Clinical, Cytological and Molecular Evidence of *Mesocestoides* sp. Infection in a Dog from Italy

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Summary

A 12-year-old, 13 kg, mixed-breed male dog was referred for anorexia and depression. The dog showed discomfort on abdominal palpation. Abdominal ultrasound examination revealed multiple, small, round anechoic cystic structures. Cystic fluid obtained with fine needle aspiration contained several 2–4 mm white motile flecks. Microscopic examination of the fluid revealed numerous irregularly shaped organisms measuring several hundred microns to 3 mm, the morphology of which was suggestive of intact and fragmented acphalic metacestodes of the genus *Mesocestoides* sp. Molecular analysis confirmed that the peritoneal infection was caused by *Mesocestoides* sp.

Introduction

Peritoneal cestodiasis represents an uncommon, likely under-diagnosed condition caused by larval forms of tapeworms from the genus *Mesocestoides* (phylum Platyhelminthes, class Cestoda, family Mesocestoididae) (Locatelli et al., 1984). It has been shown that *Mesocestoides* spp. can adapt to low-oxygen environments (Conn, 1990; Caruso et al., 2003). The life cycle of this tapeworm is indirect. It is generally thought that two intermediate hosts and one definitive host are involved in the cycle, although the complete life cycle has never been worked out and some authors have questioned the presence of a first intermediate host (von Nickisch-Rosenegk et al., 1999). Peritoneal infection can occur when the ingested third larval stage (tetrathyridium) penetrates the intestinal wall and invades the peritoneal cavity (Specht and Voge, 1965; Eckert et al., 1969; Speckmann and Webster, 1975). These larvae can cause ascites, abdominal distension, peritonitis and, occasionally, death in dogs and other definitive hosts (Voge and Berntzen, 1963; Barsanti et al., 1979; Williams et al., 1985; Crosbie et al., 1998). Cytological diagnosis of larval *Mesocestoides* infection is made by demonstration of either acphalic metacestodes or tetrathyridia in abdominal effusion (Crosbie et al., 1998; Raskin and Meyer, 2001; Caruso et al., 2003). Molecular analysis by means of polymerase chain reaction (PCR) and subsequent sequencing represent sensitive and accurate methods to confirm peritoneal cestodiasis caused by *Mesocestoides* sp. (Crosbie et al., 1998, 2000).

Case History

A 12-year-old, 13 kg, mixed-breed male dog was presented to the Clinica Veterinaria S. Angelo with anorexia, depression, lethargy and a stiff gait. The dog was routinely used for hunting and frequently travelled to mid- and southern Italy, in particular, Tuscany and Calabria. The dog was routinely vaccinated and received regular heartworm prevention drugs. One year earlier, a haemangiopericytoma was removed from the left thigh. On clinical examination, the dog was hyperthermic (40.0°C) and showed discomfort on abdominal palpation. An abdominal lateral radiograph was not clear because of a diffuse increase in radiopacity with a ground-glass appearance (Fig. 1). A complete blood count showed mild increase in band neutrophils (750 cells/μl), lymphopenia (300 cells/μl) and mild monocytosis (1800 cells/μl). Serum biochemistry revealed a high alkaline phosphatase concentration (367 U/l). The electrophoretic pattern of serum proteins showed hypoalbuminaemia (2.47 g/dl), high alpha 2 and beta fractions and low gamma globulin fraction, suggesting an acute inflammatory process. Salicylic acid (Ascriptin®; Aventis Pharma SA, Alfortville, France) and amoxicillin-clavulanate (Synulox®; Pfizer, Inc., New York, NY, USA) were given at standard dosages and the dog was scheduled for abdominal ultrasonography the following day. Multiple small (0.5–2.0 cm), round anechoic cystic structures were observed in the peritoneal cavity on abdominal ultrasonography. One of these cysts was aspirated using a 21-gauge 1.5-in. needle connected with a 10-ml syringe. Five millilitres of turbid milkish fluid containing several 2–4 mm motile white flecks were obtained (Fig. 2). The fluid was prepared using standard procedures for cytological examination and stained with a Romanowsky-type stain. A moderate number of non-degenerated neutrophils was present, mixed with numerous irregularly shaped organisms measuring several hundred microns to 3 mm. Some appeared intact, whereas others were fragmented. Closer examination showed that they were composed of an amorphous basophilic substance admixed with numerous, refractile calcareous corpuscles showing a concentric layering (Figs 3 and 4). Based on clinical findings, diagnostic imaging and cytological examination, a diagnosis of peritoneal cestodiasis was made. Some millilitres of the cystic fluid were filtrated using a 100-micron pore filter and flushed with 0.9% saline solution. The flecks were then transferred to 95% ethanol alcohol for molecular
The dog was treated with fenbendazole (Panacur®; Laboratories Hoechst SA, L’Aigle, France) at a dose of 50 mg/kg/p.o. with food, q 24 h for 10 days and with a single dose of praziquantel (Droncit®; Bayer AG, Leverkusen, Germany) at 5 mg/kg. Initially, the dog improved. However, 6 days after the end of fenbendazole treatment, the dog developed abdominal distension and fever. Abdominal ultrasonography showed peritoneal effusion. However, no cysts were evident this time. A sample of the peritoneal fluid was obtained by paracentesis and submitted for cytopathology and bacterial culture. Cytological examination revealed a severe neutrophilic inflammation associated with the presence of many intracellular and extracellular rods. Bacterial culture yielded *Escherichia coli* growth. Only a few calcareous corpuscles were present. Based on antibiotic susceptible test, enrofloxacin (Baytril®; Bayer AG) and amoxicillin-clavulanate (Synulox®) were administered at the standard dosage. Laparotomy and peritoneal lavage were performed several days later. Exploratory surgery revealed peritoneal effusion and fibrinous adhesions on visceral and parietal peritoneum compatible with chronic peritonitis. Molecular analysis of the flecks previously transferred to 95% ethanol alcohol was performed by means of PCR on a portion of 18S rRNA gene according to Crosbie et al. (2000). The amplifications obtained, of the expected size (about 1050 bp), were gel-purified (using the QIAquick® PCR Purification Kit; Qiagen Gmbh, Hilden, Germany) and directly sequenced using ABI technology (Applied Biosystem, Foster City, CA, USA). The sequence obtained was deposited in the EMBL Data Library (accession: AJ781094) and was compared with the available *Mesocestoides* spp. sequences available in the databases. Sequence AJ781094 was aligned to the available sequences of *Mesocestoides* spp. and other representatives of the class Cestoda. Our sequence showed 100% identity with the four sequences available in the databases for *Mesocestoides corti*. In addition, there were nine further 18S rRNA gene sequences in the databases that showed 100% identity with the sequence generated in the present study; these sequences were indicated as derived from *Mesocestoides* sp. Finally, there were two additional 18S rRNA sequences in the databases derived from *Mesocestoides* sp., showing 0.3% nucleotide substitutions compared with the sequence reported here.

Blood tests carried out 4 months after the initial presentation revealed non-regenerative anaemia (4 660 000 erythrocytes/μl), slight eosinophilia (1600 cells/μl), hypoalbuminaemia (2.04 g/dl) and moderate hyperglobulinaemia (5.35 g/dl). During the following 8 months, the dog experienced several episodes of recurrent peritoneal cestodiasis and bacterial peritonitis that were treated with fenbendazole at the increased dosage of 100 mg/kg q 12 h continuously (Panacur®) and enrofloxacin at the dosage of 5 mg/kg q 24 h (Baytril®) respectively. The dog was alive after 1 year of presentation and was on continuous daily fenbendazole administration to control the disease.
Discussion

Tapeworms of the genus *Mesocestoides* are characterized by the possibility of invasion of the peritoneal cavity, where they may undergo asexual reproduction and cause peritonitis in intermediate and definitive hosts (Hart, 1968; Barsanti et al., 1979; Crosbie et al., 1998; Caruso et al., 2003). A case of scrotal cestodiasis has also been reported in the literature (Zeman et al., 1988).

The entire life cycle of *Mesocestoides* is still not clear. Some authors hypothesize an indirect cycle with two intermediate hosts and one definitive host. According to this hypothesis, the first intermediate host is a coprophagous arthropod, which is ingested by the second intermediate host, usually a small rodent, a snake or a frog, where there is the development of the ingested by the second intermediate host, usually a small definitive host. According to this hypothesis, the first intermediate host is a coprophagous arthropod, which is ingested by the second intermediate host, usually a small rodent, a snake or a frog, where there is the development of the ingested by the second intermediate host, usually a small definitive host. According to this hypothesis, the first intermediate host is a coprophagous arthropod, which is ingested by the second intermediate host, usually a small rodent, a snake or a frog, where there is the development of the third larval stage (tetrathyridium). The adult form of *Mesocestoides* develops in the intestine of the definitive host. Peritoneal infection occurs when ingested tetrathyridia penetrate the intestinal wall (Specht and Voge, 1965; Williams et al., 1985; Caruso et al., 2003; Padgett and Boyce, 2004). Definitive hosts include several wild and domestic carnivores, for e.g., skunks, foxes, coyotes, dogs and cats (Specht and Voge, 1965; Eckert et al., 1969; Quintavalla et al., 1996; Crosbie et al., 2000; Padgett and Boyce, 2004). In this case report, the dog presented aspecific symptoms such as anorexia and lethargy similar to those reported by other authors (Crosbie et al., 1998; Caruso et al., 2003). Fever was likely because of related concurrent neutrophilic peritonitis, a complication that can be found during peritoneal cestodiasis (Caruso et al., 2003). Laboratory results showed progressive non-regenerative anemia, moderate eosinophilia, hypoalbuminaemia and hyper-globulinaemia. These abnormalities have also been reported by other authors and were attributed to chronic peritoneal inflammation, parasitic infection (anemia, eosinophilia, hyperglobulinaemia) and protein exudation into the peritoneal cavity secondary to inflammation (hypoalbuminaemia) (Barsanti et al., 1979; Williams et al., 1985; Caruso et al., 2003). Ultrasound examination of the abdomen performed at the initial presentation, revealed many small round anechoic cystic structures in the peritoneal cavity. These cysts represent acephalic cestode larvae attached to or within abdominal organs (Williams et al., 1985; Crosbie et al., 1998; Caruso et al., 2003). The fluid obtained from the cyst at initial presentation, and the peritoneal effusion aspirated subsequently, were grossly similar and agree with that described by others (Barsanti et al., 1979; Caruso et al., 2003). The severe inflammation observed in the following months may have been the result of an alteration in the immune system, because of chronic parasitic infection. Cytological examination of the fluid revealed larvae of *Mesocestoides* sp. of different dimensions, at various stages of reproduction. The organisms detected in the dog were acephalic metacestodes (larvae without suckers), which differ from the typical tetrathyridium, which feature a scolex with four well-developed suckers. The acephalic metacestode is the larval stage most frequently found in canine peritoneal cestodiasis caused by *Mesocestoides* spp., and has not been reported in other host species (Crosbie et al., 1998; Caruso et al., 2003). Moreover, tetrathyridia have been found in peritoneal infections in dogs (Padgett and Boyce, 2004). Another feature of peritoneal cestodiasis that was also present in our dog were calcareous corpuscles (Caruso et al., 2003). Even if they are the only form observed during cytological examination, as they represent a characteristic remnant of cestode tissue, their presence allows specific diagnosis even in the absence of intact larvae (Caruso et al., 2003).

Although the adult form of *Mesocestoides* spp. is susceptible to many taenicides, only fenbendazole appears to be effective against metacestodes and is the therapy of choice for peritoneal cestodiasis, even if precise dosages and duration of treatment are not reported (Crosbie et al., 1998; Caruso et al., 2003). Moreover, although fenbendazole may bring the resolution of clinical signs and a marked reduction in the number of parasites, it is not always effective in eliminating the parasite and preventing recurrence definitively (Barsanti et al., 1979; Barsanti, 1999; Crosbie et al., 1998; Caruso et al., 2003).

The identification of *Mesocestoides* spp. is supported only by molecular analysis, as also described by von Nickisch-Rosenegk et al. (1999) and by Crosbie et al. (2000). As sequence AJ781094 showed 100% identity with the sequences previously obtained from *M. corti* and *Mesocestoides* sp., we assumed that the isolate here reported could be identified as belonging to the genus *Mesocestoides*.

To our knowledge, this report represents the first case of peritoneal cestodiasis cytologically diagnosed and confirmed by molecular analysis in Europe.

References


