Canine peritoneal larval cestodosis in a dog
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Introduction

Mesocestoides spp. are parasites in intestines of birds and mammals, including humans (7, 9). Taxonomic classification of the cestodes belonging to the Mesocestoididae family is unclear according to recent morphological and genetical studies (12, 13, 17). This family may not take be part of the order Cyclophyllidea. In respect to biological (life cycle with three hosts), morpho-anatomical (the median location of the genital pore and vitellarium consisting of two compact masses) and sperm characters (rotation of flagellum, the number of axonemes and twisting of cortical microtubules), Mesocestoididae spp. differ from the other members of Cyclophyllidea (9, 13). Also, molecular analysis reveals that Mesocestoididae spp. are basal to the Tetrabothriidea (12).

The three species of Mesocestoides taken into consideration are as follows: Mesocestoides vogae (syn. M. corti), Mesocestoides lineatus and Mesocestoides leptoelyracus (syn. M. litteratus) (10). M. vogae is found in the intestine of dogs (8) and bobcats (18) and M. lineatus in the small intestine of wild carnivores, dogs and cats. Final hosts of M. leptoelyracus are foxes, cats and dogs (7).

The complete life cycle of Mesocestoides spp. is still obscure (7). The infective larval stage (tetrathyridium) of Mesocestoides spp. is found in the abdominal cavity and on lungs and livers of intermediate hosts such as amphibians, reptiles, birds and small mammals (7, 10). The life cycle is completed in definitive carnivorous hosts by eating intermediate hosts. Adult cestodes develop in the intestine of definitive hosts following a prepatent period of approximately 2–3 weeks (7, 10).

Infections with adult Mesocestoides spp. are reported with a quite different incidence in various carnivorous animals such as dogs (6, 19, 21), red foxes (6, 11), wolves (1) and jackals (6). Occasionally, synchronous presence of both the adult Mesocestoides spp. and its infective larval stages in the same definitive host occurs (19) and in these cases the more severe clinic and pathologic outcomes are reported (7, 19). Metacestode stages of Mesocestoides spp. in the abdominal cavity of dogs results in canine peritoneal cestodosis (20). Anorexia, peritonitis, ascites, leukocytosis, tissue damage, granuloma formation and encapsulation are observed in animals infected with metacestode stages (7).

The infection of the definitive host is simply detected by observation of proglottids with a typical paruterine organ excerted with the faeces (7, 10). In contrast, the detection of peritoneal larval cestodosis in dogs is more complex. The possible methods used for diagnosis of peritoneal larval cestodosis are necropsy (19), ultrasonography (20), histological and cytological examination (3). These methods together with molecular tools are subsequently used for identification of peritoneal larval cestodosis (2, 4, 22).

Data on Mesocestoides species responsible for peritoneal larval cestodosis in dogs are limited (2, 22). The aim of this study is to demonstrate a clinical case of peritoneal larval cestodosis in a dog combined with the determination of the parasitic larvae by molecular diagnosis.

Case report

Patient data

A 3-year-old female Doberman dog was presented to the veterinary clinic, with abdominal distension and inactivity after mating. The blood analysis (Diam’s 20-Vet Blood Analysis Equipment, Ph.diagnostics, Fransa) revealed leukocytosis (62.7 \times 10^3/\mu l) and an increased haemotocrit (56.7%, RR 37–55%). The number of red blood cells (7.13 \times 10^6/\mu l, RR 5.5–8.5 \times 10^6/\mu l), haemoglobin (16.3 g/dl, RR 12–18 g/dl) and platelets (4.1 \times 10^3/\mu l, RR 2–9 \times 10^3/\mu l) were within physiological limits.

According to the findings of the ultrasonographic examination pyometra was suspected in the dog and ovariohysterectomy was performed under xylazine/ketamine anaesthesia. During surgery, some parasitic results, creamy in colour and 2–3 cm in length, were seen on the omentum covering spleen, liver and small intestine of the dog (Fig. 1). When examined under light microscopy, all parasitic structures had a thickened head section and a tapered tail.

Parasitic DNA isolation

Parasitic DNA isolation was obtained from the alcohol-preserved parasite samples. Briefly, parasite samples were cut into small pieces and digested with 80 μg of proteinase K in 500 μl of 10 mM Tris-HCl (pH 7.5). A commercial tissue DNA isolation kit (Tissue DNA Extraction Kit-GF1, Vivantis Technologies, Malaysia) was used for DNA extraction following manufacturer-recommended protocols, with the exception of overnight proteinase K-incu-
Cestode specific primers were used to amplify a 314 bp fragment of mitochondrial 12S rDNA (15). The primer sequences used were 5' to 3' TTA AGA TAT ATG TGG TAC AGG ATT AGA TAC CC (60.for) and 3' to 5' AAC CGA GGG TGA CGG GCG GTG TGT TGT ACC (75.rev). PCR products were carried out in a final volume of 25 μL, containing 10× PCR buffer with KCl, 2.5 mM MgCl₂, 0.5 μl each of dNTP, 10 pmol of each primer, 300 ng of template DNA, and 0.2 μL of TaqDNA polymerase (MBI Fermentas). The DNA was amplified in an Amplitonx-6 Thermal Cycler (Nyx 12 Technik, California, USA) as follows: 10 min at 95 °C, followed by 30 cycles of 1 min at 93 °C, 1.5 min at 55 °C, 2 min at 73 °C and final extention of 10 min at 70 °C before storage at 4 °C. Ten microliter aliquots of the amplicons were separated by electrophoresis through 2% agarose gel containing ethidium bromide in 1× TBE buffer at 120 V for 30 min. The DNA fragments were visualized by UV transillumination. Subsequently, bands were cut from the gel and amplified DNA fragments were purified by Nucleospin Extract kit (Macharey-Nagel, Germany) and sequenced using a ABI 3130XL Genetic Analyzer (Applied Biosystem, USA). The expected fragment was found at about 314 bp and was sequenced. The resulting sequence was compared with GenBank. The highest sequence homology with 94% was found for M. vogae mitochondrial gene for 12S rRNA, partial sequence (Accession no. HM011122).

The evolutionary history was inferred using the Neighbor-Joining method. The percentages of replicated trees in which the associated taxa cluster together in the bootstrap test (1000 replicates) were shown next to the branches. The evolutionary distances were computed using the Kimura-2 parameter method and were in the units of the number of base substitutions per site. The analysis involved nine nucleotid sequences. All ambiguous positions were removed for each sequence pair. Evolutionary analyses were conducted in MEGA5 (Fig. 2).

Discussion

The infectious larva of Mesocestoides spp. is called tetrathyridium (7, 10). Tetrathyridia are found in cyst like structures in the abdominal cavity and on abdominal organs (7, 19). On the other hand, the acephalic metacestode stage is most frequently found in canine peritoneal larval cestodosis caused by Mesocestoides spp. (2–4, 22). Padget and Boyce (16) reported that the acephalic metacestode is an unusual larval form of Mesocestoides spp. and further development of these larvae is not seen. Acephalic metacestodes of Mesocestoides spp. are generally reported in multiple small, white cystic structures in the abdominal cavity of dogs in cases of peritoneal larval cestodosis (2, 3, 22). In our case, tetrathyridia were found free on the omental surfaces of a dog during surgery and determined by molecular analysis.

Peritoneal larval cestodosis can occur in definitive hosts when infective stages of larvae penetrate the intestinal wall and enter the peritoneal cavity (7, 10). Reports of the Mesocestoides species responsible

Fig. 1 Acephalic metacestodes (arrows) were seen on omentum the dog during surgery.

Fig. 2 Phylogenetic tree of Mesocestoides spp. samples based on 12S rDNA sequences of inferred by the Neighbor-Joining method with the bootstrap test (1000 replicates). Numbers at nodes represent the percentage of bootstrap replications.
for peritoneal larval cestodosis in the dog are limited. This infection has rarely been reported in dogs in some countries such as Italy (2), Germany (22), Turkey (19) and USA (3). It is well known that the morphological examination has not been solely helpful for a species differentiation of Mesocestoides spp. metacestodes. Molecular tools allow the identification of Mesocestoides species (5, 14, 15). Previous studies have shown that tetrapharyridia of *M. lineatus* and *M. vogae* are identified as by molecular analysis (2, 22).

The mitochondrial 12S rRNA gene fragment is a useful tool to differentiate between taxa of Cyclophyllidea by PCR methods (14). The sequence comparison of a reliable differentiation between most of species and families should be about 70 to 100% (14). In our case, the highest sequence homology of accephalic metacestodes obtained from a Doberman dog was observed for *M. vogae* (94%). Our Mesocestoides sample and *M. vogae* were branched in the same node, but *M. lineatus* and *M. leptothylacous* were branched in different nodes according to the phylogenetic tree (Fig. 2).

**Conclusion for practice**

Tetrapharyridia of *Mesocestoides vogae* can be found on the omental surfaces of dogs during surgery and should be determined by molecular diagnosis. Clinical signs are not significant for diagnosis of peritoneal larval cestodosis.

**Acknowledgement**

The authors would like to thank Bio. Tuba Uzunalioglu of Kirikkale University for contribution to the molecular analyses.

**Conflict of interest**

The authors confirm that they do not have any conflict of interest.

**References**