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First autochthonous case of clinical *Hepatozoon felis* infection in a domestic cat in Central Europe

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ABSTRACT

Three different *Hepatozoon* (Apicomplexa, Hepatozoidae) species have been described infecting domestic cats in Europe (i.e. *H. felis*, *H. canis* and *H. silvestris*), however, reports on clinical hepatozoonosis are uncommon and treatment protocols are not clearly defined.

A six-year-old male European short-hair cat from Austria presented poor general condition, lethargy, anorexia, icterus, a painful abdomen, fever, ruffled hair and a tick infestation, and it had never left Austria. Laboratory tests revealed leukopenia, thrombocytopenia and increased serum levels of symmetric dimethylarginine (SDMA) and bilirubin. In May Grünwald-Giemsa-stained blood smears, structures resembling *Hepatozoon* gamonts were observed inside neutrophil granulocytes. A PCR targeting a fragment of the 18S rRNA gene of *Hepatozoon* spp. and DNA sequencing allowed the diagnosis of *H. felis*-DNA in blood samples. The cat was treated with imidocarb dipropionate (6 mg/kg body weight, repeated after 14 days) and doxycycline monohydrate (5 mg/kg body weight twice a day, p.o., for four weeks) and recovered completely. A broad haematological and biochemical laboratory control after six months showed all evaluated parameters under normal ranges. Coinfection with other feline pathogens (i.e. feline leukaemia virus, feline immunodeficiency virus, feline Coronavirus, *Leishmania* and *Dirofilaria immitis*) could not be detected.

This study reveals the presence of *H. felis* in Austria and provides more evidence on the geographical distribution and pathogenicity of this parasite for domestic cats. To the authors' knowledge, this is the first autochthonous case of feline hepatozoonosis in Central Europe.

1. Introduction

The genus *Hepatozoon* (Apicomplexa, Hepatozoidae) includes more than 340 species affecting mammals, birds, reptiles and amphibians [1–4]. These apicomplexan parasites undergo a heteroxenous life cycle that involves a hematophagous invertebrate definitive host, in which sporulated oocysts are formed and a vertebrate intermediate host in which merogony and gametogony occur. The merogonic stages occur in different tissues and organs and are the ones responsible for lesions and clinical signs in the host. The gamont stages of *Hepatozoon* circulate in blood cells (i.e. erythrocytes of species infecting reptiles and leucocytes of species infecting birds and mammals) of the vertebrate hosts and are ingested by the invertebrate hosts during blood feeding [3]. Usually, the vertebrate host is infected through ingestion of an arthropod definitive host containing infective oocysts [3], but additional routes of infection were described for some *Hepatozoon* species, such as predation of a paratenic or intermediate vertebrate host harbouring cystozoites (monozoic or dizoic cysts) in their tissues (e.g. *H. americanum*) [3–5] and transplacental transmission (e.g. *H. canis*; *H. felis*) [1,6].

Hepatozoon infection in domestic cats has been first observed in India in 1908 and since then it has been recorded several times in Asia, Europe, Africa and America [3], but the involved species have been only recently identified. So far, three different *Hepatozoon* species were described in domestic cats in Europe: *H. felis*, *H. canis* and *H. silvestris* [1,7,8]. *H. felis* has been the most frequently diagnosed species in cases of feline hepatozoonosis in different countries around the world [1,9–14]. Interestingly, while some *Hepatozoon* species seem to be host-specific, *H. canis*, the worldwide distributed *Hepatozoon* species infecting domestic dogs, is also able to infect cats [3,7,15,16] and several species of wild carnivores [17–22], which may represent an infection reservoir for domestic animals.

In Europe, *H. felis*-DNA was identified by PCR in blood from

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domestic cats from Italy [7]; Spain [14,23,24]; Portugal [10,11] and Cyprus [9,12]. *H. canis*-DNA was detected in blood from cats in Italy [7]; Spain [14,16] and France [25]. *H. silvestris*, the most recently described species, was first detected in European wild cats (*Felis silvestris silvestris*) from Bosnia and Herzegovina in 2017 [2]. Soon afterwards it was also evidenced in blood from a subclinically infected domestic cat in Italy [7] and in a domestic cat with severe myocarditis in Switzerland [8].

Clinical cases of *Hepatozoon* infection in cats have been only scarcely reported and treatment protocols are not clearly defined [1]. In this study, diagnosis and therapy of a case of hepatozoonosis due to *H. felis* in a domestic cat from Austria is presented. To the authors' knowledge, this is the first autochthonous case of feline hepatozoonosis in Central Europe.

2. Case report

A six-year-old neutered male European short-hair cat from the Burgenland State in Austria (approximate coordinates: 47°50′44.3″ N 16°31′23.8″ E) was presented to a private veterinary clinic on June 14th, 2018 showing poor general condition, lethargy and anorexia. At the clinical examination, the cat was lightly icteric, presented a painful abdomen, fever (39.8 °C), ruffled hair, a bite wound in the neck, pruritus and a tick infestation, but no fleas were observed. No tick prophylaxis had been carried out during the last year. The cat had free outdoor access, but normally stayed in the surroundings of the house and it had never travelled to other countries. The owner informed that the cat had been in contact with a snake before admission, thus snake poisoning was also considered in the differential diagnosis.

A complete haematological check-up including standard profiles for kidney, liver, pancreas and muscle function was performed at a private laboratory. Before the laboratory results were obtained, amoxycillin (Betamox® long acting 150 mg/ml, Norbrook Laboratories Limited, 15 mg/kg as a subcutaneous [sc] injection) was initially administered but the clinical signs did not remit.

Hematologic and biochemical analysis of blood samples revealed leukopenia (3.7 G/l; reference range 3.9–19 G/l) characterized by lymphopenia (lymphocytes 595/µl; reference range 850–5850/µl) and mild neutropenia (segmented neutrophils 2604/µl; reference range 2620-15,170/µl); mild thrombocytopenia (149 G/l; reference range 155–641 G/l); anisocytosis, and slightly increased serum levels of symmetric dimethylarginine (SDMA) (18 µg/dl; reference range 0–14 µg/dl) and bilirubin (0.5 mg/dl; reference range 0–0.4 mg/dl). SDMA was determined using a commercially available high-throughput immunoassay (IDEXX SDMA® Test; IDEXX Laboratories Inc.) [26]. Serum levels of calcium were slightly reduced (2.1 mmol/l; reference range 2.2–2.9 mmol/l) and magnesium was elevated (1.3 mmol/l, reference range 0.6–1.1 mmol/l). Red blood cell (RBC) count, as well as further standard biochemical parameters were within normal ranges. In May Grünwald-Giemsa-stained blood smears, ovoid structures resembling *Hepatozoon* gamonts (11.2 × 5.1 µm, with ovoid nucleus) were observed inside neutrophil granulocytes (Fig. 1). To confirm the microscopic finding, a real-time polymerase chain reaction (PCR) for *Hepatozoon* spp. targeting the 18S rRNA gene [27] was performed and a positive result was obtained. Subsequently, in order to determine the *Hepatozoon* species, a conventional PCR using the primers H14Hepa18SFw and H14Hepa18SRv, which targets a fragment of the 18S rRNA gene of *Hepatozoon* spp. was performed as previously described [8]. As a positive control, DNA extracted from the heart of a naturally infected European wild cat was used [2]. A 590 bp PCR product was amplified, subsequently purified using a commercial kit (DNA Clean & Concentrator-5 Zymo Research, Irvine, USA) and sequenced (Microsynth, Balgach, Switzerland) in both directions with the same primers used in the PCR. The obtained sequence was trimmed from the primers and deposited in GenBank (accession number: MK724001). BLAST analysis revealed 100% (561/561 bp) identity with GenBank sequences

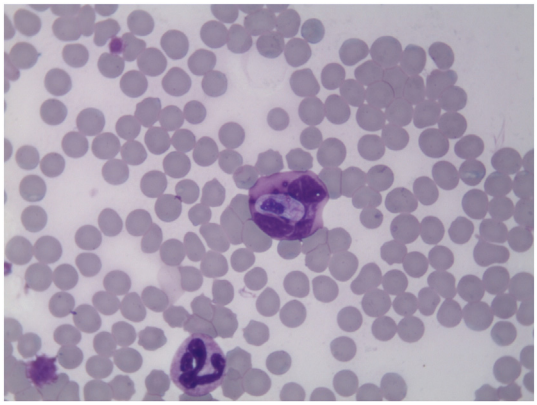

Fig. 1. *Hepatozoon felis* gamont (11.2 × 5.1 µm) inside a neutrophil granulocyte in a May Grünwald-Giemsa-stained blood smear from a domestic cat from Austria.

of *Hepatozoon felis* from European wild cat (*Felis silvestris silvestris*) from Bosnia and Herzegovina (accession Nr. KX757033.1). The sequence similarity with *H. canis* and *H. silvestris* GenBank entries was of only 97.7% and 96.3%, respectively (accession Nr. MK091088.1 and MH078194.1, respectively).

Consequently, the cat was treated with imidocarb dipropionate (Imizol®, Merck Animal Health) (two doses of 6 mg/kg body weight, sc, with an interval of 14 days) in combination with doxycycline monohydrate (Doxybene® ratiopharm Arzneimittel Vertriebs-GmbH) (5 mg/kg body weight twice a day, orally, for four weeks). At control, two weeks after beginning of the treatment, the cat showed a very good recovery and after one month the clinical signs totally disappeared.

Six months after the first diagnosis was made, a complete laboratory check-up including blood count and standard biochemical profiles for kidney, liver, pancreas and muscle function was carried out. In order to discard coinfection with other feline pathogens, the cat was tested for feline leukaemia virus (FeLV) antigen (PetChek® FeLV, IDEXX Laboratories Inc.) and antibodies against feline immunodeficiency virus (FIV) (PetChek® Plus Anti-FIV, IDEXX Laboratories Inc.) [28], and also for antibodies against feline Coronavirus (FCoV ELISA cat, Afosa, Germany) and *Leishmania* (immunofluorescent test [30] validated for cats with anti-cat conjugate), for *Dirofilaria immitis* antigen (SNAP® 4Dx® Plus, IDEXX Laboratories Inc. after heat pre-treatment of serum [31]); and by real-time PCR for *Hepatozoon* [27] and *Leishmania* [33]. All evaluated parameters were under normal ranges. All serological and PCR tests performed gave negative results (including tests for antibodies to *Anaplasma* spp., *Ehrlichia* spp. and *Borrelia* C6 antigen comprised in SNAP 4Dx® Plus test, primarily performed for detecting heartworm antigen). No blood parasites could be observed in Giemsa stained-blood smears.

3. Discussion

Many aspects about hepatozoonosis in cats are still poorly understood, such as pathogenesis, life cycle, definitive hosts and transmission routes of the *Hepatozoon* species involved. Likewise, adequate treatment protocols for feline hepatozoonosis are not clearly defined [29].

Opposite to *H. canis* infection in dogs, which primarily targets haemolymphatic organs, feline hepatozoonosis seems to be often associated with infection of skeletal muscle and myocardium [1,3,8,32], but also other organs may be affected. Although in the first reports of feline hepatozoonosis the involved species were not identified, the tropism for muscle tissues was confirmed at least for *H. felis* [1] and *H. silvestris* [8]. It is still not clear to what extent this also applies to *H. canis* infections in cats.

Prevalence studies on *Hepatozoon* infections in cats from Europe are scarce. In a study from Cyprus, 37.9% (66/174) of the tested cats

reacted positive in a *Hepatozoon* sp. PCR, and sequencing of 14 of the amplicons revealed *H. felis* in all cases [12]. In Portugal, *Hepatozoon* DNA was detected by PCR methods in blood from 8.6% (56/649) of cats from the south [11] and in 15.6% (50/320) cats from the north and centre of the country [10]. Sequencing of 13 [11] and four [10] of the amplified PCR products, respectively, revealed only *H. felis*-DNA. In southern Italy, *Hepatozoon* spp.-DNA was detected in 5.1% (10/196) of the analysed cats; further sequencing of the obtained amplicons confirmed the presence of all three described *Hepatozoon* species affecting domestic cats: *H. felis* ($n = 8$), *H. canis* ($n = 1$) and *H. silvestris* (n = 1) [7]. In Spain, both *H. felis* ($n = 9$) and *H. canis*-DNA (n = 1) were detected by PCR/sequencing in blood from 1.6% (10/644) of cats in Madrid [14]. In the Barcelona area, *H. felis* was detected by PCR and sequencing in blood from 4% (4/100) [23] and 16% (4/25) [24] of cats. In France, *H. canis*-DNA was revealed in blood from 1.7% (2/196) of the analysed cats [25].

In Austria and Central Europe, no cases of *H. felis* infection in domestic cats were reported before. Further studies are needed to assess the prevalence of this infection in this geographical region. Infections by *H. felis* may be subclinical or associated to mild clinical signs (e.g. lethargy, fever, weakness and lymphadenopathy), but also severe cases may occur [1,3,29]. In some prevalence studies, some or all cats with positive *Hepatozoon* PCR results in blood presented clinical signs that might have been associated with the infection [10–12,14,23–25], while in other studies all positive cats were apparently asymptomatic [7].

In the present case, the clinical signs and laboratory findings observed indicate impairment of the renal and liver function, as it has been described in feline hepatozoonosis [1] and is also commonly observed in canine hepatozoonosis due to *H. canis* [3]. In a retrospective study from Israel, most cats with hepatozoonosis showed elevated serum levels of the muscle enzyme creatine kinase (CK) [34]. However, in the present case, CK serum levels were in normal range and only a slight decrease in calcium and increase of magnesium serum levels were observed, suggesting that no severe injury of striated muscle was present. In this case, slightly increased bilirubin and SDMA levels, with creatinine levels within normal ranges may suggest acute hepatic and renal injury. SDMA is regarded as a more reliable and sensitive indicator of renal function than creatinine, as it is not influenced by lean body mass, especially important in patients with muscle loss [35]. In cases of renal injury, SDMA shows an earlier increase in serum than creatinine, remaining at high levels also in cases of chronic kidney disease, on average with 40% reduction of glomerular filtration rate, compared with up to 75% reduction needed to increase creatinine level [35]. At control, creatinine and SMDA serum levels were in normal ranges, suggesting a recovery of the kidney function upon specific treatment.

The level of parasitaemia in feline hepatozoonosis seems to be generally low with less than 1% of the neutrophils and monocytes containing gamonts [3]. Accordingly, in this study, only three *H. felis* gamonts were observed in the whole blood smear, and no other pathogens could be microscopically detected. Noteworthy, most prevalence studies on feline hepatozoonosis were exclusively based on molecular techniques [7,10–12,14,23,25] and the presence of circulating gamonts was only rarely reported [1,34,36].

Immunosuppression and co-infections with other pathogenic agents are assumed to contribute to the severity of feline hepatozoonosis [8]. In several of the described cases of feline *Hepatozoon* infections, coinfections with other pathogens were diagnosed, including the immunosuppressive viruses FIV and FeLV [11,12,24], Feline Panleukopenia Virus [32], *Leishmania* [9,11,12,23], *Babesia* spp. [11,37], *Cytauxzoon* [25] and hemotropic mycoplasmosis [1,3,9,25]. In the present case, no *Cytauxzoon* or other organisms like hemotropic mycoplasmas or *Anaplasma* spp. were observed in the stained blood smear and no co-infection with feline pathogens such as FeLV, FIV, FCoV, *Leishmania* or *D. immitis*, which could potentiate the clinical signs could be detected, suggesting that *H. felis* can be a primary pathogen of cats.

The life cycle, definitive host and mode of transmission of *H. felis* are still unknown. The cat in the present study had a tick infestation, but it was immediately treated with Fipronil (Frontline Spot On® Merial) before the diagnosis of hepatozoonosis was made, and unfortunately no ticks for species differentiation or further analyses were collected. A study from Israel showed a significant association between infection in cats and access to outdoors suggesting the possibility of transmission by an arthropod vector or by carnivorism [1]. Ticks, mites, sand flies, tsetse flies, mosquitoes, fleas, lice, reduviid bugs and leeches were shown to serve as definitive hosts of different *Hepatozoon* species [3,4]. *H. felis* DNA was detected in four *Rhipicephalus sanguineus* ticks collected from two cats and one dog in Portugal [38]; in one *Ixodes hexagonus* tick from a cat in Wales (with only 90% sequence identity to available GenBank sequences) [39] and in 1.9% of 685 *Ctenocephalides felis* fleas collected from 185 cats in Israel [40], suggesting the possible involvement of these ectoparasites in the life cycle; however, no *H. felis* oocysts were detected in any arthropod vector so far. Alternative ways of transmission such as predation on an infected intermediate or paratenic host were described for *H. americanum* in dogs in US [5], but it is not known if this way of transmission may also occur or have any epidemiological relevance in *Hepatozoon* species affecting cats. Besides, transplacental transmission may represent an additional way of transmission. So far, this has been shown for *H canis* in dogs [6] and *H. felis* in cats [1].

Information on treatment of feline hepatozoonosis is scarce. To the best of our knowledge, there have been no controlled studies evaluating treatment efficacy in feline hepatozoonosis apart from a few case reports [29]. Therefore, the treatment protocol used in the present case was based on some experience in dogs. Imidocarb dipropionate was frequently used to treat *H. canis* infection in dogs at 5–6 mg/kg every 14 days until gamonts were no longer observed in blood smears on 2–3 consecutive examinations [3], and it was often combined with doxycycline at 10 mg/Kg for 21 days [41]. Doxycycline was also used to treat *H. americanum* infection in dogs in combination with trimethoprim–sulfadiazine and pyrimethamine [3]. However, there is no real consensus on the drugs and times required for the treatment of canine hepatozoonosis [42]. Two longitudinal studies monitored the presence of *H. canis* in blood and buffy coat and/or *H.canis*-DNA in blood and bone marrow after treatment with either imidocarb alone; imidocarb combined with doxycycline, and toltrazuril/emodepside combined with clindamycin, and showed that these treatments did frequently not lead to a complete parasite elimination [41,42]. Nevertheless, our microscopic and PCR findings indicated that the combined treatment with imidocarb and doxycycline seemed to have been effective to clear the parasite from blood in the cat of the present report.

This study reveals the presence of *H. felis* in Austria and provides more evidence on the geographical distribution and pathogenicity of this parasite for domestic cats. In this case, the cat recovered completely after a combined therapy with imidocarb and doxycycline. Further studies are needed to elucidate its life cycle, epidemiology and clinical significance of this emerging pathogen for cats.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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