

THE WOLF (*CANIS LUPUS*), AN INDICATOR SPECIES FOR THE SYLVATIC *TRICHINELLA* CYCLE IN ROMANIA

LUPUL (*CANIS LUPUS*), O SPECIE INDICATOARE PENTRU CICLUL SILVATIC AL NEMATODULUI *TRICHINELLA* ÎN ROMÂNIA

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ABSTRACT | REZUMAT

Wildlife is the most important reservoir of *Trichinella* spp. worldwide. *Trichinella britovi* is the most widespread species in European wild carnivores, but also in wildlife of Asia and North and West Africa. *Trichinella britovi* can also infect domestic pig (*Sus scrofa domesticus*) populations and is the second most dangerous *Trichinella* species for human health. The wolf (*C. lupus*) is positioned at the top of the trophic pyramid in the wild fauna. After 2000, an increase in a number of wolf specimens was recorded in Romania due to its status as a protected species. The wolves have a varied diet that includes several wildlife species, such as red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), wild boars (*Sus scrofa*), wild rabbits (*Lepus europaeus*), and fallow deer (*Dama dama*), but also, even if more rarely, domestic animals, such as sheep (*Ovis aries*), goats (*Capra aegagrus hircus*), domestic pigs (*S. scrofa domesticus*), and, occasionally rodents like mice (*Mus musculus*) and black rats (*Rattus rattus*). The study aimed to investigate the presence of *Trichinella* spp. larvae in the muscle tissue of *C. lupus* originating from three Romanian counties. Muscle samples were examined by artificial digestion, and the obtained larvae were identified at the species level by multiplex PCR. All the *C. lupus* specimens were found infected by *Trichinella britovi*, the most frequently observed species in wild carnivores in temperate zones. The present study provides further information on the circulation of *T. britovi* in *C. lupus* and confirms its role in maintaining and transmitting *Trichinella* infection in the Romanian territory.

Keywords: *Trichinella britovi*, Romania, wolf, *Canis lupus*

Fauna sălbatică este cel mai important rezervor de *Trichinella* spp. la nivel mondial. *Trichinella britovi* este specia cea mai răspândită la carnivorele sălbatice din Europa, dar și la animalele sălbatice din Asia și Africa de Nord și de Vest. *Trichinella britovi* poate infecta și populațiile de porci domestici (*Sus scrofa domesticus*) și este a doua cea mai periculoasă specie de *Trichinella* pentru sănătatea umană. Lupul (*C. lupus*) se află în vârful piramidei trofice în fauna sălbatică. După anul 2000, în România a fost înregistrată o creștere a numărului de exemplare de lup datorită statutului de specie protejată. Lupii au o dietă variată care include mai multe specii sălbatice precum cerbul comun (*Cervus elaphus*), căpriorul (*Capreolus capreolus*), mistrețul (*Sus scrofa*), iepurele sălbatic (*Lepus europaeus*) și cerbul lopătar (*Dama dama*), dar și, chiar dacă mai rar, animale domestice precum oile (*Ovis aries*), caprele (*Capra aegagrus hircus*), porcii domestici (*S. scrofa domesticus*) și, ocazional, rozătoare precum șoarecii (*Mus musculus*) și șobolanii negri (*Rattus rattus*). Studiul a avut ca scop investigarea prezenței larvelor de *Trichinella* spp. în țesutul muscular al *C. lupus* originar din trei județe ale României. Probele musculare au fost examinate prin digestie artificială, iar larvele obținute au fost identificate la nivel de specie prin PCR multiplex. Toate exemplarele de *C. lupus* au fost infectate cu *T. britovi*, specia cea mai frecvent întâlnită la carnivorele sălbatice din zonele temperate. Studiul prezent oferă informații suplimentare privind circulația speciei *T. britovi* la *C. lupus* și confirmă rolul acestuia în întreținerea și transmiterea infecției cu *Trichinella* pe teritoriul României.

Cuvinte cheie: *Trichinella britovi*, Romania, lup, *Canis lupus*

Species of the genus *Trichinella* have a global distribution in wild carnivores and omnivores, thanks to their ability to infect a wide range of hosts that includes ma-

mmals, birds, and reptiles, and are widespread on all continents except Antarctica (37). Four species of the genus *Trichinella* are present in Europe: *T. spiralis*, *T. britovi*, *T. pseudospiralis*, and *T. nativa*. *Trichinella britovi* is the most widespread species among wild carnivores (30,38). Wild species such as the lynx (*Lynx lynx*), the jackal (*Canis aureus*), the wolf (*C. lupus*), the red fox (*Vulpes vulpes*), and the wildcat (*Felis silvestris*) have been more frequently found infected with *T. britovi* (73% to 98%) rather than with the other European *Trichinella* species (19, 38). In Romania, *L. lynx*, *V. vulpes*, *C. aureus*, *C. lupus*, and *F. silvestris* represent an important source of infection with *Trichinella* spp. larvae

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and play an essential epidemiologic role in the maintenance of sylvatic trichinellosis (5).

The *C. lupus*, due to its position at the top of the trophic pyramid, has a high probability of getting infected by *Trichinella* spp. In Romania, the *C. lupus* has benefited from the status of a protected species since 2000, and such a condition led to a notable increase in the number of specimens present in the territory (10). The *C. lupus* diet includes a variety of wildlife species (e.g., *C. elaphus*, *C. capreolus*, *S. scrofa*, *L. europaeus*, and *D. dama*), domestic animals (e.g., *O. aries*, *C. aegagrus hircus*, and *S. scrofa domesticus*), and small rodents (*M. musculus* and *R. rattus*) (12). Consumption of mice is a feeding habit common to several species of canids (e.g., *C. lupus*, *V. vulpes*, and *C. aureus*), which can represent a natural source of infection with various parasitic species, the most relevant of which is *Trichinella* (12).

The present study aimed to investigate the presence of *Trichinella* spp. larvae in some *C. lupus* specimens collected in three counties of Romania, and to identify the species involved in the infection.

MATERIALS AND METHODS

Diagnostic procedures

The research was carried out over one year on nine *C. lupus* specimens (six males and three females) aged between 2 and 5 years. For six specimens, an official derogation was issued by the Ministry of Environment, Water, and Forests in compliance with Law 407/2006 (on hunting and wildlife protection) (23), and Emergency Ordinance No 57/2007 (33) while the remaining three specimens were found dead. The animals originated from three Romanian counties, Timiș, Alba, and Cluj - Napoca (Table 1). They were examined at the Parasitic Diseases Clinic of the Faculty of Veterinary Medicine Timișoara / University of Life Sciences from Timișoara.

About 30 g of muscle from the diaphragm and foreleg muscles were sampled from each animal and tested for the presence of *Trichinella* spp. larvae by artificial digestion according to Commission Regulation (EC) no. 1375/2015 (11). After artificial digestion, larvae were collected, counted, stored in 96% ethanol, and sent to

the European Reference Laboratory for Parasites (EUR LP) (Rome, Italy) for species identification by multiplex PCR (39). Briefly, DNA purified from single larvae was extracted using a DNA IQ System kit (Promega, Madison, WI, USA) and a Tissue and Hair Extraction kit (Promega, USA). Five primer sets targeting specific regions (expansion segment V, ITS1, and ITS2) of the ribosomal DNA repeats were used in multiplex PCR to obtain a species-specific electrophoretic DNA band pattern (40).

RESULTS AND DISCUSSION

Eight out of a total of nine (88.88%) *C. lupus* specimens collected from Timiș, Alba and Cluj-Napoca County tested positive for *Trichinella* spp. Larvae. Five *Trichinella* larvae for each positive animal were individually tested by multiplex PCR and identified as *T. britovi* (Fig. 1).

Wildlife is the main reservoir of *Trichinella* and represents an important source of infection for domestic animals such as *S. scrofa domesticus*, or less frequently horses (*Equus ferus caballus*), which are in turn the primary source of infection for humans (38). In Europe, *T. britovi* has been reported in numerous sylvatic hosts: *C. lupus* (13, 38), *C. aureus* (16,38), *V. vulpes* (38, 45), *N. procyonoides* (20,28,38), *F. silvestris* (15,38), *L. lynx* (22, 25, 38), badger (*Meles meles*) (31,38), pine marten (*Martes martes*) (26), stone marten (*Martes foina*) (2, 38), European otter (*Lutra lutra*) (38), European beaver (*Castor fiber*) (42), *S. scrofa* (27, 38, 41) and bear (*U. arctos*) (21, 38).

In Romania, *Trichinella* infection is present and widespread throughout the country, affecting wild animals significantly. Three species are circulating in our country: *T. spiralis*, *T. britovi*, and *T. pseudospiralis*. The main animals affected by this parasite are *V. vulpes*, *C. aureus*, *C. lupus*, *L. lynx*, *F. silvestris*, *M. foina*, *M. meles*, ermine (*Mustela erminea*), ferret (*Mustela putorius*), mink (*Mustela lutreola*), *U. arctos*, *S. scrofa*, and, recently, *N. procyonoides* and *M. martes* (5, 7, 8, 18, 25, 26, 27, 28).

The present study correlates with previously reported results observed in both Romania and Europe. *Trichinella britovi* is described here as the only species iso-

Table 1
Counties, hunting grounds, and GPS locations of *C. lupus* specimens

Nrt. Crt.	County	Hunting fund	GPS Location	
			Latitude (N)	Longitude (E)
1.	TM	59 Giarmata	45.91878	21.40053
2.	TM	59 Giarmata	45.896512	21.411023
3.	CJ	54 Huiedin	46.871016	22.897096
4.	AB	20 Lopadea Nouă	46.263813	23.794434
5.	AB	52 Pianu	45.869849	23.798211
6.	AB	12 Geamăna	46.351796	23.2408112
7.	AB	20 Lopadea Nouă	46.253606	23.798211
8.	AB	20 Lopadea Nouă	46.247671	23.793061
9.	TM	59 Giarmata	45.91204	21.362958

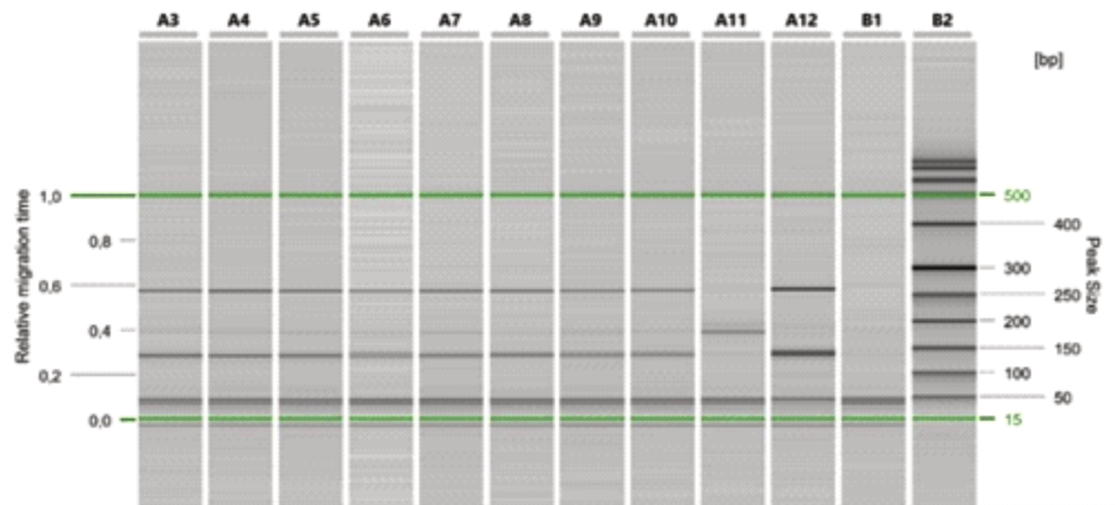


Fig. 1. Capillary electrophoresis run of multiplex PCR on *Trichinella* larvae collected from the *C. lupus*; A3 - A10 larvae isolated from the *C. lupus*; A11 *T. spiralis* positive control; A12 *T. britovi* positive control; B1 negative control; B2 size marker.

lated from the muscle tissue of *C. lupus* originating from three different Romanian counties.

Trichinella britovi, the most widespread species in wild carnivores in Europe, also affects wildlife in Asia and North and West Africa. *Trichinella britovi* can also infect domestic pig populations and is the second most dangerous *Trichinella* species for human health (1,34).

Canis lupus is the wild carnivore species considered sentinel for *T. britovi* infection in Europe (29). Carnivore-carnivore transmission, influenced by incorrect hunting practices adopted in some regions of the globe, supports the high prevalence of *Trichinella* infection observed in northwestern Europe in *V. vulpes*, *C. lupus*, *C. lupus familiaris*, mustelids, and *N. procyonoides*, with the highest infection rate in *C. lupus* (2,35).

The role as an indicator of *T. britovi* infection attributed to this wild carnivore has been reported in Spain (43), Balkan countries (44, 47), Poland (4, 9), Turkey (6, 14), and Croatia (3).

In Romania, *Trichinella* sp. larvae were reported by direct trichinelloscopy and artificial digestion in the past by Nesterov et al. (32), Gherman et al. (17), and Marian et al. (24), but the first molecular characterisation of *T. britovi* species in *C. lupus* was made by Blaga et al. (5), in 2009. Blaga in his study identified *C. lupus* as the main wild host for *Trichinella*, followed by *F. silvestris*, and *V. vulpes* (5).

The present study confirms the presence of *T. britovi* in *C. lupus* in Romania.

CONCLUSIONS

The present study provides current information on the circulation of *T. britovi* in *C. lupus* and underlines its important role in the maintenance of the infection in wildlife as well as in the transmission of the parasite from the wild to the peripheral domestic ecosystem, which in turn represents a direct risk of human infection.

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