

# Molecular Identification and Clinicopathological Findings of *Hepatozoon* sp. Infection in a Cat: First Report from Turkey

Bir Kedide *Hepatozoon* sp. Enfeksiyonunun Moleküler İdentifikasyonu ve Klinikopatolojisi: Türkiye'den İlk Rapor

Gülten Emek Tuna<sup>1</sup> , Serkan Bakırcı<sup>2</sup> , Ceren Dinler<sup>1</sup> , Gizem Battal<sup>3</sup> , Bülent Ulutaş<sup>1</sup> 

<sup>1</sup>Department of Internal Diseases, Adnan Menderes University School of Veterinary Medicine, Aydın, Turkey

<sup>2</sup>Department of Parasitology, Adnan Menderes University School of Veterinary Medicine, Aydın, Turkey

<sup>3</sup>Therapy Pet Hospital, İzmir, Turkey

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## ABSTRACT

*Hepatozoon* is a genus of protozoa belonging to the phylum Apicomplexa. Ticks are the vectors for the members of this genus. The protozoans infect a wide variety of mammals, birds, reptiles, and amphibians. The domestic and wild felids are also susceptible to *Hepatozoon* infection. A five-year-old female cat was presented to the Adnan Menderes University, Faculty of Veterinary Medicine Animal Hospital, with a 1-month history of inappetence and weight loss. In a physical examination, weakness, depression, anorexia, lymphadenopathy, icteric mucous membranes, abdominal distension, and fever were detected. Laboratory analysis revealed anemia, neutrophilic leukocytosis, and increased serum total bilirubin and indirect bilirubin concentrations. Hepatozoonosis was diagnosed by the observation of *Hepatozoon* spp. gamonts within neutrophils in Giemsa-stained blood smears and confirmed by polymerase chain reaction (PCR). This is the first report about the molecular identification of hepatozoonosis in a cat from Turkey.

**Keywords:** Cat, *Hepatozoon* sp., PCR, Turkey

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## ÖZ

*Hepatozoon* türleri, keneler tarafından bulaştırılan ve çok sayıda memeli, kuş, sürüngen ve amfibiyenleri enfekte eden Apicomplexa filumuna ait protozoalardır. Evcil ve vahşi kedigiller de *Hepatozoon* türlerine duyarlıdır. Beş yaşında dişi bir kedi, Adnan Menderes Üniversitesi Veteriner Fakültesi Hayvan Hastanesi'ne bir aydır devam eden iştahsızlık ve kilo kaybı şikayeti ile getirildi. Fiziksel muayenede güçsüzlük, depresyon, zayıflama, lenfadenopati, ikterik müköz membranlar, abdominal distansiyon ve ateş tespit edildi. Laboratuvar analizleri anemi, nötrofilik lökositoz, artmış serum total bilirubin ve indirekt bilirubin konsantrasyonlarını ortaya koydu. Hepatozoonosis tanısı, Giemsa boyalı periferik kan yaymalarındaki nötrofiller içerisindeki *Hepatozoon* gametositlerinin görülmesiyle konuldu ve PCR ile teyit edildi. Bu olgu, Türkiye'de bir kedide hepatozoonosis'in moleküler tespitinin ortaya konulduğu ilk rapordur.

**Anahtar Kelimeler:** Kedi, *Hepatozoon* sp., PCR, Türkiye

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## INTRODUCTION

*Hepatozoon* species are parasites from the phylum of Apicomplexa with a definitive host (a blood-sucking invertebrate) and an intermediate host (a vertebrate) (1-3). There are more than 340 species of *Hepatozoon* and about 46 of them infect mammals (2, 3). Despite a few differences in the ecology and biology of these protozoa, the primary route of *Hepatozoon* species transmission to the vertebrate hosts is represented by the ingestion of the invertebrate host carry-

ing mature oocysts (1, 4). Although the main vector of *Hepatozoon* spp. is the ixodid ticks, other blood-sucking invertebrates, fleas, and mosquitoes have the potential to transmit the infection. However, the vectors of feline hepatozoonosis are not exactly known (1).

Feline hepatozoonosis has been described from various countries, including Brazil, France, India, Israel, Nigeria, Portugal, Thailand, South Africa, Spain, USA, and Italy (3, 4). Three *Hepatozoon* species have been reported in felines:

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**Corresponding Author / Sorumlu Yazar:** Gülten Emek Tuna E.mail: emektuna@adu.edu.tr

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*Hepatozoon felis*, *H. canis*, and *H. silvestris* (2, 4, 5). A small number of epidemiological surveys have also been published (4, 6-8). Depending on the cat lifestyle, geographical area, diagnostic method used, and type of samples tested, the prevalence of infection was found to range from 0.6% to 36.8% using different methods across various regions (2-5).

Two forms of the parasite have been found in the cat: intracellular gamonts in neutrophils and monocytes and meronts in several tissues (3). Hepatozoonosis is usually diagnosed by detecting the gamonts in blood smears and by identifying meronts in biopsy specimens (3). However, the parasitemia degree is low in cats (less than 1%) (9). Hence, it is often overlooked in blood smears. In recent years, with the development of molecular tech-

niques, PCR has been started to be used for the diagnosis of *Hepatozoon* spp. in cats. Molecular methods are more sensitive and specific than the blood smear method for the detection of *Hepatozoon* spp. (1, 3).

In Turkey, although hepatozoonosis is widespread in dogs (10-13), feline hepatozoonosis has not yet been documented. This case is the first report of molecular identification of *Hepatozoon* spp. infection in a domestic cat from Turkey.

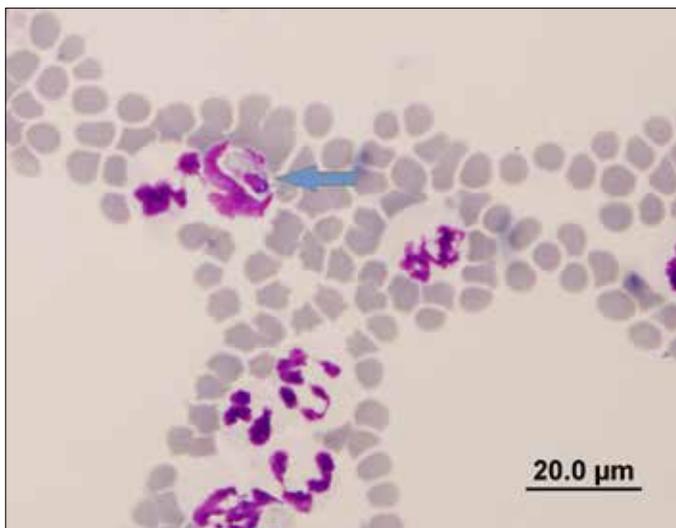
### CASE REPORT

A five-year-old female cat was presented to the Veterinary Teaching Hospital, Adnan Menderes University, with a 1-month history of inappetence and weight loss. The cat was living indoors with access to the outdoors. Vaccination and antiparasitic treatment program of the cat were irregular. The cat was fed with commercial food and did not have any serious health problems until 3 weeks ago. Three weeks before the presentation, the cat was

**Table 1.** Hemato-biochemical findings of the cat naturally infected with *Hepatozoon* spp.

Parameters	Values
WBC ( $\times 10^3$ cells/ $\mu$ L)	59.10
Lymphocytes ( $\times 10^3$ cells/ $\mu$ L)	0.50
Monocytes ( $\times 10^3$ cells/ $\mu$ L)	2.83
Neutrophils ( $\times 10^3$ cells/ $\mu$ L)	54.38
Lymphocytes (%)	0.8
Monocytes (%)	4.4
Neutrophils (%)	92.0
RBC ( $\times 10^6$ cells/ $\mu$ L)	3.84
Hemoglobin (g/dL)	6.4
Hematocrit (%)	18.19
MCV (fL)	47
MCH (p/g)	16.5
MCHC (g/dL)	34.9
Platelets ( $\times 10^3$ cells/ $\mu$ L)	771
Glucose (mg/dL)	75
AST (U/L)	13
ALT (U/L)	21
ALP (U/L)	170
GGT (U/L)	9
Total protein (g/dL)	6
Albumin	2.4
Direct Bilirubin (mg/dL)	1.9
Total Bilirubin (mg/dL)	4.76
Urea (mg/dL)	42
Creatinine (mg/dL)	0.75

WBC: white blood cells; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltransferase



**Figure 1.** *Hepatozoon* spp. gamont in neutrophils from Giemsa stained peripheral blood smear. Scale bar=20  $\mu$ m



**Figure 2.** PCR amplification of a partial sequence of the *Hepatozoon* spp. from the cat

1: Marker, 2: Sample, 3 and 4: positive control

treated for a hepatic/post hepatic icterus in a private clinic, but no improvement was observed. In a physical examination of the cat presented to our hospital, weakness, depression, anorexia, fever (40.5°C), lymphadenopathy, tachycardia, icteric mucous membranes, and abdominal distension were observed.

Three blood samples were collected from the cephalic vein for hematological, biochemical, and molecular examinations and for the detection of feline leukemia virus (FeLV) antigen and antibodies to feline immunodeficiency virus (FIV). The hematological abnormalities were moderate anemia and neutrophilic leukocytosis (Table 1). The serum biochemical abnormalities included elevated direct and total bilirubin concentrations (Table 1). The FIV antigen test by using rapid diagnostic commercial test kit (Fassisi® FeLFIV, Germany) was positive. To assess the cause of the abdominal distension, abdominal radiography and ultrasonography were performed. The radiography revealed increased soft tissue density in the proximal abdomen and numerous dilated bowel loops filled with gas and feces. The ultrasonography of the cranial abdomen showed marked hepatomegaly and a normal gallbladder structure. Caudal abdominal ultrasonography was unremarkable. Giemsa-stained blood smears were prepared and examined under the light microscope. The blood smears revealed *Hepatozoon* spp. gamonts within the neutrophils (Figure 1). The gamonts measured  $9.72 \pm 0.80 \mu\text{m}$  in length and  $4.17 \pm 0.09 \mu\text{m}$  in width. The slides were also used to examine leukocyte differentiation, erythrocyte morphology, and other infectious agents.

Written informed consent was obtained from cat's owner who participant in this case. PCR was performed to confirm *Hepatozoon* sp. infection (Figure 2). The DNA was extracted from each of two ethylenediaminetetraacetic acid-containing blood samples using the Promega Wizard genomic DNA extraction kit (Madison, WI, USA) following the manufacturer's instructions. Two microliters of extracted DNA were used for PCR amplification. Primers HepF (5'-ATA-CAT-GAG-CAA-AAT-CTC-AAC-3') and HepR (5'-CTT-ATT-ATT-CCA-TGC-TGC-AG-3'; Thermo Electron GmbH, Germany), which amplify a 666 bp fragment of the 18S rRNA gene of *Hepatozoon* spp., were used according to Inokuma et al., (14). The PCR product was electrophoresed on a 1.5% agarose gel and visualized under ultraviolet light.

Following the diagnosis, intravenous fluid therapy, doxycycline (5 mg/kg/once a day), lactulose (5 mL/cat, PO, twice a day), ursodeoxycholic acid (10 mg/kg once a day), B complex vitamins (1.0 mL/cat, once a day), and vitamin C (200 mg/cat, once a day) were prescribed. Prescribed drugs were not administered by the cat owner because the cat had escaped and she died. A postmortem pathological examination could not be performed.

## DISCUSSION

Tick-borne diseases are caused by a vast number of agents, including viruses, bacteria, and parasites. Although these diseases are more common in dogs, they are rarely reported in cats because cats are more resistant to tick-borne diseases. This situation is related to the specific behavior of cats and the mechanical removal of ticks from their body surface during grooming. Also, cats are likely to have innate resistance or adaptation to infec-

tion, restriction of the disease progression, or the transmission of the infectious agents (15, 16).

*Hepatozoon* infection in a domestic dog was first described by the Nevzat (17) in Turkey. The first clinical case of *H. canis* infection in a dog was reported by Voyvoda et al. (18). Several epidemiological surveys have revealed a high prevalence of *H. canis* in dogs in Turkey (10-13). The studies indicated that the prevalence of *H. canis* infection in dogs varies as 22.3-54.3%. Although canine hepatozoonosis caused by *H. canis* occurs commonly, *Hepatozoon* spp. in cats have not been reported in Turkey. We report the first case of *Hepatozoon* sp. infection in a cat in Turkey in this case.

Feline hepatozoonosis is usually associated with outdoor access where contact with ticks is more frequent compared to strictly indoor cats (2, 3, 5). In our case, we found no ticks on clinical examination, but since the cat lived in outdoor access, she might have had contact with ticks. The transmission of *Hepatozoon* in cats is not yet fully understood. However, Aktas (19) detected *H. felis* DNA in *Rhipicephalus sanguineus* tick in Turkey. Unlike other tick-borne protozoan and bacterial disease, *Hepatozoon* infection in a dog occurs by the ingestion of the infected ticks, and it is presumed that cats are infected by the same route (15).

In cats, the infection has a low virulence, mostly subclinical, and there is scarce clinical information on the disease (2, 5, 20). As in dogs with hepatozoonosis, the identification of a characteristic clinical syndrome in feline hepatozoonosis is difficult. The most common clinical findings of feline hepatozoonosis are nonspecific, including fever, anorexia, weakness, weight loss, lymphadenopathy, lethargy, and icterus (2, 14, 15). The clinical signs of the cat presented here were similar to those reported for cats with *Hepatozoon* sp. infection by other investigators (9, 20). The presence of co-infections with FeLV or FIV, other causes of immunosuppression, and other vectorborne pathogens have frequently been described in feline hepatozoonosis (5, 21). Similar with those reports, FIV and possible immunosuppression related to FIV could also play an important role in this case. A few studies have indicated that co-infections may enhance the virulence of *Hepatozoon* spp. (9, 22). Also, immunosuppression may increase the degree of parasitemia (2, 23).

In the present case, the results of laboratory tests revealed neutrophilic leukocytosis, moderate anemia, and bilirubinemia. Anemia and leukocytosis with neutrophilia in our case were similar with those previously reported (3, 9), and those alterations could be related to secondary necrosis and inflammation in the spleen, lymph nodes, liver, and lungs because of meronts observed in many tissues, such as the skeletal muscle, myocardium, lung, liver, pancreas, bone marrow, and lymph node (3, 9). Elevated serum total and direct bilirubin levels detected in our case may be related to the presence of developmental stages of the agent in the liver and hemolysis.

Nonspecific and broad clinical findings of the disease make it difficult to confirm the diagnosis based exclusively on clinical assessment. Hence, definitive diagnosis of feline hepatozoonosis is mainly performed by the determination of *Hepatozoon* spp. gametocytes within neutrophils and monocytes in stained blood smears. Because of low parasitemia, PCR should be considered

the diagnostic test of choice for confirming *Hepatozoon* infection (3). In this case, the diagnosis was confirmed by the detection of gamonts in Giemsa-stained blood smears and by PCR.

## CONCLUSION

This case is the first molecular identification of *Hepatozoon* sp. in a cat from Turkey. This infection should be considered in cats with inappetence, weight loss, anemia, icterus, and leukocytosis. Further studies are needed to understand hepatozoonosis in cats in Turkey.

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