Parasitology Research
https://doi.org/10.1007/s00436-021-07141-8

HELMINTHOLOGY - SHORT COMMUNICATION

First description of peritoneal and pleural metacestodosis caused by *Mesocestoides vogae* in a European wild cat (*Felis silvestris silvestris*)

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Received: 13 October 2020 / Accepted: 24 March 2021

Abstract

Tapeworms of the genus *Mesocestoides* (Cestoda: Cyclophyllidea: Mesocestoididae) are still enigmatic to scientists, due to their high morphological variability, low host specificity, and unknown details of their life cycle. They are found worldwide, with carnivorous mammals as the main definitive hosts, and the disease is potentially zoonotic. After ingestion by a definitive host, the tetrathyridium can occasionally migrate through the intestinal wall and reach the peritoneal cavity or abdominal organs causing peritoneal metacestodosis. Here, we report on a case of metacestodosis of a European wild cat (*Felis silvestris silvestris*) found dead in Croatia. At necropsy, a large number of white, rice-like structures were found free in the abdominal and thoracic cavities, as well as along the serous surfaces and in the lungs. DNA isolated from the nodules was genotyped and based on a 320-base pair long 12S fragment classified as *Mesocestoides vogae*. Although post-mortem changes were advanced, severe emaciation due to the severe parasitic infection and gastrointestinal bleeding was diagnosed as the likely cause of death. Intestinal cestodosis was previously reported in wild cats, but according to our knowledge, this is the first description of peritoneal and pleural metacestodosis caused by *M. vogae* tetrathyridia (metacestodes) in any wild carnivore species.

Keywords *Mesocestoides* · European wild cat · Necropsy · 12S rDNA gene

Section Editor: David Bruce Conn

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Introduction

Tapeworms of the genus *Mesocestoides* (Cestoda: Cyclophyllidea: Mesocestoididae) are still enigmatic to scientists, due to their high morphological variability, low host specificity, and unknown details of their life cycle (Skirnisson et al. 2016). It is assumed by some authors that the life cycle of *Mesocestoides* requires three hosts, but recently, McAllister et al. (2018) suggested that a simple 2-host cycle might be possible. Ants (Padgett and Boyce 2005) and coprophagous arthropods (Foronda et al. 2007) were hypothesized as the possible first intermediate hosts; however, these findings have been refuted as infection in these hosts has never been demonstrated, and transmission was never proved (Loos-Frank 1991; McAllister et al. 2018). The presumed second intermediate hosts are insectivorous vertebrates (mammals, birds, reptiles, and amphibians) that will develop metacestodes (tetrathyridium) in their serous cavities, various internal organs, and subcutaneous tissues including the mammary glands upon ingestion of either an infected first

Published online: 31 March 2021

intermediate host or infective oncospheres (Conn and Etges 1983). Detailed descriptions of post-larval pre-tetrathyridia and tetrathydrium metacestodes stage were provided by McAllister et al. (2018) and Conn et al. (2010, 2011).

The adult tapeworms are found worldwide, with the exception of Australia and Antarctica, in intestines of placental mammals and rarely birds (Padgett et al. 2013). Main definitive hosts are carnivorous mammals (canids, felids, and mustelids). As potentially zoonotic, human mesocestoidosis can occur following the consumption of raw or undercooked viscera of an intermediate host (Padgett and Boyce 2004; Padgett et al. 2005).

After ingestion by a definitive host, the tetrathyridium can occasionally migrate through the intestinal wall and reach the peritoneal cavity, other coelomic cavities, or abdominal organs (Crosbie et al. 2000). Proliferative *Mesocestoides* tetrathyridia were found in the peritoneal cavity of dogs (*Canis familiaris*), domestic cats (*Felis catus*), and primates (reviewed by Padgett et al. 2013) causing peritoneal metacestodosis. Both intestinal infection and peritoneal metacestodosis are usually asymptomatic, so detection of tetrathyridia in the peritoneum is usually incidental during the treatment for unrelated issues or at necropsies (Papini et al. 2010; Boyce et al. 2011). However, in dogs, tetrathyridia can occasionally cause peritonitis with chronic ascites and even fatal outcome (Wirtherle et al. 2007; Papini et al. 2010).

Identification of *Mesocestoides* species based on the morphology alone can be difficult. Most significant characteristics of adult tapeworms are a morphology of median ventral position of the genital atrium, a bipartite vitelline gland, and a parauterine organ (Georgiev and Kornyushin 1994; Hrckova et al. 2011). Morphological identification of the tetrathyridia to the species level is impossible (Zaleśny and Hildebrand 2012). Molecular tools have significantly helped in species identification, and so far, only several representatives of the genus can be reliably identified using both morphological and molecular characteristics—*Mesocestoides litteratus* (Batsch 1786), *M. lineatus* (Goeze 1782), *M. vogae* (Etges 1991), and *M. canislagopodis* (Rudolphi 1810) (Nickisch-Rosenegk et al. 1999; Padgett and Boyce 2005; Literák et al. 2006; Hrckova et al. 2011; Skirnisson et al. 2016)—but recently, indications of possible new species were published (Montalbano Di Filippo et al. 2018; Varcasia et al. 2018; McAllister et al. 2018).

Here, we report on a case of metacestodosis of a European wild cat affecting the thoracic and abdominal cavities and viscera. Genetic analysis revealed *M. vogae* as causative agent.

Materials and methods

In the year 2018, an adult female European wild cat (*Felis silvestris silvestris*) was found dead in Gračac, Lika region, Croatia, and brought to the Faculty of Veterinary Medicine University of Zagreb for post-mortem examination, as part of a wildlife health monitoring program.

During necropsy, stomach and intestines were opened and flushed with water, gastrointestinal mucosa was scraped, and content was checked for parasites under the stereo-microscope and compound light microscope. Feces were analyzed using flotation with a saturated $ZnSO_4$ solution (S.G.=1.3). Collected parasites were examined morphologically under the stereo-microscope and using molecular methods. DNA was extracted using a Wizard Genomic DNA Purification Kit (Promega, USA), following the manufacturer's protocol. The mitochondrial 12S rDNA gene was amplified using primers 60fov (5′ TTAAGATATATGTGGTACAG GATTAGATACCC 3′) and 375rev (5′ AACCGAGG GTGACGGGCGGTGTGTACC 3′) (Nickisch-Rosenegk et al. 1999). PCR was carried out using a total volume of 20 μl Platinum® PCR SuperMix (Invitrogen) (consisting of *Taq* DNA polymerase with Platinum® *Taq* antibody, 22 mM Tris-HCL (pH 8.4), 55 mM KCl, 1.65 mM $MgCl_2$, 220 μM dNTP mix), 5 μl of extracted parasite DNA, and 0.2 mM of each primer. PCR conditions were 2 min at 95 °C, 40 cycles at 94 °C for 30 s, 30 s at 45 °C, 72 °C for 30 s, and a final elongation stage at 72 °C for 7 min. The amplicons were sequenced by the commercial company Macrogen Europe B.V. Sequence alignment was performed using the BioEdit software (Hall 1999). Alignments were manually proofed and compared with sequences available in GenBank using the BLAST program (https://blast.ncbi.nlm.nih.gov).

Samples of lung tissue collected during necropsy were fixed in 10% neutral buffered formalin and processed routinely for histopathological examination. Tissue samples were cut into 4-μm thick sections and stained with hematoxylin and eosin (H&E).

The mitochondrial 12S rDNA gene sequence obtained in this research was deposited in the GenBank under the accession number MT974335, while specimens of the Mesocestoides were archived in Collection of Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Zagreb under catalog number 2802.

Results and discussion

Gross examination revealed advanced post-mortem changes, severe emaciation, dehydration, stomach erosions and melena. A large amount of white, rice-like structures were found free in the abdominal and thoracic cavity, as well as along the serous surfaces and in the lungs. No other significant macroscopic lesions were observed. Histologically, few cross sections of adult parasites measured 200–300 μm were found in lung parenchyma, mostly close to the surface (Fig. 1a) and compressing surrounding lung parenchyma. They were characterized by thick outer tegument surrounding a loose parenchymatous matrix and numerous calcareous corpuscles (Fig. 1b). Morphological identification of the parasites found in the lungs was not possible based on these histopathological findings. Although post-mortem changes were advanced, severe emaciation, possibly due to the severe parasitic infection and gastrointestinal bleeding, was diagnosed as the likely cause of death.

Taenia taeniaeformis was found in the intestine, while eggs of *Capillaria* sp. were identified in the feces. Stereomicroscopic examination of the white rice-like structures revealed juvenile stages (tetrathyridia) of different sizes, compatible with those of the *Mesocestoides* genus, as previously described by Eleni et al. (2007).

Sequences obtained from the nodules were all identical and confirmed that the parasite belongs to the *Mesocestoides* genus (deposited in the GenBank under the accession number MT974335). A 320-base pair long 12S fragment showed 98.8% similarity with sequences from Turkish dogs deposited

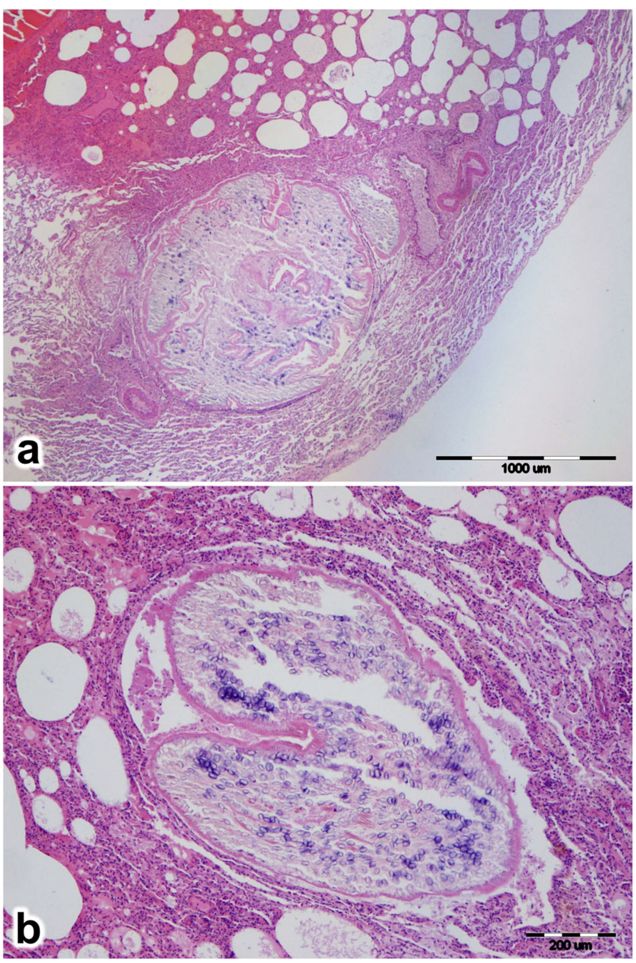

Fig. 1 **a** Lung, histological sections of the adult parasite in the lung parenchyma close to the surface with compressed surrounding parenchyma (H&E ×4). **b** Cross-section of parasite composed of outer tegument surrounding a loose parenchymatous matrix and numerous calcareous corpuscles. (H&E ×10)

in the GenBank under the accession numbers JN572111 (Aypak et al. 2012) and HM011122 (Yildiz and Tong 2011). Yildiz and Tong (2011) identify their specimen as *Mesocestoides vogae*, while Aypak et al. (2012) erroneously equals *M. vogae* and *M. corti* (Etges 1991). Hence, the parasite observed in the present case was classified as *M. vogae*.

Until now, *M. lineatus* was found in wild cats as definitive hosts in Slovenia (Brglez and Železnik 1976), Germany (Schuster et al. 1993; Krone et al. 2008), Hungary (Takács et al. 2011), and Croatia (Martinković et al. 2017). *M. litteratus* was confirmed in Italy (Brianti et al. 2012) while *Mesocestoides* sp. was identified in wild cats in Bulgaria (Kirkova et al. 2011). However, studies on parasites of European wild cats are scarce and usually based on a limited sample size (Martinković et al. 2017). In addition, the use of coproscopy and parasite identification solely based on morphology challenges the scope of our knowledge about this parasite in wild cats. Széll et al. (2015) and Karamon et al. (2018) emphasized low sensitivity of flotation compared to the sedimentation and counting technique (SCT), where the pre-mortem/post-mortem examination of the feces and amount of examined feces/intestinal content differ significantly. It is important to note that flotation can be done in living and dead animals while SCT only in dead animals. Also, the amount of feces and intestinal content largely differs (couple of grams vs. whole intestinal content). Although there is possibility to find some segments during the coprological examination, utmost important fact is that eggs when leaving the host's intestine are still within the paruterine organ. Usually, those segments stay whole after the process of coprological examination preparation, and this is also one of the reasons why the coprological examination stays egg-negative, even if the tapeworm segments were present in the fecal sample. Therefore, it is possible that the prevalence of *Mesocestoides* spp. in wild cats is underdiagnosed when using scat samples vs whole animal gastrointestinal system (Napoli et al. 2016; Martinković et al. 2017). The conflicts among key morphological characters and a high degree of host-induced non-specific morphological variations result in taxonomical confusion (Padgett et al. 2005; Skirnisson et al. 2016). Thus, the necessity for using both morphological characteristics and molecular analysis is further emphasized by recent reports of possible new *Mesocestoides* species (Eleni et al. 2007; Montalbano Di Filippo et al. 2018; Varcasia et al. 2018; McAllister et al. 2018).

Both intestinal cestodosis and peritoneal metacestodosis were previously reported in domestic cats but are rarely diagnosed in live animals due to the lack of clinical signs. According to our knowledge, this is the first description of peritoneal and pleural metacestodosis caused by *M. vogae* metacestode in any wild carnivore species. Having in mind that wild cats prey on birds, reptiles, and amphibians (Lozano et al. 2006), which were reported as hosts of tetrathyridia (Padgett and Boyce 2004; McAllister et al. 2014), the source of infection is more accessible to wild than domestic cats. Considering the observed necropsy findings, we can speculate that the investigated wild cat must have developed certain clinical signs of the disease. Clinical metacestodosis is usually linked with immunosuppressive disorders, including neoplastic and hormonal diseases, and viral infections such as feline leukemia virus and feline immunodeficiency virus infection. In this case, potential underlying mechanisms of the observed condition were not further investigated due to advanced post-mortem changes of the carcass and therefore remain unknown. Nevertheless, presented data contribute to better understanding of epidemiology of the *Mesocestoides* genus, but also expand our knowledge on the parasite diversity of European wild cats.

Author contribution MS carried out parasite PCR diagnostics and drafted the manuscript; AGK and DK performed the necropsy; FM carried out parasite morphological diagnostics; MB, ML, AR, HBH, SRRP, and SG assisted during necropsy; all authors contributed to the final version of manuscript.

Declarations

Ethics approval Committee of Faculty of Veterinary Medicine University of Zagreb approved the research.

Conflict of interest The authors declare no competing interest.

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