect wild animals. Animals parasitized by this nematode are fox, jackal, raccoon dog, wolf, wild cat, lynx, stone marten, badger, mink, ermine, ferret, bear, and wild boar. *T. britovi* is the most widespread species in the sylvatic cycle in Romania (3, 4, 5, 11, 15, 16, 17, 18, 20). The presence of the species *T. britovi* in wild carnivores found in different peripheral habitats of rural localities may represent a way of transmission of this zoonosis to species of wild fauna of hunting interest, such as wild boar, and, through it, to humans (6, 11, 17, 19).

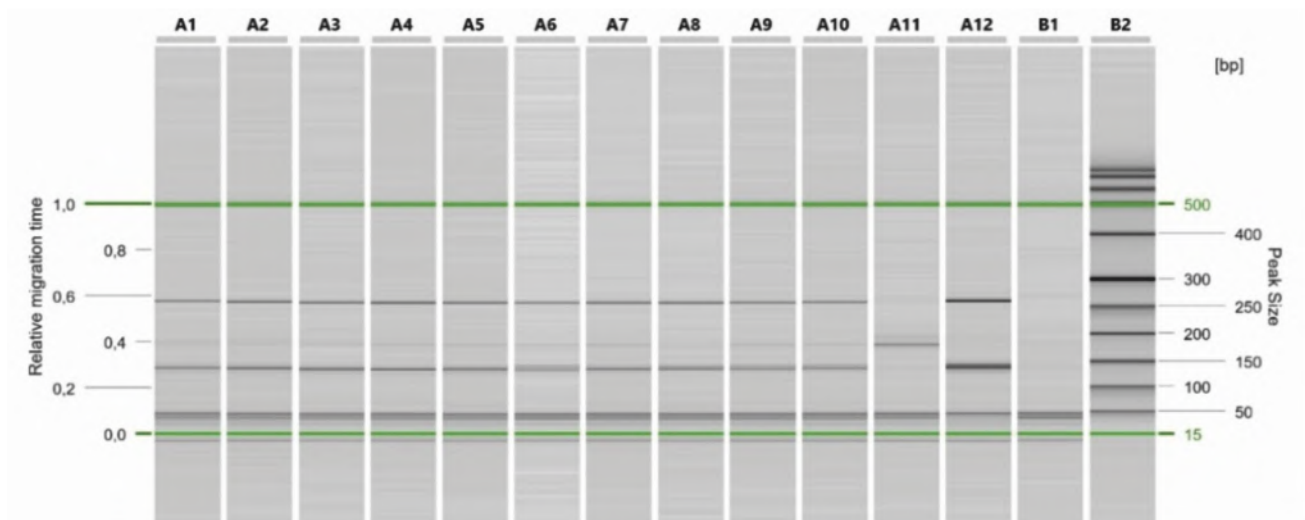


Fig. 2. Capillary electrophoresis run of multiplex PCR on *Trichinella* larvae collected from the lynx; A1 - A10 larvae isolated from the lynx; A11 *T. spiralis* positive control; A12 *T. britovi* positive control; B1 negative control; B2 size marker

CONCLUSIONS

The presence of the species *T. britovi* in the lynx muscle emphasizes the vector role of this wild carnivore in the transmission of trichinellosis in the sylvatic environment and confirms that the nematode *T. britovi* remains a permanent presence in the wild fauna of Romania. We conclude that the lynx can have direct repercussions on consumption species, in particular wild boar and bear, at risk of human infestation.

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