

PERITONEAL LARVAL CESTODIASIS FROM *MESOCESTOIDES* SP. IN A RED FOX (*VULPES VULPES*) IN GRAN SASSO MONTI DELLA LAGA NATIONAL PARK (ITALY)

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Introduction

Tapeworms of the genus *Mesocestoides* (Cestoda, Cyclophyllidea, Mesocestoididae) are unique in several aspects of their biology. The complete life cycle of *Mesocestoides* spp. has not yet been conclusively demonstrated. Rausch (1) suggests it requires at least 3 hosts. The supposed role of coprophagic arthropods as first intermediate hosts has been presumed by some authors, however there is no convincing evidence for this part of the cycle (2). Recently it has been suggested that *Mesocestoides* spp. might develop through a simple two-host life cycle rather instead of an obligate three-host cycle, only using vertebrates as intermediate hosts (3).

The second stage larvae, called tetrathyridia, are hypothesized to have little host specificity and have been reported from the peritoneal cavity and organs of a large diversity of mammals, birds and reptiles, causing a potentially life-threatening peritonitis. (1; 4; 5). Adult worms live in the small intestines of carnivorous mammals, i.e., the dog, cat, fox, wolf, coyote, jackal, raccoon, badger, lynx and some species of wild felines and rarely birds and humans (6; 7).

Foxes, dogs and cats are known to act as both definitive and intermediate hosts. The tetrathyridial metacestodes will multiply asexually by longitudinal scission of the scolex, penetrate the intestinal wall and invade the peritoneal cavity of the hosts; massive infections in snakes, lizards and other animals have been recorded (8). This is the only genus of tapeworms presenting this phenomenon.

Several studies document the presence of *Mesocestoides* spp. in the intestines of foxes in Europe, with a variable prevalence: 41.9% in Slovak Republic (9), 75.87% in the Iberian Peninsula (10), 45.8% in Hungary (11), 78.2% in Poland (12) and 17.5% in Emilia-Romagna, Italy (13). Rare are reports of infection by the larval forms in this host species.

To our knowledge, tetrathyridia have only been found in the thoracic and abdominal cavities in three adult foxes (10) in Spain, in the peritoneal cavity in one fox in Spain (14) and in one fox in Turkey (15) and encysted in the hepatic parenchyma in one fox in Italy (13).

The present work describes a case of peritoneal cestodiasis caused by larvae of *Mesocestoides* sp. in an adult female fox (*Vulpes vulpes*) found agonizing with uncontrolled limb movements near Gran Sasso Monti della Laga National Park (L'Aquila, Italy), caused by tetrathyridia of *Mesocestoides* sp. This is the first report of proliferative peritoneal larval cestodiasis by *Mesocestoides* sp. in a fox in Italy.

Materials and methods

In December 2021, the carcass of a female fox from the municipality of L'Aquila, Gran Sasso Monti della Laga National Park, was subjected to necropsy.

Abdominal cavity was invaded by hundreds of white bodies similar to grains of rice, with a soft consistence, adhering to the omentum, and serosa of the intestine, liver and kidney.

These formations, suspected larval forms of parasites, were removed and washed in 0.85% saline, fixed in ethyl alcohol 70% and 95% and subsequently cleared with glycerin for microscopy studies; some of them were sent to the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana for molecular identification.

Total DNA was extracted with DNeasy Blood & Tissue Kit after a lysis step with protein-ase K. After PCR amplification (ITS-D1 / D2 regions) and sanger sequencing, obtained sequences were analysed using BLAST (nucleotide collection nr/nt).

Lung, brain, liver, small intestine, spleen and kidneys were also collected for virological investigations; all tissues were fixed in 10% neutral buffered formalin, embedded in paraffin and routinely processed for histology (Hematoxylin and Eosin stain, HE).

Results

Parasites were 0.3-1 cm long, flattened and white (Figures 1 and 2), with an enlarged cephalic region characterized by a deep invagination. They showed many transverse superficial folds along the whole body and had two types of shapes, one oval and globular and the other one piriform with the elongated caudal end (Figure 3); they contained large homogeneous white corpuscles in the matrix. In accordance with these characteristics, the larval forms found at necropsy were identified as acephalic metacestodes belonging to the order Cyclophyllidea.

Figure 1. Abdominal cavity of the fox was invaded by hundreds of white bodies.



Figure 2. Detail of the omentum of the fox.



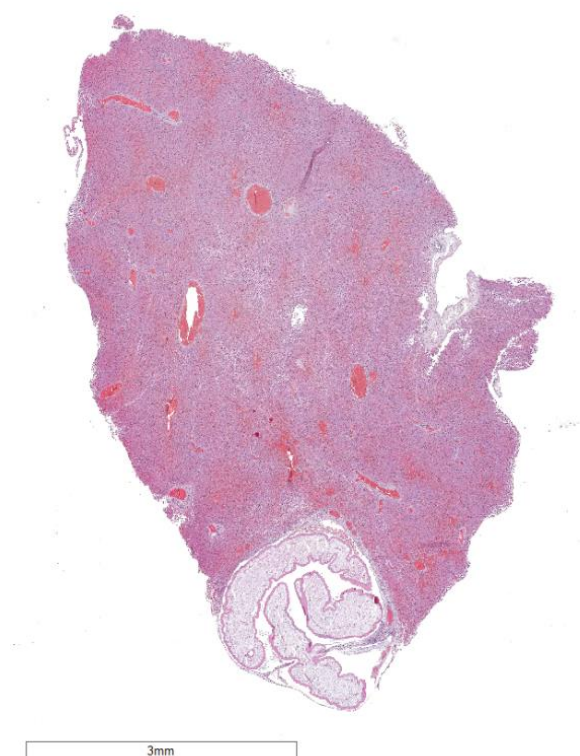
Figure 3. Tetrathyridia of *Mesocestoides* sp. collected from the omentum with two types of shapes, one oval and globular and the other one piriform with the elongated caudal end.



The recovered immature worms were identified as tetrathyridia of *Mesocestoides* sp. (Vailant, 1863) (Cestoda, Cyclophyllidea, Mesocestoididae) by molecular analyses. Total DNA was extracted with DNeasy Blood & Tissue Kit after a lysis step with protein-ase K. After PCR amplification (ITS-D1 / D2 regions) and sanger sequencing, obtained sequences were analysed using BLAST (nucleotide collection nr/nt).

At necropsy the fox presented tracheitis, large areas of pneumonia, catarrhal gastritis and enteritis. No adult intestinal parasites, including adult tapeworms, were found. Virological investigations on lung and brain revealed viral molecular signals of Canine Distemper Virus (CDV). Histopathological examination of liver showed a parasitic cystic formation containing single or multiple larvae, surrounded by a thin fibrous capsule (Figure 4). The capsule was infiltrated by inflammatory cells mainly composed of lymphocytes. The entire liver parenchyma was strongly hyperemic.

Figure 4. Red foxes, liver. Parasitic cyst.



Conclusions

Although foxes can serve both as the second intermediate and the definitive hosts for *Mesocestoides* spp., only larval stages were observed. This is the first report of proliferative peritoneal larval cestodiasis by *Mesocestoides* sp. in a fox in Italy. Further investigations are necessary for the identification of the specie and for the genomic and phylogenetic definition of the specimens under study. Varcasia et al. (16) investigated the taxonomy and the molecular characterization of *Mesocestoides* in the Mediterranean region and results showed three well-supported clusters of *Mesocestoides* spp. in southern Italy and Tunisia, which were strongly divergent from *Mesocestoides litteratus*, *M. corti* and *M. lineatus*, and therefore the existence of new species of the genus *Mesocestoides* in the Mediterranean area is assumed.

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