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First report of *Hepatozoon felis* infection in a domestic cat (*Felis catus*) in Iran

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Abstract

A 6-year-old male domestic short-haired cat (*Felis catus*) was presented with weakness, anorexia, fever, icterus, a painful abdomen, ruffled hair and a tick infestation, and it had no prior surgery. Laboratory analysis revealed left-shifted neutrophils, thrombocytopenia, low albumin content and high serum bilirubin concentration as well as activities of hepatic enzymes including alanine aminotransferase and aspartate aminotransferase. Azotemia and increased serum levels of creatinine and urea were also recorded. In Giemsa-stained blood smear, *Hepatozoon* gamonts were observed within neutrophils. Species-specific polymerase chain reaction assay was used to amplify an approximately 590 bp fragment of *18S rRNA* gene and confirmed *Hepatozoon felis* infection. The cat was treated with imidocarb dipropionate and doxycycline and recovered completely. Six-month follow-up showed no recurrence. This study reveals the presence of *H. felis* in Iran and it should be considered in differential diagnosis in febrile and icteric cats. To the authors' knowledge, this is the first description of *H. felis* infection in a cat in Iran.

Introduction

Hepatozoon species are protozoal, apicomplexan vector-borne infectious pathogens being transmitted by blood-feeding arthropods to mammals, birds, reptiles and amphibians. The transmission of *H. felis* in cats and other carnivores usually occurs by ingestion of a hematophagous arthropod, such as a tick, which is a definitive host containing sporulated oocysts.[1] Schizogony occurs in various organs of intermediate vertebrate hosts, and merozoites invade leukocytes and become gametocytes.[2] There is also a possibility that infection may occur by predation.[3] *Hepatozoon felis* infection varies from being subclinical and identified incidentally in apparently healthy cats to being a severe and life-threatening clinical disease.[2] Hepatozoonosis of the domestic cat was first observed in India in 1908 and this infection has been reported from different countries around the world.[4] In Iran, *Hepatozoon* infection is well-recognized in dogs and *H. canis* is the common species.[5,6] In 2009, *Hepatozoon* spp. was reported from a stray dog and Persian leopard in the northeast regions of Iran by microscopical examination.[7,8] However, there is yet no report about hepatozoonosis in cats from Iran either by cytological examination or molecular assay. Therefore, the present report was the first case of *H. felis* infection in a domestic short-haired cat in Iran.

Case Description

In May 2022, a 6-year-old male domestic short-haired cat from a rural area around Karaj, located in the north of the central plateau of Iran (approximate coordinates: 35°56′N 50°54′E), was admitted to a private veterinary clinic due to a 1-week history of weakness and anorexia. Fever (39.50 ˚C), a painful abdomen, ruffled hair, mild jaundice and a tick infestation were also recorded in clinical examination. There were no detectable parasites in the fecal examination, and no helminths/flukes eggs and coccidian oocysts were seen in the feces. An adult tick (*Rhipicephalus sanguineus*) was found on the cat. It had no prior surgery. The blood samples from the femoral vein were collected in ethylenediaminetetraacetic acid-containing blood collection tubes (Kendall Co., Mansfield, USA) for hemato-biochemical and molecular analyses. First, Giemsa-stained blood smears were prepared for

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hematological cytology under a light microscope (SZ61; Olympus, Tokyo, Japan) using morphological features[1] and molecular assays. Also, the cat was tested for feline infectious peritonitis virus (FIPV) antigen (Biotech Co., Ltd., Shanghai, China) and antibodies against feline immunodeficiency virus (FIV; Biotech Co.).

Genomic DNA from the blood sample was extracted using a DNA extraction kit (Favorgen, Ping Tung, Taiwan). A pair of primers, H14Hepa18SFw and H14Hepa18SRv, was used to amplify an approximately 590 bp fragment length of *18S rRNA* gene of *Hepatozoon* spp. as described by Pereira *et al*.[9] Polymerase chain reaction (PCR) was conducted in a thermocycler (CP2-003; Corbett Research, Mortlake, Australia) programmed for an initial denaturation and activation step at 94.00 ˚C for 10 min. This step was followed by 35 cycles at 72.00 ˚C (94.00 ˚C 30 sec, 64.00 ˚C 45 sec and 72.00 ˚C 30 sec) for 7 min. The PCR product was purified from the agarose gel (1.50 %) using purification kit (Bioneer, Daejeon, South Korea) according to the manufacturer's instructions. The 15.00 µL of purified PCR product and 10.00 µL of forward (5'-GAAATAACAATACAAGGCAGTAAAATGCT-3') and reverse (5'-GTGCTGAAGGAGTCGTTTATAAAGA-3') primers (5.00 µM each) were sent for sequencing (Pishgamn, Tehran, Iran). The obtained *18S rRNA* sequence was compared to GenBank® entries using the BLAST tool provided by National Center for Biotechnology Information. The *H. felis* positive control was confirmed by GenBank® under the accession No. of OQ976011. The negative control contained all essential components of the amplification reaction except the DNA template. The cat was treated with two doses of 3.50 mg kg^{-1} subcutaneous imidocarb dipropionate (Aburaihan Pharmaceutical Co., Tehran, Iran), with an interval of 14 days in combination with 5.00 mg kg^{-1} oral doxycycline (Arad Arisman Co., Qom, Iran), twice a day, for 4 weeks. Hematological and biochemical assessments were performed using automatic cell counter (Exigo, Stockholm, Sweden) and automated biochemistry analyzer (BT1500; Biotechnica, Rome, Italy).

Results

In Giemsa-stained blood smears, ovoid and elongated cytoplasmic structures similar to *Hepatozoon* gamonts (mean length of 10.30 µm and mean width of 4.20 µm) were observed inside neutrophils (Fig. 1). The serological examinations for FIPV and FIV were negative.

The sequences of the partial *18S rRNA* gene for *Hepatozoon* spp. (accession No. OQ976011) demonstrated the highest homology of 98.00 - 100 (Fig. 2).

Hematological and biochemical analyses of blood samples revealed left-shifted neutrophils and thrombocytopenia. Results of serum biochemical analyses also indicated low albumin content and high bilirubin concentration as well as activities of hepatic enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Further biochemical analyses of the sample disclosed azotemia and increased serum levels of creatinine and urea (Table 1).

Two weeks after beginning of the treatment, clinical signs totally disappeared. Six months after the first diagnosis, blood smear, serological test and PCR were negative for the parasite.

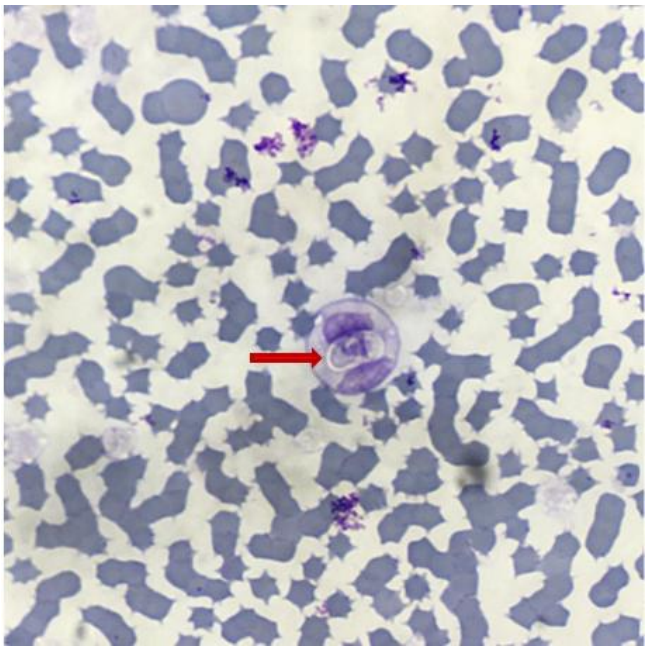

Fig. 1. Blood smear showing gamont of *Hepatozoon* spp. (arrow) in a neutrophil (Giemsa staining, 100×).

Fig. 2. Agarose gel electrophoresis of *Hepatozoon felis 18S rRNA* gene products by polymerase chain reaction (PCR). Lane M: 100 bp DNA ladder; Lane 1: Negative control; Lane 2: PCR product of *H. felis* sample.

Table 1. The hemato-biochemical parameters measured in this study.

Parameters	Patient's values	Reference range[7,10]
Band neutrophils (cells µL^{-1})	1,760	0.00 - 300
Thrombocyte (× 10^5 cells µL^{-1})	1.75	2.30 - 6.80
Albumin (g dL^{-1})	2.10	3.00 - 4.60
Bilirubin (mg dL^{-1})	0.81	0.10 - 0.70
Alanine aminotransferase (IU L^{-1})	257	23.00 - 109
Aspartate aminotransferase (IU L^{-1})	132	14.00 - 41.00
Blood urea nitrogen (mg dL^{-1})	162	18.00 - 41.00
Creatinine (mg dL^{-1})	9.30	0.70 - 2.20
Urea (mg dL^{-1})	346	21.00 - 65.00

Discussion

This study represents the first case report of *H. felis* infection in a cat in Iran and provides an evaluation of clinical cases along with morphological and molecular findings. The clinical signs and hemtological examination observed in the affected cat were consistent with previous studies.[1,2] The observed thrombocytopenia and left-shifted neutrophils can be attributed to platelet aggregation along with parasite invasion and multiplication in the animal tissues.[11,12] This case demonstrated that *H. felis* infection caused liver damage being significantly evidenced by increased levels of AST, ALT and bilirubin as well as a decreased level of albumin; as being commonly observed in dogs hepatozoonosis.[13,14] It has been shown that measurement of ALT level is very important for the diagnosis of liver disorders in dogs and cats.[15] In the current report, a significant elevation was evident in blood urea nitrogen (BUN) and creatinine levels. Similar results were reported by other researchers.[1,16] It is known that renal involvement occurs in *H. felis* infection. The elevation in BUN and creatinine levels might have related to the encapsulation of *H. felis* meronts in renal and muscular tissues.[1,2] In the present case, imidocarb dipropionate and doxycycline combination was prescribed to treat *H. felis* infection.[2,17] *Hepatozoon* was eliminated from the cat blood two weeks after drug administration. This study revealed the presence of *H. felis* infection in Iran and provided more evidence about the pathogenicity of this parasite for domestic cats. In this case, the cat was treated with combined imidocarb and doxycycline therapy. More studies are required for better understanding of hepatozoonosis epidemiology and pathogenesis in cats in Iran.

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Conflict of interest

The authors declare no probable conflicts of interest.

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