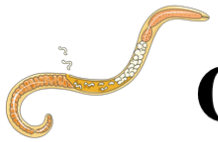
Trichinosis in Romania, updates regarding *Trichinella* spp. infections in wild animal species

Zsolt BOROS, Calin GHERMAN, Vasile COZMA

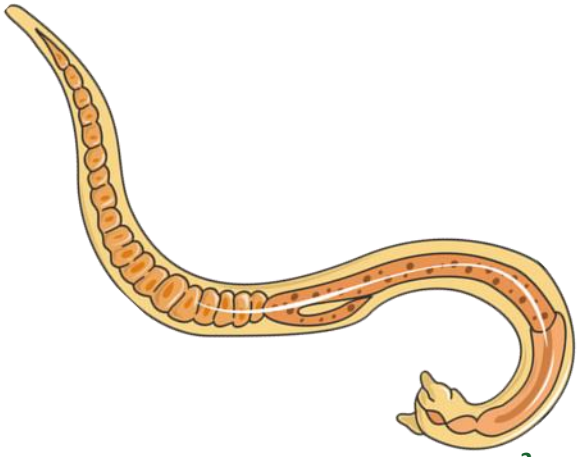
EMOP 21The 13th European Multicolloquium of Parasitology
Date of the event (12th-16th October 2021)

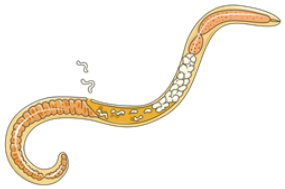
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 816172



Objectives

The main objective of this study is to present the evolution of the two *Trichinella* species in wild animals from Romania in the last 30 years, with the help of the literature plus original studies.





1991-2000

Implicatii ale activitatii umane in epizootologia trichinelozei silvatice

(Implications of human activity in the epizootiology of silvical trichinosis)

Vadim Nesterov, I. Colofan, A. Nitulescu, F. Costiov, C. Dumitrescu, C. Milla, Sandra Popescu. 1991, Rev.Rom. Paraz.

- **The objective** of this study was to indentify the presence of this parasite in several animal species present in the country.

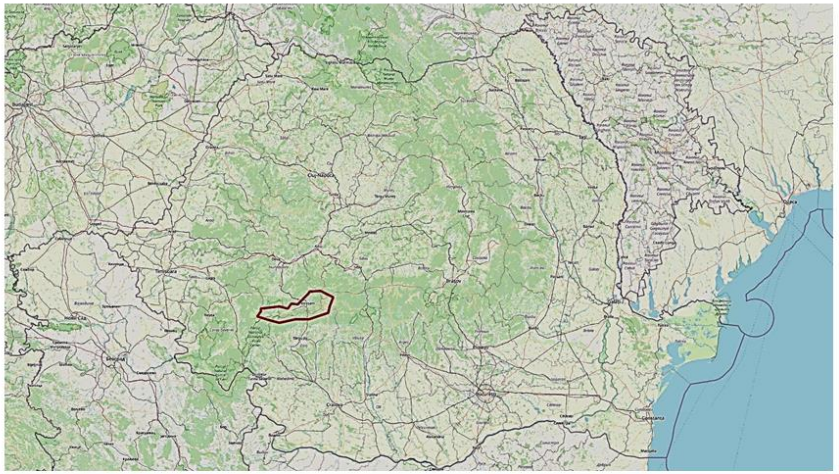
- **Materials and methods -**The study was done over a period of 20 years.
  - 50 bears (*Ursus arctos*), 399 wolfs (*Canis lupus*), 972 foxes (*Vulpes vulpes*), 158 wild cats (*Felis silvestris*), 166 badgers (*Meles meles*), 38.908 wild boars (*Sus scrofa*), 157 polecats (*Mustela putorius*);

- **Results** -positive animals were: bears 18.5%, wolfs 30.5%, foxes 15.8%, wild cats 21.5%, badgers 6%, wild boars 0.1%, polecats 5.2%;

Măsuri de supraveghere, combatere şi prevenire a trichinelozei în Valea Jiului – cel mai mare focar din România (1987-2001)(Surveillance, control and prevention measurestrichinosis in the Jiu Valley - the largest outbreak inRomania (1987-2001)

Gheorghe Cristea, Ironim Suteu. 1996. Sci Paras.

- **The objective** of this study was to indentify the presence of this parasite in several animal species present in Jiu valley.
- **Materials and methods -**The study was done between 1991-1994.
  - 492 rats, 31 wild boars, 46 nutria, 16 fox, and 38 mice were examined with trichinoscopy and artificial digestion.
- **Results** -positive animals were: 204 rats (41.5%); 7 wild boars (23.58%); 1 nutria (2.1%) 5 fox (31.5%), and 4 mice (10.5%).



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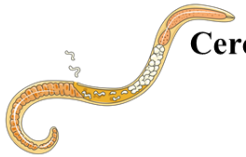
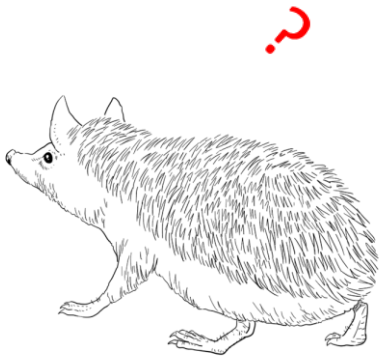
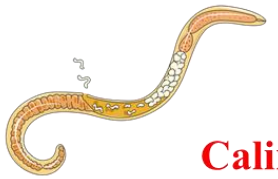


Cercetari privind intensivitatea infestatiei experimentale cu larve de Trichinella la arici (*Erinaceus europeaus*) Research on the intensity of experimental infestation with Trichinella larvae in hedgehogs (*Erinaceus europeaus*)

Gheorghe Cristea, 1996, Sci parasitol

- **The objective** of this study was to observe the experimental infestation with Trichinella spp, in hedgehogs.
- **Materials and methods** – 5 hedgehogs were experimentally infected with Trichinella larvae (500 larvae/animal) obtained from rats for 2 days.
- **Results** – 7 days latter two of the animals died- nu parasites found in the muscles.
  - 40 days post infestation all the 3 animals were positive for Trichinella spp.
  - the highest intensity =forearms and back muscles 20%; cervical and dorsal muscles 18.2%; inferior abdominal muscles 17%; maseter muscles 15%; foot muscles 12% midriff muscles 7.8%; tounge muscles 5.1%; pelvian muscles 3.2%.

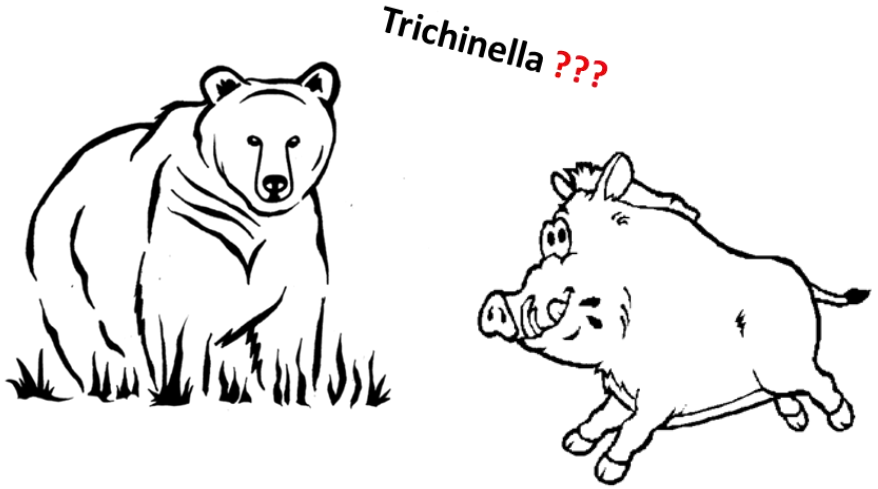




Epizootologia trichinelozei in Transilvania (Epizootology of trichinosis in Transylvania)

Calin Gherman, Ironim Suteu, 1992-1997, PhD thesis

- **The objective** of this study was to indentify the presence of this parasite in several wild animal species present in Transilvania.
- **Materials and methods -**The study was done between 1992-1997.
  - 17.053 wild boars, 503 bears were examined with trichinoscopy and artificial digestion.
- **Results** -positive animals were: 68 wild boars (0.38%); 61 bears (12.12%).

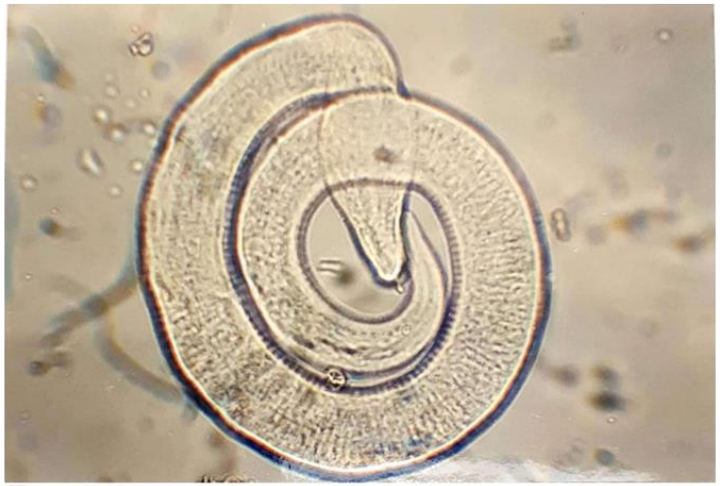


Cercetari privind transmisibilitatea suselor silvatice de Trichinella, specii sinatorpe (Research on the transmissibility of wild Trichinella strains, sinatorpe species)

Calin Gherman, Ironim Suteu, 1998. PhD thesis

- **The objective** of this study was the verification of the transmissibility of isolated wild strains in domestic animals.

- **Materials and methods -Strain M,** isolated from wild boars.
  - **Strain V,** isolated from foxes.
  - **Strain U**, isolated from bears.
  - experimental infestation in pigs and other animal species.
- **Results** – **Strain M** 100% infestation rate;
  - **Strain V** 100% infestation rate;
  - **Strain U** 100% infestation rate;



Muscle larvae of M strain (Gherman, 1998).

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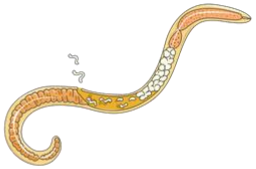
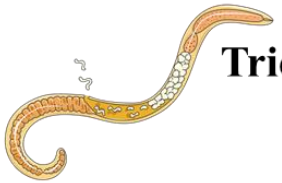
1997-2007



The prevalence of trichinelosis in Covasna county between 1997- 2007

I. Oprescu, Gh. Dărăbuş, S. Morariu, Narcisa Mederle, M. Ilie, K. Imre, Mirela Imre, A. Mako, I. Nincov, 2007. Lucrări ştiinłifice medicină veterinară vol. xlii

- **The objective** was to present the cases of trichinelossis observed between 1997 and 2007, in Covasna County, in wild boars and bears.
- **Materials and methods** –trichinelloscopy and artificial digestion
  - 210 meat samples of wild boar
  - 60 meat samples of bears
- **Results** -20 samples of wild boars were positive, which represents 9.52% of the total samples. The higher proportion of positive samples was 26.3% in 1997.
  -regarding the bears only 23 were positive (38.3%). The highest proportion of positive samples was found in 2000 (66.6%).

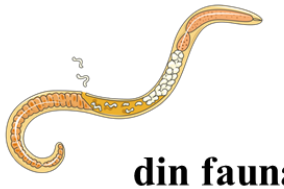


Trichinellosis in Romania: a short review over the past twenty years

Olteanu G. 2001. Parasite

- **The objective** of this study was to emphasized the evolution of trichinellosis in man and animals during the last two decades in Romania.
- **Materials and methods** – pigs and wild boars were examined by artificial digestion
- **Results**

Year	Pigs			Wild boars			Number of human cases
	Number			Number			
	Examined	Infested	%	Examined	Infested	%	
1990	377,386	477	0.12	48	–	–	–
1991	328,492	218	0.06	22	–	–	–
1992	230,657	99	0.04	22	–	–	37
1993	292,254	276	0.09	45	–	–	201
1994	212,672	399	0.19	12	–	–	41
1995	185,435	2,504	1.35	77	1	1.30	55
1996	217,510	738	0.34	33	–	–	9
1997	2133,722	1,874	0.87	18	–	–	25
1998	123,898	829	0.66	23	–	–	67
1999	123,661	349	0.27	19	–	–	146



2001-2019

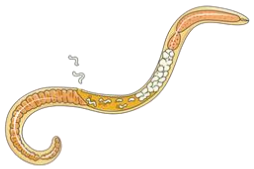
Zoonoze helmintice la specii de carnivore sălbatice din fauna României (Helminthic zoonoses in species of wild carnivores from the Romanian fauna)

Gherman Calin, V. Cozma, Viorica Mircean, F. Brudaşcă, N. Rus, A. Detesan. 2002. Sci Parasitol

- **The objective-** assessment of the zoonotic risk of some species of wild carnivores from the Romanian fauna: the fox (*Vulpes vulpes*), the wolf (*Canis lupus*) and the cat wild (*Felis silvestris*).
- **Materials and methods-** 1999-2002

  - Fox (50), wolf (7), wild cat (6) carcases
- **Results-** Foxes- *Trichinella* spp. 16%;

  - Wolfs- *Trichinella* spp. 71.4%;
  - Wild cats- Trichinella spp. 16.6%;



A dramatic increase in the incidence of human trichinellosis in Romania over the past 25 years: impact of political changes and regional food habits.

Radu Blaga, Durand, B., Antoniu, S., Gherman, C., Cretu, C. M., Cozma, V., & Boireau, P. 2007. Vet Parasitol

- **The objective** was to present an analysis of passive surveillance data of human trichinellosis in Romanian counties over the last 25 years.
- **Materials and methods-** Epidemiologic data for each county were analyzed from two different time periods: before (1980–1989) and after (1990–2004) political changes.
- **Results**- During the past 25 years, 28,293 human cases.
  - Increase in the incidence was observed from 1980 to 1989 compared with the 1990–2004 period.

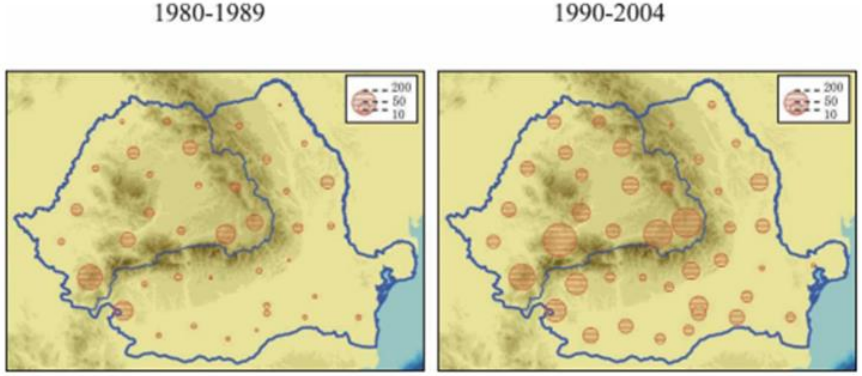


FIGURE 2. Cumulated incidence rate of trichinellosis in Romanian counties, 1980–1989 and 1990–2004 (cases per 10^6 persons per year). Thin blue line = Transylvanian border. This figure appears in color at www.ajtmh.org.

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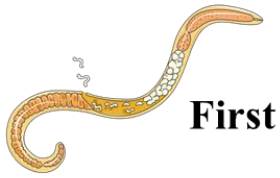


First Identification of *Trichinella* sp. in Golden Jackal (*Canis aureus*) in Romania

Radu Blaga, Gherman, C., Seucom, D., Cozma, V., & Boireau, P. (2008). J wildlife diseases

- **The objective-** identification of *Trichinella* spp in golden jackals.
- **Materials and methods-** a golden jackal that was collected in July
- trichinelloscopy, artificial digestion, and PCR.
- **Results -***Trichinella britovi*

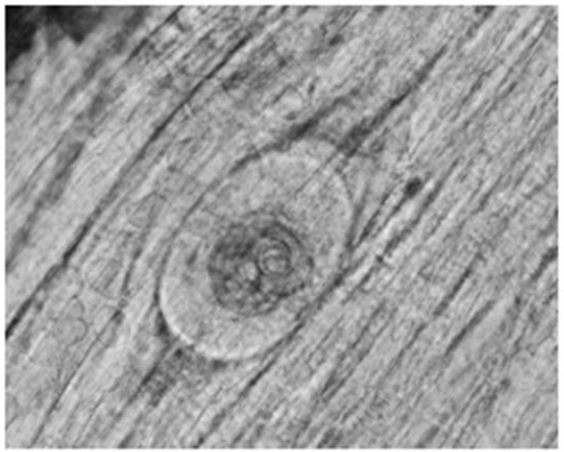
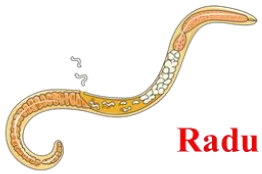


FIGURE 1. Encysted larva of *Trichinella britovi* in the tibial muscle of golden jackal (*Canis aureus*) (trichineloscopy 40×).



Trichinella species circulating among wild and domestic animals in Romania

Radu Blaga, C. Gherman, V. Cozma, A. Zocevic, E. Pozio, P. Boireau. 2009. Vet. Parasitol.

- **The objective** for this study was to identify *Trichinella* spp. circulating among wild and domestic animals in Romania, using PCR method.
- **Materials and methods-** larvae from 54 wild and 23 domestic mammals were examined.
- **Results-** *T.britovi* was more prevalent (n=26.65%) than *T. spiralis* (n=14.35%). *T. spiralis* was more prevalent in domestic animals (n=9), *T. britovi* was more prevalent in wildlife (n=24).

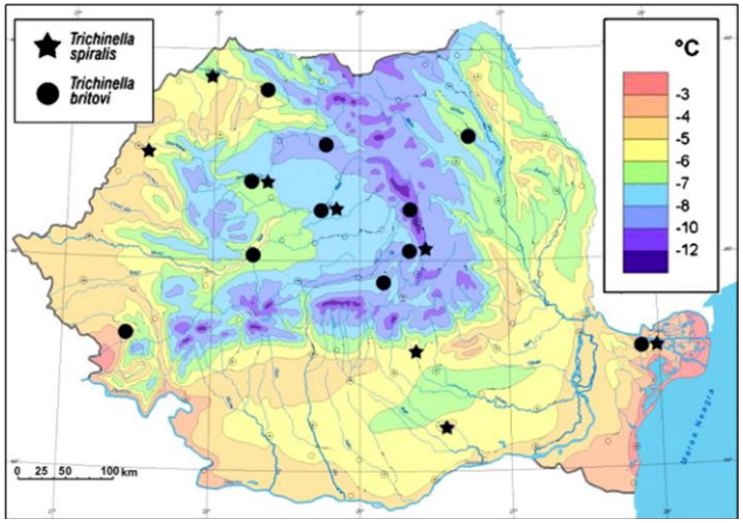


Fig. 1. Distribution of *Trichinella spiralis* (solid star) and *Trichinella britovi* (solid circle) infected animals in Romania by climate zones. Temperatures displayed are the lower average temperatures detected in January during the period 1960–2000. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

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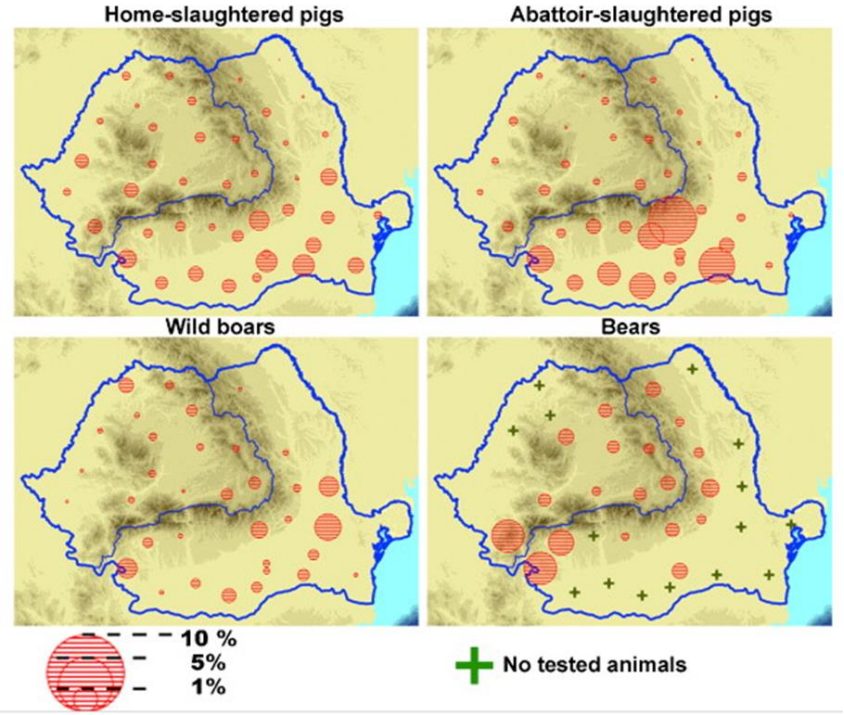
Animal Trichinella infection in Romania: Geographical heterogeneity for the last 8 years

Radu Blaga, Durand, B., Stoichici, A., Gherman, C., Stefan, N., Cozma, V., & Boireau, P. 2009. Vet. Parasitol.

- **The objective-** was true routine Trichinella test data in pigs and game animals, to investigate the extent of the infection in sluagheterd animals in Romania, during 1997-2004.
- **Materials and methods-** trichinelloscopy of domestic and wild animals.
- **Results:-** accumulative prevalence of 8 cases/10/4 for pigs, 9 cases/10/3 for wild boars, and 13.1 cases/10/2 for bears for the 8 years period.

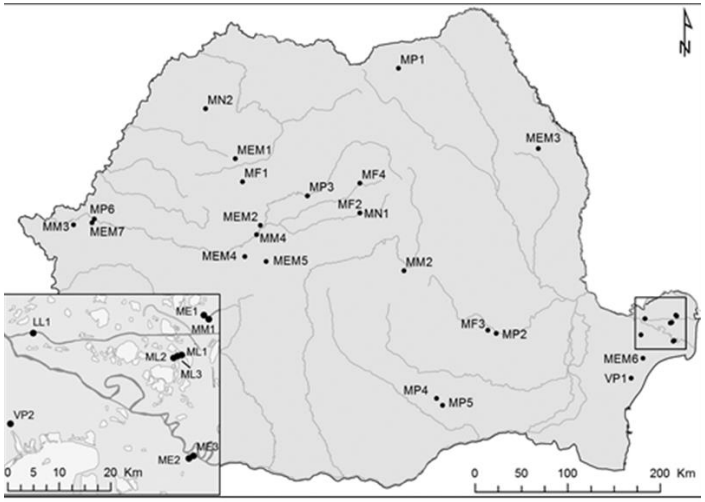


European Mustelids occupying Pristine wetlands in the Danube delta are infected with Trichinella likely derived from domesticated swine

Miruna Oltean, Kalmár, Z., Kiss, B. J., Marinov, M., Vasile, A., Sándor, A. D., & Rosenthal, B. M. 2014. J. wildlife diseases

- **The objective** was to help clarify the relationship between wildlife, agricultural production, and public health, we sought to describe the *Trichinella* species distribution in mustelids in Romania.
- **Materials and methods-** 32 specimens from nine species of Mustelidae;

  - artificial digestion; PCR;
- **Results-** *Martes foina* (2)- *T.britovi; Mustela ermine (2) T.britovi (1) T. sprialis; Mustela lutreola (1);*



Geographic area for sample collections. LL=*Lutra lutra*; ME=*Mustela erminea*; MEM=*Meles meles*; MF=*Martes foina*; ML=*Mustela lutreola*; MM=*Martes martes*; MN=*Mustela nivalis*; MP=*Mustela putorius*; VP=*Vormela peregusna*. Inset: Danube Delta zone.

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Prevalence of *Trichinella* spp. Infection in large wild carnivore species from Romania between Jan 2014 and July 2015.

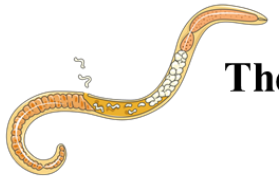
Marian Ionuţ, I., Mihalca, A. D., & Gherman, C. M. 2015. Bulletin USAMV.

- **The objective** was to update the data on the prevalence of *Trichinella* spp. infection in large carnivores from Romania between 2014 and 2015.
- **Materials and methods-** 67 examined cadavers, 54 were golden jackals, 5 wolves, 5 wildcats and 3 Eurasian lynxes.

  - artificial digestion
- **Results-** the prevalence was 40.0% in wolf, 33.3% in wildcat, 53.7% in golden jackal and 66.6% in Eurasian lynx.

Tab. 1. The frequency, prevalence and 95% CI of Trichinella spp. infection in wild carnivores

Species	Frequency	Prevalence (%)	95%CI
Wild cat	2/6	33.3	4.3 - 77.7
Wolf	2/5	40.0	5.2 - 85.3
Golden Jackal	29/54	53.7	39.6 - 67.4
Lynx	2/3	66.6	9.4 - 99.2



The red fox (*Vulpes vulpes*) plays a minor role in the epidemiology of the domestic cycle of Trichinella in Romania

Kalmar Imre, Pozio, E., Tonanzi, D., Sala, C., Ilie, M. S., Imre, M., & Morar, A. 2015. Vet. Parasit.

- **The objective** was s to provide data about the prevalence of *Trichinella* spp. infections in red foxes (*Vulpes vulpes*)
- **Materials and methods-** 121 animals from 45 hunting grounds

  - artificial digestion
- **Results**- Infections were detected in 26 (21.5%) foxes from 18 (40%) hunting grounds of the three

  counties (13/67 in Arad, 1/3 in Hunedoara, and 12/51 in Timis).

  - The mean larval density was 10.5 larvae per gram., *T. britovi* (96%), and the larvae from one isolate were identified as *T. spiralis* (4%).

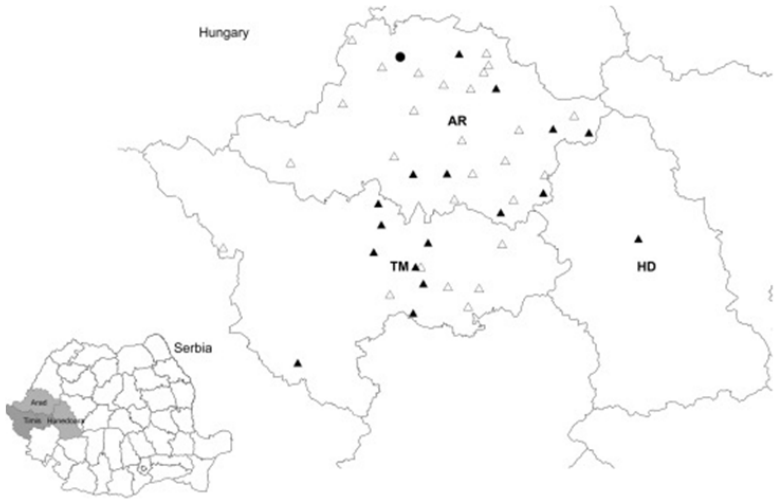


Fig. 1. Map of Romania showing the locations of the red fox (*Vulpes vulpes*) sampling in three western counties, i.e., Arad (AR), Hunedoara (HD) and Timiș (TM). Black triangle, locality in which one or more *Trichinella britovi*-infected foxes were detected; empty triangle, locality in which one or more *Trichinella* spp.-negative foxes were detected; black circle, locality in which only a single *Trichinella spiralis* infected fox was detected.

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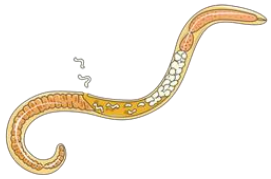


Epidemiological study on Trichinella infection in pigs and wild boars in Hunedoara county (Romania), during of 2010-2014 period

Flaviu Otniel Ciobotă, Gh. Cristea, Mariana Ioniță, I. L. Mitrea, 2015, Supplement 1/2015, 4th ISAA

- **The objective** of this study was to was to determine the incidence of Trichinella infection in domestic pigs and wild boars from Hunedoara county, between 2010 and 2014.
- **Materials and methods -**The study was done between 2010-2014.
  - 59.759 domestic pigs raised in households and from 973 wild boars were examined with trichinoscopy and artificial digestion.
- **Results** –positive pigs were: in 2010, 2 (0.016%); in 2011, 4(0.02%); in 2012 , 2 (0.016%); in 2013,5 (0.048%), and in 2014, 2 (0.017%).
- - In wild boars: in 2010, 1(0.82%); in 2011, 0 (0%); in 2012, 2 (1.30%), In 2013, 2 (1.11%), and in 2014, 3 (0.74%).

Figure1. *Trichinella* spp. Larvae collected from striated muscles of domestic pig (80x)

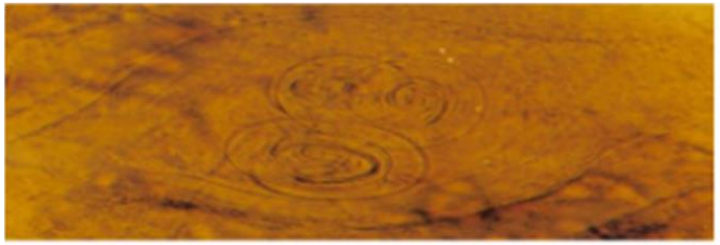
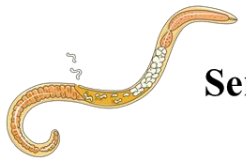


Figure 2. *Trichinella* spp. cyst, along the striated muscle fibers in domestic pig (80x)



2020-2021

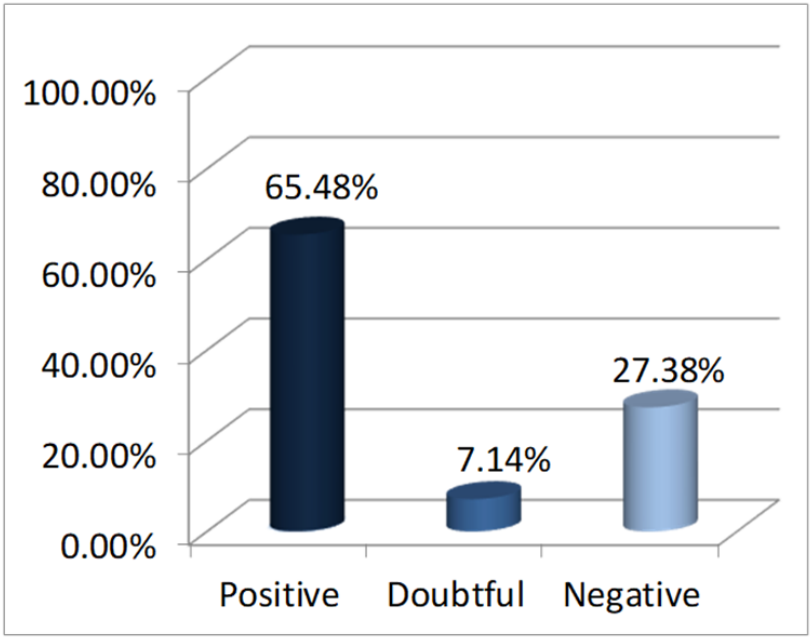
Seroprevalance of *Trichinella* spp. in wild boars (*Sus scrofa*) from Bihor county, western Romania

Zsolt Boros, I. Vallée, L. C. Panait, C. M. Gherman, A. Chevillot, P. Boireau, V. Cozma, 2020. Helminth

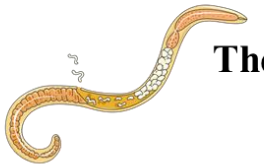
- **The objective** of this study was to identify the presence of *Trichinella* spp. in wild boars from Bihor County, Romania.
- **Materials and methods -** 84 plasma and diaphragm samples were collected from Bihor county,
  - artificial digestion -5 g of diaphragm tissues
  - Indirect ELISA- 84 samples of plasma were tested
  - Western blot- 26 random plasma samples were tested

Figure 1. The map of Romania with counties.

- **Results -** Artificial digestion-all diaphragm samples were negative for *Trichinella* larvae
  - Indirect ELISA- **65.48 %** (n=55) were positive; **7.14 %** (n=6) were doubtful; **27.38%** (n=23) were negative;
  - Western blot -Total of 26 samples:- **23.74%** (n=6) were positive; **-76.92%** (n=20) were negative;
  - Statistical analysis -no inter-rated agreement between these tests were found Kappa value.



ELISA results



The European badger, *Meles meles*, as a new host for *Trichinella britovi* in Romania

Boros Zsolt, Angela Monica Ionica, Georgiana Deak, A. D. Mihalca, G. B. Chisamera, Adriana Gyorke, C. Gherman, Vasile Cozma. 2020. Vet. Parasitol

- **The objective** of the research was to investigate the occurrence of these parasites in badgers and to identify the involved *Trichinella* species.
- **Materials and methods** –61 carcasses of European badgers (32 males, 29 females; 47 adults, 14 young);

-14 counties from Romania

-trichinoscopy and artificial digestion

-Multiplex PCR

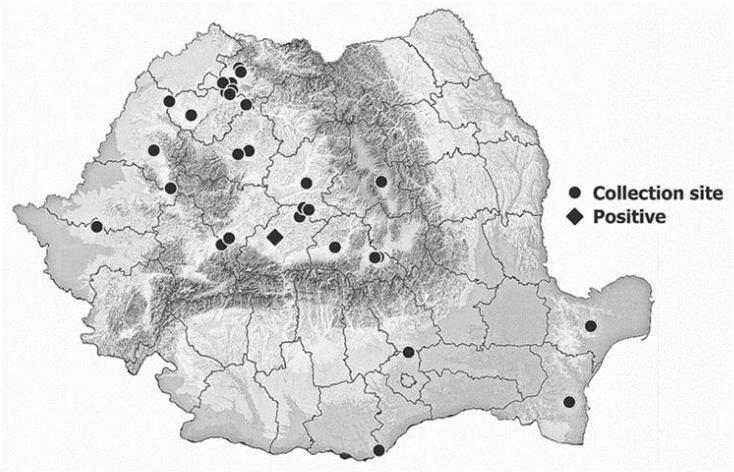


Figure 1. Map showing the collection sites, including the positive animal.

- **Results** -A single badger (1.6%; 95% CI: 0.3-8.7) was found positive for *Trichinella* spp. The animal originated in Sibiu County, central Romania.

  - Five cysts => trichinoscopy.

  - Artificial digestion => 70 larvae/100 g of muscle.

  -The electrophoretic profile of both infested muscle and larval isolates included two bands (~127 and 253 bp respectively), => *Trichinella britovi* in this animal.

- **Conclusion -**The present study describes the first report of Trichinella britovi infection in the European badger from Romania.

  -However, the low prevalence indicates a minor reservoir role for this species

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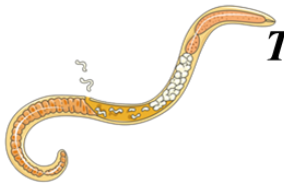

Trichinella spp. infection in European polecats (*Mustela putorius* Linnaeus, 1758) from Romania

Boros Zsolt, Angela Monica Ionică, Georgiana Deak, A.D. Mihalca, G. Chișamera, Ioana Cristina Constantinescu, C. Adam, C. Ghermana, Vasile Cozma. 2021. Helminth.

- **The research objective** was to investigate the occurrence of these parasites in European polecats from Romania and to identify the involved *Trichinella* species.
- **Materials and methods** -75 carcasses of European polecats (54 males, 21 females; 60 adults, 15 young);

  -9 counties from Romania;

  -trichinoscopy and artificial digestion

  -Multiplex PCR

  -Statistical analysis

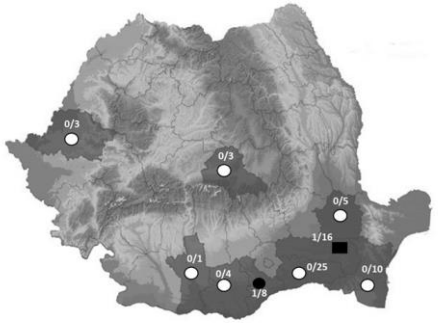

Figure 1. Distribution of *Trichinella* spp. infection in European polecats in nine counties in Romania

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- **Results** -Two European polecats (2.67%; 95% CI 0.32-9.30%) were positive for *Trichinella* spp. by both methods from Ialomița and Giurgiu counties.

  -Artificial digestion revealed an infection rate of **2466 larvae/100 g** of muscle in the polecat from Ialomița and **254 larve/100g** in the animal from Giurgiu.

  -The polecat from Ialomița (infected muscle and larval isolates) co-occurrence of ***Trichinella britovi*** (~127 and 253 bp respectively) and ***Trichinella spiralis*** (173 bp). The polecat from Giurgiu county (infested muscle and larval isolates) included only one band of ~173bp, -occurrence of only ***T. spiralis***.

	Polecat 1 (Ialomița County)	Polecat 2 (Giurgiu County)
	Nr of cysts	Nr of cysts
Diaphragm	15	13
Foreleg	46	12
Posterior leg	17	23
Total	78	48

Table 1. The number of identified cysts in the two positive polecats.

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. This parasitic infections distribution in Romania is influenced by the wild animal's habitat as well as by the eating habits of the local population. The habit to consume game meat like the one from wild boars is only increasing the risk for humans to acquire this parasitic infection. Infections with *Trichinella spp.* in wildlife create outbreaks, in which transmissibility can be produced in domestic, free-range pigs.

This research was supported by University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (USAMV Cluj-Napoca), and by the project PPILOW. The project PPILOW has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°816172.

PPILOW **PARTNERS**

Thank you for your attention

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 816172