sciendo

DOI 10.2478/helm-2023-0015

HELMINTHOLOGIA, 60, 2: 161 – 165, 2023

Intestinal endoparasitism in wild cat (*Felis silvestris*) from Banat area (Romania)

N. MEDERLE[1], G. DARABUS[1], A. STANCU[1], M. PENTEA[1], M. IMRE[1], I. LUCA[1], I. PAVLOVIC[2], N. ZDRAVKOVIĆ[2]

[1]Faculty of Veterinary Medicine of Timisoara, Romania, Calea Aradului 119, 300645 Timisoara, Romania,
E-mail: *narcisa.mederle@yahoo.com*, *gheorghe.darabus@fmvt.ro*, *astancu2002@yahoo.com*, *marius.pantea@e-uvt.ro*, *mirela.imre@gmail.com*, *iasminaluca0@gmail.com*; [2]Scientific Veterinary Institute of Serbia, Janisa Janulisa 14, 1100 Belgrade, Serbia,
E-mail: *dripavlovic58@gmail.com*, **nemanja.zdravkovic@nivs.rs*

Article info

Received September 22, 2022
Accepted May 23, 2023

Summary

The wild cat (*Felis silvestris*), spread in Romania from the Danube Delta to the mountain range is present in the Banat area, on the hunting ground that can be contaminated with different stage developmental forms of parasites, some of them having real zoonotic potential. The wild cat is an animal protected by the Romanian law of protection animals.

Coprological samples from 88 wild cats from 16 hunting grounds, as well as the gastrointestinal tract collected from six wild cats cadavers and the molecular characterization of the cestodes identified in their intestines, allowed us to establish intestinal parasitic fauna. During coprological examination *Isospora* oocysts, tapeworm eggs, eggs of *Toxocara cati*, *Ancylostoma spp*. and *Capillaria spp* were found. At the same time, the form of genera Mesocestoides, Taenia, Toxocara/Toxascaris and Ancylostoma were identified at necropsy. Further polymerase chain reaction (PCR) identification revealed the species of *Taenia taenieformis*, and *Mesocestoides litteratus*, the latter providing a zoonotic potential.

This study, the first in the western part of the country (Banat area, Timis County), provides information about the parasitic fauna of wild cats and underlines the importance of the human contamination risk.

Keywords: wild cat; endoparasitism; zoonotic risk

Introduction

The European wild cat (*Felis silvestris*) is a small wild cat species native to primarily inhabits broad-leaved and mixed forests from Scotland, the Iberian and Apennine Peninsula, Continental Central and Eastern Europe Turkey and the Caucasus (Castelló, 2020). In Romania, wild cat inhabits the Danube Delta, forests and mountain range, territories much wider than the lynx that prefers the quiet forests, as wide as possible, with many old trees and logs. In Romania, the wild cat is a species protected by Romanian law for animal protection No. 205/2004 (the Republic of Romania Official Gazzete, 2014).

It resembles the domestic cat but has longer legs, a larger, flatter head, and a full, relatively short tail ending in a rounded (not pointed) tip. The coat is yellowish gray with dark stripes and bands in the striped tabby pattern; the tail is black-ringed. It measures 45 – 80 cm in head-to-body length and 25 – 40 cm in tail length. Weighs typically 3 – 8 kg. The European wildcat is a lonely animal, but it can also be found in groups during mating. The wild cat mates in February – March and the gestation is about 70 days, and each female gives birth to 2 – 4 kittens. Hunting tactics are similar to those of the house cat (Hunter & Barrett, 2015).

The parasitic fauna of wild and domestic cats is quite similar, which is not surprising because they can be infected with same types of

* – corresponding author

endoparasites belonging to the *Protozoa* or parasitic worms, and some of them have zoonotic potential. Due to the urbanization of natural game habitats, there has been close contact between wild and domestic felids, the spread of parasitic infections in semi-urban areas which reportedly increased the opportunity for the expansion of certain zoonotic parasites in the urban environment and endangering the health of both animals and people (Takács & Takács, 2002; Barutzki & Schaper, 2003; Pavlović *et al*., 2007; Moskvina *et al*., 2018).

In this paper, we identify and evaluate the parasitic load in wild cats from 16 hunting grounds in the Banat region (western Romania) and molecularly characterize the species of cestodes isolated from their intestines and their zoonotic potential.

Materials and Methods

Collection and storage of materials for examination

Coprological samples and the cadavers were collected, transported and processed following the biosecurity rules. The documents regarding the preparation of the necropsy were carried out according to the procedure of the internal regulation of the veterinary clinic (COD USAMVBT – PG 001- R045). Prior to the examination, the gastrointestinal content samples were frozen for 14 days for safety reasons.

Fecal samples

We sampled the fecal matter of 120 wild cats collected from 16 hunting grounds in the Banat region (Timis County) between 2017 – 2019 (Fig. 1). In addition to feces, intestinal contents obtained during necropsy were examined too. Examinations were performed with the conventional flotation method with a saturated $ZnSO_4$ solution (Euzéby, 1981). Parasite oocysts and eggs were determined by morphology characteristics.

Necropsy examination

We performed a necropsy on seven dead wild cats, four males and three females. All animals were collected from the same hunting grounds in the Banat region, from where the feces samples were taken. According to the occlusal surface and the teeth, the dental assessed age of the animals ranged between 2 – 4 years. Necropsy examination included predilection sites of internal parasites: trachea, lung, heart, complete gastrointestinal tract, liver, kidney and urinary bladder. The stomach, intestine and the other organs were slit opened and visible helminths removed with contents washed

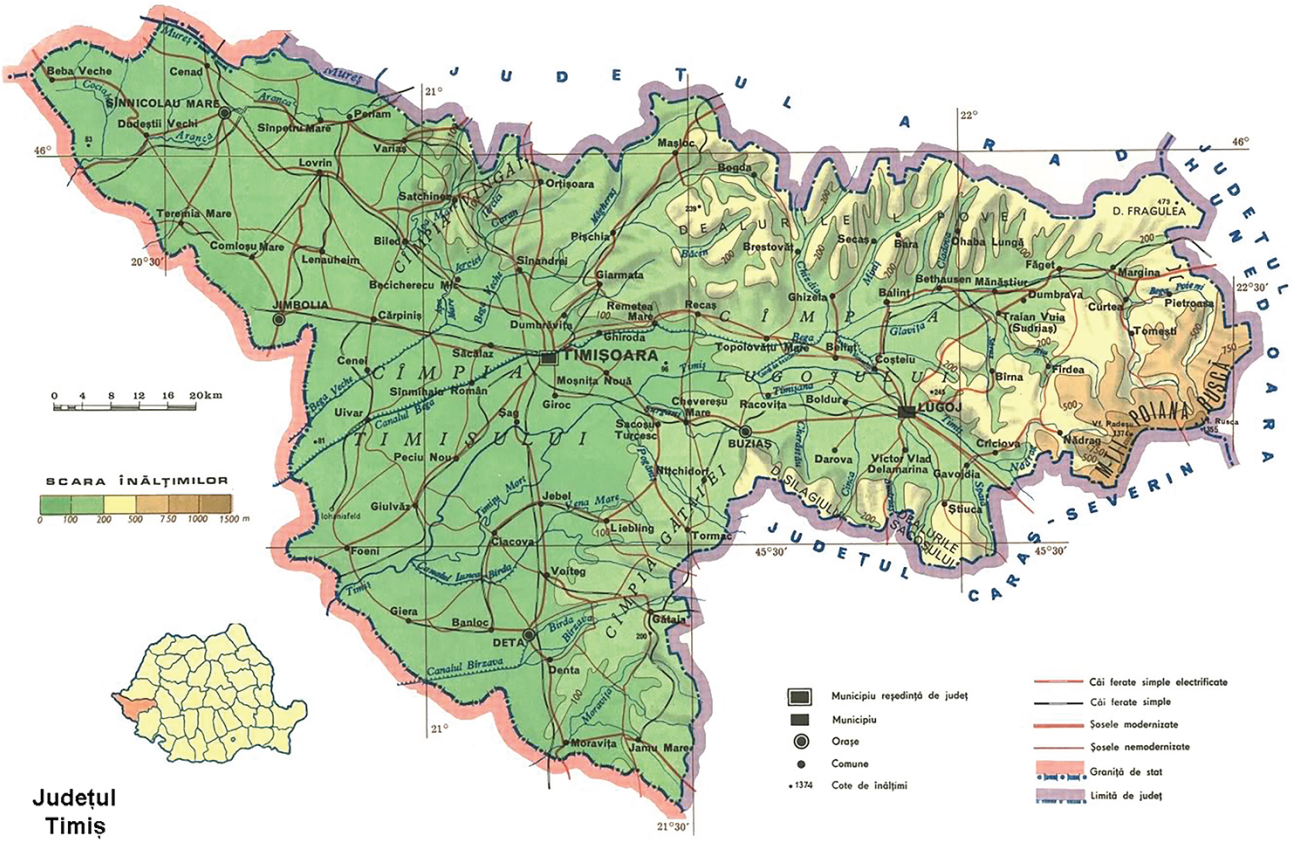

Fig. 1. The map of Timis county (https://pe-harta.ro/judete/Timis.jpg)

out (thoroughly intestine). The contents and washing were percolated over a gauze sieve mesh aperture 150 mm under jet water. The retained material was examined in small quantities at a time in a large whistle enamel tray. The contents of the stomach and intestines were examined in Petri dishes on a dark background by a microscope and found parasites we stored for analysis in 2 % formalin.

Polymerase chain reaction

Fragments of mature forms of tapeworms were identified by morphology characteristics where applicable. On the other hand, the polymerase chain reaction (PCR) performed further identification. Extraction of DNA was performed by a commercial kit (Isolate II Fecal DNA Kit, Bioline) and kept in the freezer at –20 °C until the analysis. The actual amplification targets 267bp of *Taenia* genus *rrnS* gene by a previously published procedure (Trachsel *et al*., 2007) with the following primers CEST 3 (5'-YGA YTC TTT TTA GGG GAA GGT GTG-3') and CEST5 (5'- GCG GTG TGT ACM TGA GCT AAAC-3'). The amplification program was carried out with the My Cycler thermocycler (BioRad) at 95 °C 2 min, followed by 40 cycles at 95 °C 30s, 58 °C 90 s, 72 10 s °C and a final step at 72 °C for 10 min. Amplicon analysis and control were performed by horizontal electrophoresis on a 1.5 % agarose gel electrophoresis submersion system, with the addition of the *Midori Green* fluorescent dye (Nippon Genetics Europe). PCR products were sequenced and compared for species confirmation with those available in the GenBank database using BLAST alignment. With doubtful and low discriminatory results, the mitochondrial 12S rDNA 314bp partial gene sequence was targeted (von Nickisch-Rosenegk *et al*., 1999; Padgett *et al*., 2005). In brief: for reactions, primer pair 60 forward (5'-TTA AGA TAT ATG TGG TAC AGG ATT AGA TAC CC-3') and 375 reverse. (5'-AAC CGA GGG TGA CGG GCG GTG TGT ACC-3'), were used with master mix (MyTaq Mix, Bioline). Amplification program was carried out with the MyCycler thermocycler (BioRad), temperature conditions: 95 °C, 2 min, then 35 cycles of 95 °C, 30 s; 55 °C, 30 s; 72 °C, 30s, followed by a final step at 72 °C for 10 min.

Results

With coprological examination, we found oocysts of *Cystoisospora* sp. in 39.16 % of fecal samples, tapeworm eggs were found in 59.16 %, *Toxocara cati* eggs in 30.83 %, eggs of *Ancylostoma spp*. in 36.66 % and eggs of *Capillaria spp*. in 14.16 % samples.

During the post-mortem examination, we found *Cystoisospora rivolta* in all examined animals*, Mesocestoides litteratus* and *Taenia taeniaeformis* in 6*, Ancylostomae tubaeforme* in 5*, Toxocara cati* in 4, and *Capillaria aerophila* in one wild cat. The most diverse infection included four different species of parasites. It was found in one animal, with three in 2, with two in 3 and mono infections only with *Cystoisospora rivolta* in one wild cat.

A total of 6 samples of tapeworms were found and put through PCR analysis. Consequent sequencing revealed samples 1,2 and 6 were identical with the sequence *Taenia taeniaeformis* (GenBank nr. JQ663994.1 and EU219554.1), one (sample 3) with 12S rRNA gene sequence resembled *Mesocestoides* sp. (GenBank nr. LT635737.1), and two (samples 4 and 5) in mitochondrial partial genome, *Mesocestoides litteratus* (GenBank nr. JF268581.1).

Discussion

The results show that wild cats from the Banat area (Timis) were infected with a larger number of zoonotic parasites, potentially dangerous to human health. These parasites are also found in domestic cats, especially non-owner cats, which move freely in this area. Likely, the infections with the same endoparasite species do not occur directly from wild to domestic cats or vice versa but because of intermediate hosts; so, there could be an indirect exchange of parasites between wild and non-owner domestic cats. The role of these parasitic elements in the environment and the potential risk of human contamination is unanimously accepted eggs. *Toxocara cati*, *Ancylostoma spp*. and etc. (Pavlović *et al*., 2007). Parasitic eggs can be found in both soil and fecal samples, which may imply significant epidemiological importance (Szwabe & Blaszkowska, 2017). Leple (2001), in his Ph.D., where he did a comparative analysis of parasites of wild and domesticated cats, confirmed their similarity. These zoonotic species were established in wild cats during numerous research in Europe and also in other parts of the world (Patton & Rabinowitz, 1994; Scholz *et al*., 2003). The prevalence of 59.15 % endoparasitic infections observed in our study was similar to levels of infections found in other parts of Europe, like in Hungary (Takács & Takács, 2002), Slovakia (Mituch, 1972), some parts of Germany Germany (Schuster *et al*., 1993; Barutzki & Schaper, 2003; Krone *et al*., 2007) and in Scotland (Burt *et al*., 1980). At the same time, it is smaller than the findings in the Mediterranean basin (Greece, Spain and Italy), where the prevalence was up to 100 % (Papadopoulos *et al*., 1997; Gaglio *et al*., 2010). A high prevalence of infection greater than 90 % was also found in the Balkan region in Bulgaria (Kirkova *et al*., 2011), Croatia (Martinković *et al*., 2017) and Slovenia (Brglez & Železnik, 1976) (Brglez & Železnik, 1976). The higher parasite burden and diversity in wild cats can be explained by the broader spectrum of prey species as potential intermediate hosts in the wild diet than in domestic cats.

In addition to morphological identification, the presence of *Taenia taeniaeformis* and *Mesocestoides litteratus* was confirmed by molecular PCR testing. Our study was supported by 3 molecular confirmations of *Taenia taeniaeformis* (similar to GenBank nr. JQ663994.1 și EU219554.1), while 3 other molecular findings out to be *Mesocestoides* sp. (similar to GenBank LT635737.1 and JF268581.1) Molecular identification of the *Taenia taeniaeformis* isolated from the intestine of the examined cats is found, similarly, in molecular biology studies conducted in Italy on groups of wild cats, domestic cats, and hybrid populations. These studies

have highlighted *Taenia taeniaeformis* as a representative species of the population parasites from the digestive tract of the animals introduced into the study (Galimberti *et al*., 2012). The first molecular biology study on domestic and wild carnivores in the Mediterranean area was reported in 2018 (Varcasia *et al*., 2018). This study was conducted on adult and larval forms of *Mesocestoides* spp., collected from domestic and wild hosts in Italy and Tunisia. The genes of subunit 1 (cox1) and the subunit of NADH dehydrogenase (nad1) of the mitochondrial genome were used as molecular markers. According to this study, three distinct species were registered: *Mesocestoides litteratus*, *M. corti* and *M. lineatus*. High levels of genetic variation were identified and no evidence of geographical structuring between clusters (Padgett *et al*., 2005).
Molecular studies performed by Literák *et al*. (2006) proved the existence of three distinct species of the genus *Mesocestoides*, and *M. litteratus* was accepted as the main species. *Mesocestoides litteratus*, a species identified molecularly in wild cats examined, is also reported in other wild carnivores. Some epidemiological aspects of identified polyparasitism were pointed out in a study on endoparasitism in Spanish wolves (Segovia *et al*., 2001), foxes and jackals in Serbia (Pavlović *et al*., 2008; Cirovic *et al*., 2015). Comparative studies of foxes in Belarus, Serbia, and Montenegro alongside the risk to human health (Shimalov *et al*., 2005) were performed. According to this and other studies, there is a possible relationship between the prevalence of fox infections infection with zoonotic parasites species and the risk of contamination of the human population in urban environmental conditions (Pavlović *et al*., 1997; Reperant *et al*., 2007). Those potential zoonoses findings urge for the educative control programs for hunters and nature hiking groups as Turkey Echinococcus targeted one (Altintas *et al*., 2021).

Conflict of Interest

The authors have no potential conflict of interest in this submission to Helminthologia.

Conclusions

The present research is the first study in the western part of Romania (Banat region). It brings information about the parasitic fauna of wild cats and highlights the importance of the human contamination risk.
Finally, this investigation reinforces that management and surveillance of zoonosis in remote areas require a one-health approach incorporating the public and veterinary services at the local level.

References

Altintas, N.A., Altintas, N.U., Sarica Yilmaz, O., Akil, M., Akdur Ozturk, E., Unver, A. (2021): Educational intervention for the awareness improvement and control programme design on Echinococcosis in Izmir, Turkey. *Helminthologia*, 58(2), 152 – 161. DOI: 10.2478/helm-2021-0013

Barutzki, D.,Schaper, R. (2003): Endoparasites in dogs and cats in Germany 1999 – 2002. *Parasitol Res*, 90 Suppl 3, S148 – 150. DOI: 10.1007/s00436-003-0922-6

Brglez, J.,Železnik, Z. (1976): Ein Übersicht über die Parasiten der Wildkatze (*Felis silvestris* Schreber) in Slowenien [An overview of the parasites of the wild cat (*Felis silvestris* Schreber) in Slovenia]. *Z Jagdwiss*, 22, 109 – 112 (In Germany)

Burt, M.D., Pike, A.W., Corbett, L.K. (1980): Helminth parasites of wild cats in north-east Scotland. *J Helminthol*, 54(4), 303 – 308. DOI: 10.1017/s0022149x00006817

Castelló, J.R. (2020): *Felids and hyenas of the world*. Princeton and Oxford, Princeton University Press.

Cirovic, D., Pavlovic, I., Penezic, A., Kulisic, Z., Selakovic, S. (2015): Levels of infection of intestinal helminth species in the golden jackal *Canis aureus* from Serbia. *J Helminthol*, 89(1), 28 – 33. DOI: 10.1017/S0022149X13000552

Euzéby, J. (1981): *Experimental diagnosis of animal helminthoses: practical work in veterinary helminthology*. Paris, Informations techniques des services vétérinaires français (In French)

Gaglio, G., Brianti, E., Brucato, G., Risitano, A.L., Otranto, D., Lia, R.P., Mallia, E., Anile, S.,Giannetto, S. (2010): Helminths of wildcat (*Felis silvestris silvestris*) in Southern Italy. *Parassitologia* 52(1-2), 274 – 275

Galimberti, A., Romano, D.F., Genchi, M., Paoloni, D., Vercillo, F., Bizzarri, L., Sassera, D., Bandi, C., Genchi, C., Ragni, B., *et al*. (2012): Integrative taxonomy at work: DNA barcoding of taeniids harbored by wild and domestic cats. *Mol Ecol Resour*, 12(3), 403 – 413. DOI: 10.1111/j.1755-0998.2011.03110.x

Hunter, L., Barrett, P. (2015): *Wild Cats of the World*. London, Bloomsbury Natural History

Kirkova, Z., Raychev, E., Georgieva, D. (2011): Studies on feeding habits and parasitological status of red fox, golden jackal, wild cat and stone marten in Sredna Gora, Bulgaria. *J. Life Sci*, 5, 264 – 270

Krone, O., Guminsky, O., Meinig, H., Herrmann, M., Trinzen, M., Wibbelt, G. (2007): Endoparasite spectrum of wild cats (*Felis silvestris* Schreber, 1777) and domestic cats (*Felis catus* L.) from the Eifel, Pfalz region and Saarland, Germany. *Eur J Wildlife Res*, 54(1), 95 – 100. DOI: 10.1007/s10344-007-0116-0

Leple, D. (2001): *Parasitologie compareé du Chat forestier (Felis silvestris silvestris, Schreber 1777) et du Chat domestique (Feliscatus, Linne 1758)* [*Comparative parasitology of the forest cat (Felis silvestris silvestris, Schreber 1777) and the domestic cat (Feliscatus, Linne 1758)*]. Ecole Nat. These de Doctorat Vét, Lyon (In French)

Literák, I., Tenora, F., Letková, V., Goldová, M., Torres, J., Olson, P. (2006): *Mesocestoides litteratus* (Batsch, 1786) (Cestoda: Cyclophyllidea: Mesocestoididae) from the red fox: Morphological and 18S rDNA characterization of European isolates. *Helminthologia*, 43(4), 191 – 195. DOI: 10.2478/s11687-006-0036-7

Martinković, F., Sindičić, M., Lučinger, S., Štimac, I., Bujanić, M., Živičnjak, T., Stojčević Jan, D., Šprem, N., Popović, R.,Konjević, D. (2017): Endoparasites of wildcats in Croatia. *Vet Arh*, 87(6), 713 – 729

Mituch, J. (1972): Die Helminthenfauna der Fleischfresser in der Slowakei. *Folia Venatoria II*, 161 – 172

Moskvina, T.V., Izrailskaia, A.V., Tsybulsky, A.V. (2018): Parasites of stray and client-owned domestic cats in urban areas in Russia during 2000 – 2015 years. *Trop Biomed*, (35), 267 – 279

Padgett, K.A., Nadler, S.A., Munson, L., Sacks, B., Boyce, W.M. (2005): Systematics of mesocestoides (Cestoda: Mesocestoididae): evaluation of molecular and morphological variation among isolates. *J Parasitol*, 91(6), 1435 – 1443

Papadopoulos, H., Himonas, C., Papazahariadou, M., Antoniadou-Sotiriadou, K. (1997): Helminths of foxes and other wild carnivores from rural areas in Greece. *J Helminthol*, 71(3), 227 – 231. DOI: 10.1017/s0022149x00015960

Patton, S., Rabinowitz, A.R. (1994): Parasites of wild felidae in Thailand: a coprological survey. *J Wildl Dis*, 30(3), 472 – 475. DOI: 10.7589/0090-3558-30.3.472

Pavlović, I., Kulišić, Z., Milutinović, M. (1997): The role of foxes (*Vulpes vulpes* L.) in the epizootiology and epidemiology of nematoda parasitic zoonoses. *Acta Vet (Beogr)*, 47(2-3), 177 – 182

Pavlović, I., Kulišić, Z., Momčilović, J., Mišić, Z., Krstić, D. (2007): *Basic measure to control and sanition of parasitic contamination of green areas in urban environmental condition.* pp. 78 – 79, Beograd.

Pavlović, I., Tambur, Z., Doder, R., Kulišić, Z., Jakić-Dimić, D. (2008): The role of red foxes (*Vulpes vulpes* L.) in the epidemiology of zoonotic tapeworms of the genus *Mesocestoides* (Cestoda: Cyclophilidae). *Veterinaria* 57(1-2), 109 – 117

Reperant, L.A., Hegglin, D., Fischer, C., Kohler, L., Weber, J.M., Deplazes, P. (2007): Influence of urbanization on the epidemiology of intestinal helminths of the red fox (*Vulpes vulpes*) in Geneva, Switzerland. *Parasitol Res*, 101(3), 605 – 611. DOI: 10.1007/s00436-007-0520-0

Republic of Romania (2014): Lege nr. 205 Privind protecţia animalelor [Law on animal protection no. 205]. *Official J.*: 320 (In Romanian)

Scholz, T., Uhlirova, M., Ditrich, O. (2003): Helminth parasites of cats from the Vientiane province, Laos, as indicators of the occurrence of causative agents of human parasitoses. *Parasite*, 10(4), 343 – 350. DOI: 10.1051/parasite/2003104343

Schuster, R., Heidecke, D.,Schierhorn, K. (1993): Beiträge zur Parasitenfauna autochthoner Wirte. Mitteilung: Zur Endoparasiten fauna von *Felis silvestris* [Contributions to the parasitic fauna of autochthonous hosts. Communication: On the endoparasite fauna of *Felis silvestris*]. *Appl Parasitol*, 34, 113 – 112

Segovia, J.M., Torres, J., Miquel, J., Llaneza, L., Feliu, C. (2001): Helminths in the wolf, *Canis lupus*, from north-western Spain. *J Helminthol*, 75(2), 183 – 192

Shimalov, V.V., Pavlović, I.,Kulišić, Z. (2005): Uchastie lisits (*Vulpes vulpes* L.) v epidemiologii cestod roda Mesocestoides (Cestoda:Cyclophillidea) v Belarusi, Serbii i Chernogorii [Participation of foxes (*Vulpes vulpes* L.) in the epidemiology of cestodes of the genus Mesocestoides (Cestode: Cyclofellidea) in Belarus, Serbia and Montenegro]. *Vestnik Brestskogo Universiteta*, 2(23), 84 – 87 (In Russian)

Szwabe, K., Blaszkowska, J. (2017): Stray dogs and cats as potential sources of soil contamination with zoonotic parasites. *Ann Agric Environ Med*, 24(1), 39 – 43. 10.5604/12321966.1234003

Takács, A., Takács, P. (2002): Data on worm infestation of domestic cats (*Felis catus*) in Hungarian hunting areas. *Magy Allatorvosok*, 124(1), 26 – 30

Trachsel, D., Deplazes, P., Mathis, A. (2007): Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. *Parasitology*, 134(Pt 6), 911 – 920. 10.1017/S0031182007002235

Varcasia, A., Sanna, D., Casu, M., Lahmar, S., Dessi, G., Pipia, A.P., Tamponi, C., Gaglio, G., Hrckova, G., Otranto, D., *et al*. (2018): Species delimitation based on mtDNA genes suggests the occurrence of new species of Mesocestoides in the Mediterranean region. *Parasit Vectors*, 11(1), 619. DOI: 10.1186/s13071-018-3185-x

von Nickisch-Rosenegk, M., Silva-Gonzalez, R., Lucius, R. (1999): Modification of universal 12S rDNA primers for specific amplification of contaminated *Taenia* spp. (Cestoda) gDNA enabling phylogenetic studies. *Parasitol Res*, 85(10), 819 – 825. DOI: 10.1007/s004360050638

Supplementary Materials are available online at https://doi.org/10.2478/helm-2023-0015.