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CASE REPORT

A Case of Diffuse Peritoneal Larval *Mesocestoides corti* (syn. *M. vogae*) Cestodiasis in A Dog in Turkey [1]

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Summary

A laparotomy was performed in an eleven years-old female Doberman with loss of appetite, abdominal distension and signs of pain revealed multiple structures of a size reaching 1.5 cm, composed of small (6 mm or less), white, cystoid structures, with a total volume of approximately 2 liters. To follow the development of the parasitic organisms, under microscopic observation, their body fragments were also moving without changing place which came out of the abdominal cavity, these masses were transferred to tissue culture flasks containing RPMI containing 20% fetal calf serum and placed in a humidified incubator with 5% CO_2 at 37°C. The dog survived only one week after the surgery and the autopsy showed macroscopic parasitic material, either free-floating in the peritoneal cavity or adhering to the peritoneum and viscera. A perforation one cm in diameter was observed in pyloric area on the major curvature, covered by the omentum. No parasites were present in the intestines. Microscopically there were multiple pyrogranulomatous peritonitis, hepatitis and splenitis. The morphologic features of the parasitic material collected at laparotomy were compatible with that of *Mesocestoides* spp. larvæ. A PCR amplification was performed with mitochondrial 12S rDNA cestod-specific primers. The sequence of the isolates was 89-100% compatible with the reference *Mesocestoides corti* isolates published in the National Center for Biotechnology Information (NCBI) GenBank.

Keywords: *Mesocestoides corti, Tetrathyridiosis, Dog*

Türkiye'de Bir Köpekte *Mesocestoides corti*'ye (Syn. *M. vogae*) Bağlı Yaygın Peritoneal Larval Cestodiasis

Özet

İştahsızlık, abdominal gerginlik ve ağrı belirtileri bulunan 11 yaşında dişi bir dobermana uygulanan laporotomi sonucunda, köpeğin karın boşluğunda toplam hacmi 2 litreye yakın olan, beyaz, küçük (6 mm'ye kadar) kist benzeri oluşumları bir arada bulunduran, büyüklükleri 1.5 cm'yi bulabilen çok sayıda yapıya rastlanılmıştır. Karın boşluğundan çıkarılan ve mikroskop altında hareketlilikleri izlenen parazitik yapıların olası gelişmelerini takip etmek için söz konusu kitleler 37°C'de CO_2'li etüvde, %20'lik fötal buzağı serumlu RPMI içeren doku kültürü şişelerine aktarılmıştır. Operasyon sonrası ancak bir hafta yaşatılabilen hayvanın yapılan otopsisinde makroskobik olarak, serbest veya karın boşluğu organları ve peritona yapışık halde parazitik materyallere rastlanılmıştır. Midenin plorik bölümü kurvatura majorunda, 1 cm çapında, üzeri omentum tarafından örtülü bir perforasyon görülmüştür. Bağırsaklarda herhangi bir parazite rastlanmamıştır. Mikroskobik olarak multiple pyogranülomatoz peritonitis, hepatitis ve splenitis dikkati çekmiştir. Laporatomi sırasında toplanan parazitik materyalin mikroskobik morfolojisi *Mesocestoides* spp. larva dönemi morfolojisi ile uyumlu bulunmuştur. Mitokondrial 12S rDNA'nın cestod spesifik primerleri ile PCR yapılmıştır. Elde edilen PCR ürününün sekansı NCBI® GenBank'da mevcut *Mesocestoides corti* referans izolatları ile %89-100 oranında identik bulunmuştur.

Anahtar sözcükler: *Mesocestoides corti, Tetrathyridiosis, Köpek*

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INTRODUCTION

The adult forms of the genus *Mesocestoides* are found in the small intestine of dogs, cats, wild carnivores and sometimes in humans. Although their life cycle is not entirely known, they need two intermediate hosts. The first intermediate hosts are oribatid acarians, and the second, including the definitive hosts, are mammals, reptiles and amphibians [1,2]. Reports from Turkey to date include larvae that could be diagnosed as belonging to *Mesocestoides* sp. in their second intermediate host in dogs, cats, chickens, lizards and peacocks [3-7].

CASE HISTORY

An eleven years-old female dog of the Doberman breed was taken to the clinics of the Adnan Menderes University Faculty of Veterinary Medicine for loss of appetite, abdominal distension and signs of abdominal pain in March 2009. The laboratory tests showed that the serum biochemistry values were within the normal ranges (alanine aminotransferase, aspartate aminotransferase, urea, creatinine, total protein); RBC and hemoglobin were low (3.7 x 10^{12}/l and 7 g/dl, respectively), indicating a normocytic normochromic anemia, while total WBC (29.39 x 10^{9}/l) and neutrophil (23.24 x 10^{9}/l) counts were elevated, i.e. in neutrophilic leukocytosis. X-ray and ultrasound examination of the case showed diffuse ascites and diffusely scintillating abdominal hyperechoic foci. Laparotomy revealed severe peritonitis and multiple, white structures of a size reaching 1.5 cm, which were aggregates of white, cystoid structures of a diameter of less than 6 mm *(Fig 1-A, B)*. The structures taken out of the abdominal cavity were microscopically observed to be mobile. The cysts contained only a transparent liquid, without structures elements. To follow the development of the parasitic structures, the masses were transferred to tissue culture flasks containing RPMI-1640 containing 20% fetal calf serum and placed in a humidified incubator with 5% CO_2 at 37°C [8]. Due to intervening bacterial and fungal infection, they could only be kept alive for about two weeks. Although the dog had received post-operative treatment with oral and intraperitoneal fenbendazol and praziquantel as well as balanced electrolytic solutions, it died after one week from the operation.

An autopsy was performed on the dog. Samples from all viscera and parasites collected in the abdominal cavity were fixed in 10% neutral formaldehyde. All paraffin sections were stained with hematoxylin-eosin (HE), those suspect for mycosis additionally with the periodic acid Schiff (PAS) method and studied in light microscopy. The autopsy findings included fibroproliferative peritonitis, chronic gastritis with stomach ulcer and perforation, mycotic pyogranulomatous inflammation of spleen, proximal tubular epithelial degeneration and interstitial plasma cell infiltration of the kidneys and passive hyperemia and fibrosis of the liver; no parasites were present in the intestines. Cytologic examination of the mobile masses were obtained during laparotomy established that these structures consisted of larvae of different sizes, at different stages of asexual reproduction, of the *Mesocestoides* species. The parasitic material sections did not show the scolex and sucker structures expected in tetrathyridia, corresponding to the definition of acephalic metacestodes found in the literature *(Fig 2-A, B)* [9-11]. The larvae were covered by a thick eosinophilic integument. Numerous calcareous corpuscles were present

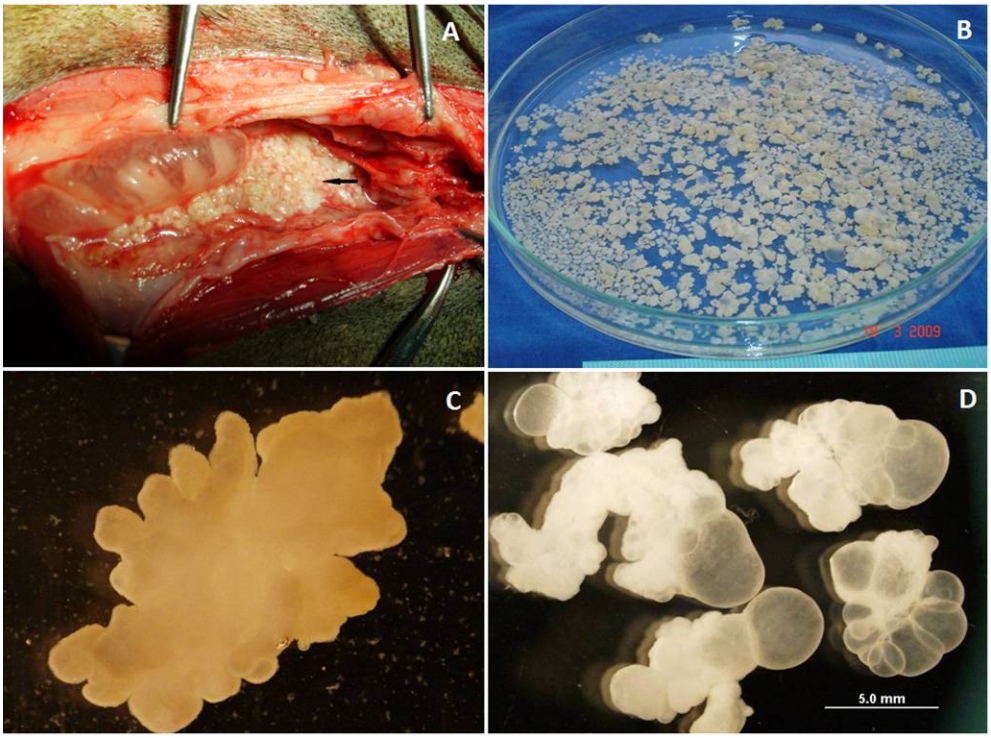

Fig 1. A- Exploratory abdominal surgery revealed with large numbers of free cestode cysts *(arrows)*, **B-** Larvae removed from the abdominal cavity, **C, D-** Budding like reproduction of larvae during the acephalic phase

Şekil 1. A- Deneysel laparotomide ortaya çıkan çok sayıda serbest cestod kisti *(ok)*, **B-** Abdominal boşluktan toplanan larvalar, **C, D-** Acephalik dönemde tomurcuklanmayla bölünmeye benzeyen görünümler

in the parenchymal tissue *(Fig 2-C)*. No larvae were present inside the cystic formations.

Although there is information indicating that the tetrathyridia of *M. corti* multiply by asexual reproduction by dividing along their long axis [2], we observed images suggesting reproduction by budding during the acephalic phase of larval development *(Fig 1-C, D)*.

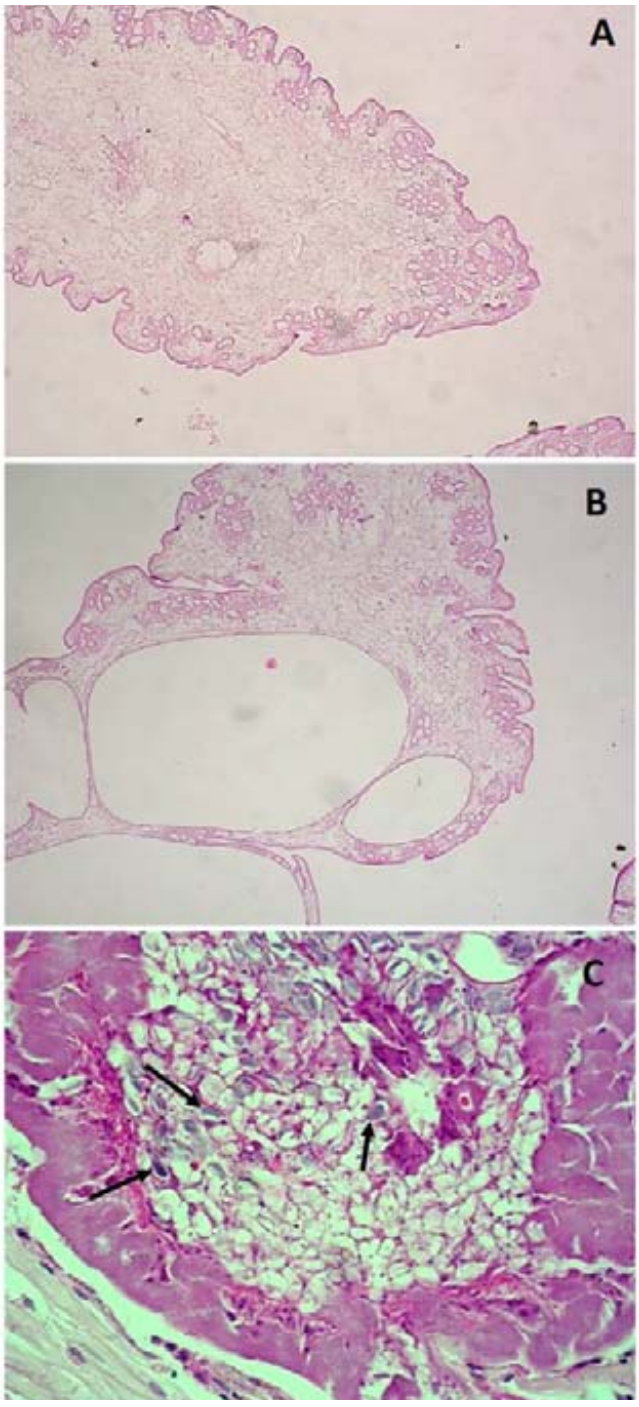

Fig 2. A, B- Histopathological view of HE stained section of a formalin-fixed parasitic cyst, **C-** Numerous calcareous corpuscles in paranchymatous tissue (arrows)

Şekil 2. A,B- Formalinle fikse edilen Hematoksilen Eozinle boyalı parazitik kist kesitinin histopatolojikal görünümü, **C-** Parankimatöz dokuda çok sayıda kalkaröz korpuskullar (ok işaretleri)

The parasitic material collected during laparotomy was stored at -80°C. DNA was extracted by repeated freezing and thawing of frozen samples. The DNA was extracted using the Wizard® Genomic DNA purification Kit (Promega Corporation, Madison, USA) in accordance with the manufacturer's protocol. PCR amplification was performed with mitochondrial 12S rRNA cestod-specific primers (F: 5'-TTA AGA TAT ATG TGG TAC AGG ATT AGA TAC CC-3' and R: 5'-AAC CGA GGG TGA CGG GCG GTG TGT ACC-3') [12] which amplify 387 bp fragment of the gene. The PCR mixture contained 1X PCR buffer, 6 mM $MgCl_2$, 200 mM (each) deoxynucleoside triphosphate,100 nM (each) primer, 2.0 U of HotStartTaq DNA polymerase (Fermentas, EU), and 1 µl of DNA template in a total 25 µl reaction mixture. Each PCR cycle consisted of 49 cycles of denaturation at 93°C for 1 min, annealing at 55°C for 90 s, and extension at 73°C for 2 min; an initial denaturation step consisting of incubation at 95°C for 10 min and a final extension step consisting of incubation at 72°C for 5 min were also included. The amplified PCR product was around 300-400 bp *(Fig 3)*. To confirm the results of PCR, one of the amplicon was sequenced with the same primers in both directions. The obtained sequence

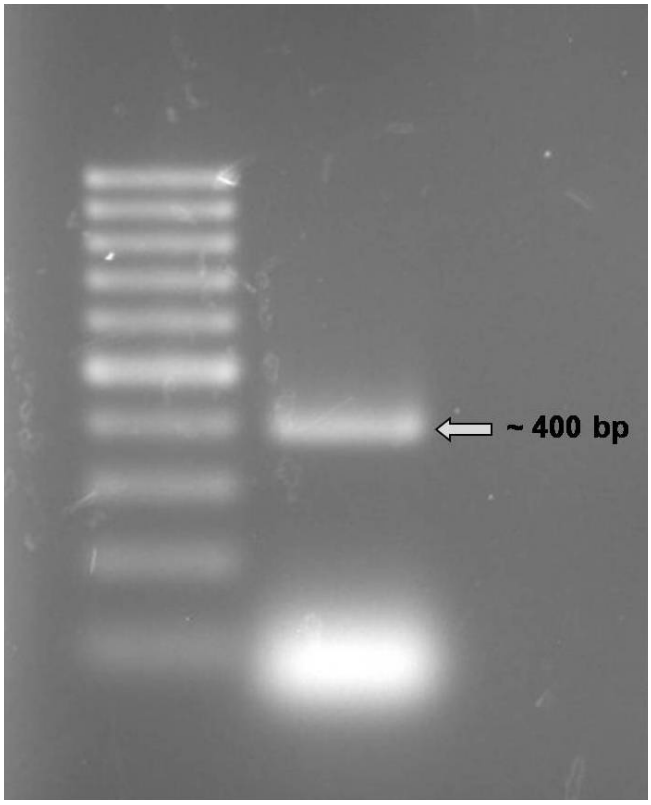

Fig 3. PCR analysis of the small 12 S rRNA gene from the *Mesocestoides corti* in a dog in Turkey. 1: Marker (100 bp), 2: Sample, 3: Negative control. The PCR products were electrophoresed on 1.5% agarose gel and stained by ethidium bromide

Şekil 3. *Mesocestoides corti*'ye ait 12 S rRNA gen bölgesinin PCR analizi. 1: Marker (100 bp), 2: Örnek, 3: Negatif kontrol. PCR ürünü %1.5 agoroz jel üzerinde elektroforeze tabii tutulmuş ve ethidium bromide ile boyanmıştır

was submitted to GenBank® database with the accession number JN572111. When the sequence result of amplified fragment was compared with reference sequences using blastn algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi), 89 and 100% similarity were found with the isolates from Japan and Turkey, respectively (AB031363, HM011122).

DISCUSSION

There are various case reports indicating that the disease caused by larval forms of cestodes belonging to the *Mesocestoides* genus in cats and dogs are characterized by non-specific signs like abdominal distension, loss of appetite, diarrhea, depression as we observed in the present case [4,5,11,13-18].

Anemia and leucocytosis observed in the present case are in accordance with previous findings of Bonfanti *et al*.[11] and Wirtherle *et al*.[14] respectively. Nevertheless, there were discrepancies between the previous cases and our case as for the eosinophil, neutrophil and platelet counts, hemoglobin, and serum albumin, globulin, urea, phosphate, creatinine and bilirubin levels. These differences lead to a speculation that in infections due to *Mesocestoides* species, blood test results may vary according to the severity of accompanying peritonitis and the presence of other infections. The cause of severe peritonitis could be due to the gastric perforation which might have occurred by a sharp angled foreign object, although no foreign body was found during autopsy. Detection of the diffuse ascites together with scintillating following abdominal radiography and ultrasonography suggest that the case could be a larval stage parasitosis as reported previously in related cases [4,11,14].

Due to the fact that parasites belonging to cysticercus, strobilocercus, coenurus, hydatic cysts and acephalic larval stage of mesocestoides cannot be distinguished based on morphology or histopathology at the beginning of developmental stage. Therefore PCR followed by sequence analysis were performed for a accurate diagnosis.

The presence of large calcareous corpuscles in the larval matrix on cytological examination and other histological findings indicated the presence of larvae of the *Mesocestoides* species as described previously [19,20].

This is a case report demonstrating the presence *Mesocestoides corti* in a dog in Aydin, Turkey using clinical, cytological and molecular methods.

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