doi: 10.17221/6/2017-VETMED

Mesocestoidosis and multivisceral tetrathyridiosis in a European cat

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ABSTRACT: This report describes the clinical, parasitological and pathological findings in a 6-year-old intact female European cat with thoracic and peritoneal tetrathyridiosis, characterized by genital involvement. Physical examination and X-ray evaluation revealed laboured breathing and several pulmonary nodules suggestive of cancer. However, necropsy demonstrated a parasitic aetiology of the disease. Histologically, multifocal granulomas were detected in the lungs, uterus and ovary. Parasitological examination permitted identification of the intestinal parasites as *Mesocestoides lineatus*, which was later confirmed by molecular examination. The larval forms in the peritoneal and chest cavity were identified as the second stage of the *Mesocestoides* sp. cestode named *Tetrathyridia* spp. The chronic injuries observed and the rapid course of the disease from the onset of the symptoms until death suggested a long period without clinical signs and indicate that overt disease can potentially be triggered by a failure of the immune system. The observed oophoritis and metritis identify tetrathyridiosis as a possible differential diagnosis in genital dysfunction.

Keywords: feline; tetrathyridiosis; necropsy; histopathology; PCR

Parasitic diseases are a serious problem for public health and represent a significant financial burden for health care systems (WHO Expert Committee 1987). Humans can contract parasitic infections from several sources, such as water, food and arthropod bites; moreover, small domestic animals, e.g., cats that become infected with parasites, can constitute a zoonosis risk in urban environments (Manfredi and Felicita 1993; Fuentes et al. 2003). Intestinal parasitic infections caused by nematodes, particularly by roundworms such as *Toxocara cati*, are frequently recorded in cats (Beugnet et al. 2014). In contrast, the tapeworm species *Taenia taeniaeformis*, *Diphyllobotrium latum* as well as *Mesocestoides* spp. are reported with lower frequency in domestic cats in Italy (Bourdeau and Beugnet 1993; Chauve 1993; Riggio et al. 2013; Spada et al. 2013); however, control of such infections remains particularly important as it ensures low levels of infestation in humans.

Mesocestoides is an enigmatic genus of the Cestoda class widespread worldwide, whose development from the larval to the adult form incorporates features of both pseudophyllidean and cyclophyllidean orders (Markowski 1934; Freeman 1957; Voge 1967). Although its life cycle and the first intermediate host are not yet fully understood, a model of transmission involving two intermediate hosts and one definitive host is widely accepted (Padgett and Boyce 2004). The first intermediate host is an arthropod, probably an oribatid mite, in which the oncosphere reaches the hemocoel stage and develops further to the cysticercoid stage. A wide range of species may act as the second inter-

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mediate host, including small mammals such as rodents or birds and reptiles that are not infected directly by eggs. They must ingest the first stage of the metacestode in the first intermediate host; the cysticercoid larval stage then develops into a tetrathyridium in the second intermediate host. Finally, dogs, cats, wild carnivores and occasionally humans, act as definitive hosts in which the larvae become adult tapeworms. After ingestion of the infected second intermediate host by a cat or dog, the tetrathyridium can occasionally migrate through the intestinal wall, reach the peritoneal cavity and abdominal organs and may be able to engage in asexual and/or sexual reproduction (Eckert 1969; Speckmann and Webster 1975; Barsanti et al. 1979; Quintavalla et al. 1996; Crosbie et al. 2000).

Although this condition is uncommon, it is likely to be underdiagnosed. While feline infection is usually asymptomatic (Quintavalla et al. 1996), in dogs the presence of tetrathyridia can cause serious peritonitis with parasitic chronic ascites and several non-specific clinical signs (Barsanti et al. 1979; Bonfanti et al. 2004; Wirtherle et al. 2007; Papini et al. 2010). Therefore, diagnosis of this infection is often an incidental finding during necropsy or laparotomy (i.e., ovariohysterectomy). However, some symptomatic clinical cases have recently been reported (Haziroglu et al. 2005; Venco et al. 2005; Eleni et al. 2007; Dahlem et al. 2015). In the literature, most reports in dogs and cats concern thoracic and/or peritoneal tetrathyridiosis with liver, spleen, abdominal wall, kidney and scrotal involvement. To the best of our knowledge, this paper is the first to describe oophoritis and metritis caused by *Tetrathyridium* spp. in a cat.

Case description

In February 2015, a 6-year-old skinny (1.7 kg in weight) intact female European cat was brought by its owner to an animal ambulance in the district of Agrigento (Sicily, Italy) for physical examination. The anamnesis revealed anorexia, moderate abdominal swelling, cough and dyspnoea. The owner also stated that the patient lived outdoors in the garden and had not been vaccinated or treated against parasites or other infectious agents. X-ray evaluation was performed for diagnosis using a Multimage Univet 120LX 1° with code: RXU.AP.467 radiograph. Clinicians attempted to treat the animal with drip, butorphanol and wide-spectrum antibiotics, but ten days later, due to a worsening of the symptoms and upon the request of the owner, the cat was euthanised.

Necropsy was performed on the same day at the Department of Veterinary Sciences of the University of Messina, Italy.

Macroscopic and physical examinations. Physical examination of the cat also revealed abdominal pain, whereas blood tests demonstrated non-regenerative anaemia and eosinophilia.

X-ray examination carried out in right lateral view showed a pulmonary mixed pattern, characterized by spread nodular pattern (0.7–1 cm) and interstitial pattern, the latter being more evident in the caudal lobes. Abdominal radiographic details were not clear because of a diffuse radiopacity with a low radiographic contrast hampering the evaluation, probably due to the presence of a moderate abdominal effusion, so that the bowel loops appeared enlarged with the presence of gas and faeces.

The mammary gland was healthy. After opening the abdominal cavity, examination of the viscera revealed a small amount of colourless fluid among meteoric bowel loops and many motile and viable worms on the surface of the latter. These worms were solid, globoid, with enlarged anterior extremities, flat, whitish and approximately 0.5–4 cm long. Free tapeworms were also spread out and adherent to the omentum and serosal surface of the abdominal wall and viscera, which was diffusely reddened due to hyperaemia resulting in peritoneal phlogosis. Numerous flat parasites were found in the lumen of the small intestine. These parasites were 5 to 40 cm in length, segmented, milky-white in colour and were recognised as belonging to the class Cestoda. This part of the intestinal tract appeared inflamed with abundant yellowish material revealing catarrhal enteritis.

A cyst-like 0.5-cm-sized, whitish, translucent nodular bulge, with smooth surface and which was elastic on palpation, was located under the spleen serosa. The cystic nature of the lesion was confirmed on section; in fact, it was characterized by a likely fibrous wall, and was replete with colourless fluid. Whitish, solid, globoid tapeworms, approximately 2 cm in length, with enlarged anterior extremities ploughed by a median groove were extracted from the cavity of the nodule. No lesions were observed in the liver. Several nodular bulges with smooth surfaces, ranging in size from 0.5 to 2 cm, were

doi: 10.17221/6/2017-VETMED

adherent to ovaries, to the uterine horns and to the uterus body below the perimetrium, which appeared to be reactive and was glowing red. The nodules were of the same structure as the cyst-like lesions from the spleen; they crossed the uterus wall and some of them jutted into the lumen of the organ. Moreover, petechial and ecchymotic haemorrhages were widely scattered over the uterus mucous membrane. No further lesions were found in the remaining abdominal organs, including the kidneys.

The lungs did not collapse upon opening of the thoracic cavity. These organs were moderately increased in volume with rounded edges; further, they quivered at the slightest air movement and the pleura was taut and shiny. The surface of lungs was variegated in colour with the presence of dark red, bright red, whitish-grey areas and vesicular-like bulges. The latter were sub-pleural, ranging in size from 0.5 to 1.5 cm, translucent and with smooth surfaces; they were localised in each lobe (apical, middle and caudal lobe). Lungs were pasty on palpation except in the portions where they increased in volume and were grey or white-grey in colour and crackling and cottony on palpation. The parenchyma appeared humid on sections and showed abundant white-pink foam upon pressure. Vesicular changes were also found inside the parenchyma, showing pyriform shape and cystic structure; moreover, inside each nodule a tapeworm similar to the other larvae observed in the uterus, spleen and peritoneal cavity, was present. Therefore, macroscopically, the respiratory system showed features of pulmonary oedema, granulomatous inflammation and emphysema that were responsible for the death of the cat.

Histological examinations. Tissue samples collected from the lungs, uterus, ovary and spleen during necropsy were fixed in 10% neutral buffered formalin solution, dehydrated by gradual transition in an increasing series of ethyl alcohol, embedded in paraffin and sectioned on a rotary microtome. Sections of 5 µm in thickness were placed on previously cleaned and degreased slides. Tissue sections were stained with haematoxylin and eosin (HE), dehydrated in 95% and 100% ethyl alcohols (two changes of alcohol at 100%), cleared, released into xylene for four minutes and mounted for routine pathological examination. The slides were observed under a Zeiss Axiophot optical microscope.

Histologically, multifocal granulomas were detected in the lungs, uterus and ovary. Granulomas were characterized by cystic formations surrounded by a fibrous capsule infiltrated by lymphocytes, plasma cells, macrophages and on rare occasions giant cells (Figure 1). Multifocal calcifications were also seen in the granulomas of the ovary. These cysts, corresponding to metacestodes, showed irregular borders representing the tegument of the parasite and a loose mesenchymal stroma with different organs.

In the lungs, the granulomas were surrounded by severe hyperaemia and accompanied by multifocal moderate alveolar oedema and emphysema. Severe multifocal hypertrophy/hyperplasia of the tunica media of the small arteries and moderate multifocal hypertrophy of the smooth interalveolar muscle septa were also observed.

A severe and multifocal depletion of the white pulp was also detected in the spleen.

Morphological and molecular study of parasites. A number of adult tapeworms and larvae were stored in 70% alcohol, then placed in Petri dishes and observed with a stereomicroscope (Zeiss Discovery V12). Larval stages of flatworms, meanwhile, were dehydrated in alcohol solutions of increasing concentration, subsequently dried according to the critical point method and metallised with a palladium-gold layer (20 nm ± 5%) for observation with a scanning electron microscope (Cambridge Stereoscan 240 SEM).

Adult (n = 1) and larval (n = 1) tapeworms stored in 90% ethanol were sent to the Department of Veterinary Sciences at the University of Turin, Italy,

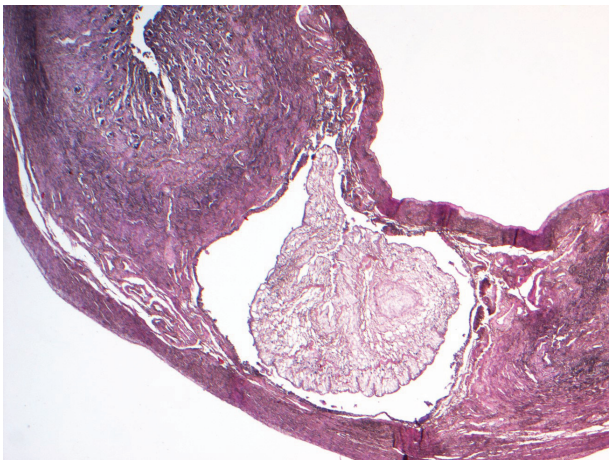

Figure 1. Histological section of the cat uterus with a parasitic granuloma containing a single large larva surrounded by a fibrous capsule and mononuclear inflammatory infiltrate. (HE, × 2.5)

doi: 10.17221/6/2017-VETMED

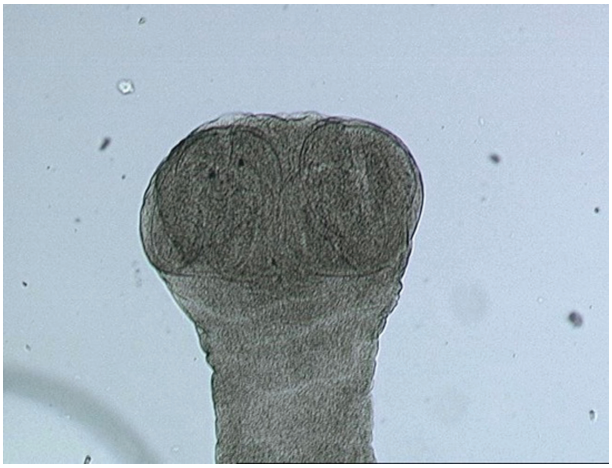

Figure 2. *Mesocestoides* spp. unarmed scolex

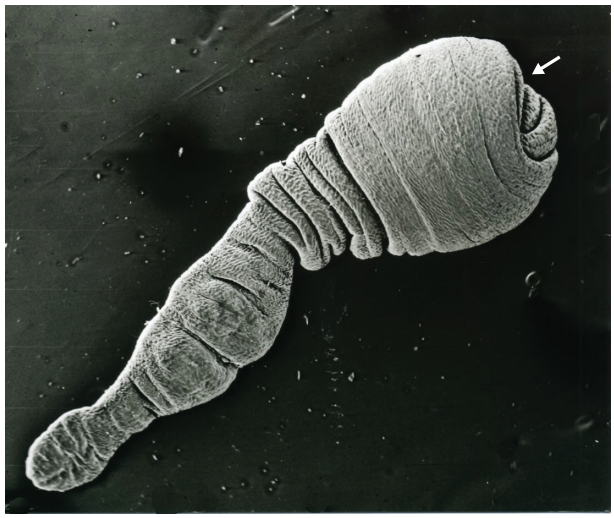

Figure 3. Scanning electron micrograph showing a *Tetrathyridium* spp. larva collected from the abdominal cavity with an enlarged anterior extremity crossed by a deep apical invagination (arrow). (× 20)

to confirm morphological identification by means of PCR amplification and sequencing. Total Genomic DNA was extracted using the GenElute Mammalian Genomic Miniprep Kit (Sigma-Aldrich, Milan, Italy) following the manufacturer's instruction, with the exception that 30 mg of parasitic tissues were pre-homogenised with a manual pestle and digested overnight in 10 mg/ml proteinase K. A fragment of approximately 400 bp of the cytochrome c oxidase subunit 1 (*COX-1*) gene was amplified using CycloCox1 FA and Cyclo16SRc primers (Littlewood et al. 2008; Hrckova et al. 2011). PCR amplification was carried out in a final volume of 25 µl containing approximately 100 ng of DNA template, 2.5 µl 10X PCR buffer, 5 µl of Q Buffer, 2.5 IU of HotStarTaq DNA Polymerase (Qiagen, Milan, Italy), 0.5 µl of dNTP mix (10 mM of each dNTP, Sigma-Aldrich, St Louis, USA), and 12.5 pmol of each primer. An initial denaturation step of 15 min at 95 °C was followed by 40 cycles of 1 min at 94 °C, 1 min at 52 °C, and 1 min at 72 °C and a final elongation step of 10 min at 72 °C. PCR amplicons were visualised on a 2% agarose gel with a UV transilluminator (GelDoc 72 1000, Bio-Rad, Hercules, USA). PCR-positive amplicons were purified (Nucleospin Extract II kit, Macherey-Nagel, Duren, Germany) and sequenced (Macrogen Europe, The Netherlands). All standard precautions were taken to minimise the risk of cross-contamination. The resulting sequences were aligned using MUSCLE and compared to those available in BLAST (http://blast.ncbi.nlm.nih.gov). Homologous closely-related sequences were used to construct a maximum-likelihood tree using the Hasegawa-Kishino-Yano nucleotide substitution model with 2000 Bootstrap replications.

The parasites found in the intestinal lumen were comprised of ten adult cestodes ranging in size from 5 to 40 cm, and presenting with large, unarmed scoleces with four oval suckers (Figure 2). Microscopic examination of the mature proglottids showed that their length was higher than their width and that the central genital pore opening was on the ventral surface. According to these features, the parasites were identified as *Mesocestoides lineatus*. The 52 larval forms found in the peritoneal and chest cavity during necropsy ranged from 0.5 to 4 cm in size and were morphologically identified as the second stage of the cestode belonging to the *Mesocestoides* species, named *Tetrathyridium* spp. These parasites exhibited an enlarged cephalic region characterized by a deep invagination and showed many transverse superficial folds, mainly close to the anterior extremity. The caudal region was tapered and pointed at the tip (Figure 3).

The sequences obtained from the two parasites were identical with no polymorphisms detected between the larval and adult forms. The resulting sequence was deposited in GenBank under accession No. KU821650. The most similar sequences present in BLAST are from *Mesocestoides* spp. (JQ740884; maximum identity 95%) and *Mesocestoides lineatus* (JF268500 and JF268501, maximum identity 93%). The phylogenetic analysis using maximum-likelihood (Figure 4) confirmed that the sequence reported in the present study can, with the strong-

doi: 10.17221/6/2017-VETMED

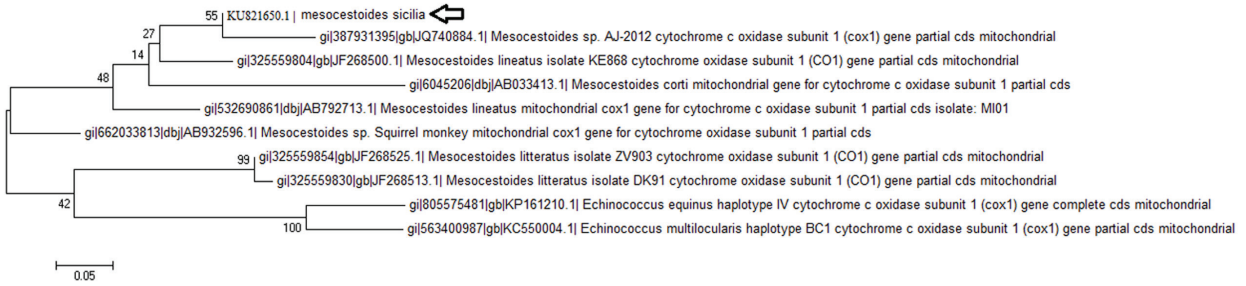

Figure 4. Maximum likelihood phylogenetic analysis was conducted using the Hasegawa-Kishino-Yano nucleotide substitution model. Numbers above branches are percent bootstrap values based on 2000 bootstrap replicates. The sequences obtained from the larval and adult forms were identical with no polymorphisms detected. The resulting sequence was deposited in GenBank under accession No. KU821650 (arrow)

est statistical support, be grouped with that of a *Mesocestoides* sp. (GenBank accession No. JQ740884) isolated from an infected cat originating from the same geographical region as the cat in the present study (Sicily, Southern Italy), and with a sequence of *Mesocestoides lineatus* (GenBank accession No. JF268500), which was isolated from a red fox in Slovakia (Hrckova et al. 2011).

DISCUSSION AND CONCLUSIONS

Peritoneal tetrathyridiosis is a disease that is rarely diagnosed in cats due to the lack of specific and non-specific symptoms. Although both larval forms and adults of *Mesocestoides* spp. can infest cats, the latter are found more frequently. The disease is characterized by a long asymptomatic period that might suggest an incompatibility between the host and the parasite and also that the cat is an unusual host of these cyclophyllidean tapeworms (Henry 1927; Schwartz 1927; Witenberg 1934; Carta 1939; Voge and Specht 1965). However, the different intestinal architecture of the cat that is characterized by a thick muscle layer and low, small and numerous villi (James 1968), together with the likely distinctive immune reaction in response to antigenic stimulation by the parasite, could explain the long prepatent period and the fact that *Mesocestoides* tapeworms are only occasionally detected in cats compared to dogs.

In the literature, the prepatent period can be seen to vary widely: from two or three weeks (Loos-Frank 1987), to 109 days (Schwartz 1927; Joyeux and Baer 1932; Carta 1939). There is only a small number of reports on peritoneal or pleural tetrathyridiosis in cats (Berg and Andersen 1982; Quintavalla et al. 1996; Haziroglu et al. 2005), even though cats have been reported to harbour both the adult tapeworms and the intermediate stage at the same time (Guralp 1974). From this reason, it is important to emphasise that this case is one of the few reports of clinically overt tetrathyridiosis in a cat (Quintavalla et al. 1996), and is, to the best of our knowledge, the only report of parasitic peritonitis associated with meteoric parasitic oophoritis and metritis due to *Mesocestoides* sp. metacestodes.

The clinical, anatomical and histopathological findings allow epidemiological and etiopathogenetic observations that could be useful for clinicians, since early diagnosis and appropriate therapeutic treatment determine the patient's prognosis (Boyce et al. 2011). It is known that this disease occurs in stray animals as a result of their eating habits (Srivastava 1939; Wirtherle et al. 2007). Also in this case, although the patient had an owner, it lived outdoors in the garden, and was not vaccinated or treated against infectious agents and parasites. These data suggest that, in addition to eating habits and environmental factors, immune and physiological status (Novak 1974; Novak 1975) could also play an important role in the onset of disease, severity of clinical signs and anatomical and histopathological findings caused by the migration of larval forms (Hermanek 1991).

Immunosuppressive disorders with iatrogenic, hormonal, neoplastic or infectious causes, such as viral infections by retroviruses like FELV or FIV in cats, which occur more frequently in stray animals, may play an important role. In any case, given the small number of cases reported in the literature and the lack of serological results that confirm FELV and/or FIV infection, we can only speculate regarding the role of the functional integrity of the immune system in the pathogenesis of the disease. The pathogenic mechanisms underlying the clini-

doi: 10.17221/6/2017-VETMED

cal, anatomical and histopathological findings that characterize and differentiate tetrathyridiosis in the cat are not yet clearly understood.

The features of oophoritis and metritis due to parasitic infestation with tetrathyridia, could be interesting for clinicians, since despite the lack of reproductive history, on the basis of the observed lesions, it is possible to imagine reproductive function disorders such as oestrus disorders or persistent anoestrus, infertility, early embryonic mortality or primary uterine inertia due to injuries to the ovaries and uterine wall. Moreover, haemorrhages detected in the uterine mucous membrane, in accordance with what was suggested by Quintavalla (1996), demonstrated that the anaemia observed during peritoneal larval cestodiasis is due to bleeding micro-ulcers resulting from the migration of tetrathyridia.

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doi: 10.17221/6/2017-VETMED

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Received: January 10, 2017
Accepted after corrections: May 8, 2017