Ticks and Tick-borne Diseases 16 (2025) 102556

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Short communication

Infection caused by a parasite most closely related to *Hepatozoon luiperdjie* in cats from Hungary

Gergő Kürtös [a,*], Nóra Rácz [b], Viktória Kassay [c], Krisztián Olaszy [b], Zsuzsanna Vizi [d], Anna Szilasi [e], Nóra Takács [f,g], Gergő Keve [f,g], Barbara Tuska-Szalay [f], Sándor Hornok [f,g]

[a] *VetMedLabor, Budapest, Hungary*
[b] *Rákosligeti Veterinary Clinic, Budapest, Hungary*
[c] *Zöldövezet Veterinary Clinic, Budakalász, Hungary*
[d] *Department and Clinic of Internal Medicine, University of Veterinary Medicine, Budapest, Hungary*
[e] *Department of Pathology, University of Veterinary Medicine, Budapest, Hungary*
[f] *Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary*
[g] *HUN-REN UVMB Climate Change: New Blood-sucking Parasites and Vector-borne Pathogens Research Group, Budapest, Hungary*

ARTICLE INFO

Keywords:
Apicomplexa
Tick-borne
Protozoan parasite
Hepatozoon silvestris
18S rRNA gene

ABSTRACT

Feline hepatozoonosis, due to *Hepatozoon felis*, has been recently reported in wild cats from Central Europe. At the same time, only two isolated cases were reported in domestic cats, one in Austria, and another in Hungary. In the southern part of Central Europe, Hungary, three cats were presented at two small animal clinics in the course of 1.5 months, their most important clinical findings included anaemia (for all three cats), malignant neoplasia (case #1), chyloabdomen, splenomegaly and hepatic nodules (case #2), and dyspnoea (case #3). *Hepatozoon* gamonts measuring 12.3 × 6 μm were present in 0.1–0.3 % of the neutrophilic granulocytes. A nearly 1700 bp-long-part of the 18S rRNA gene of this species showed only 98.3 % and 97.7 % sequence identities with *H. felis* and *H. silvestris*, respectively. Based on the phylogenetic analysis of this long part of the 18S rRNA gene, this protozoon clustered separately, with moderately high (87 %) support, from *H. felis* and was a sister species to *H. silvestris*. In a shorter part of the 18S rRNA gene, the *Hepatozoon* species in this study had 99.6 % sequence identity to *Hepatozoon luiperdjie*, and they clustered together phylogenetically, although with low (56 %) support. Based on morphological characteristics and phylogenetic relationships, the protozoan parasite emerging among domestic cats in the southern part of Central Europe is a species most closely related to *H. luiperdjie*. The emergence of this protozoan parasite in the region warrants monitoring among domestic cats in other countries of Central Europe.

1. Introduction

Hepatozoon species (Eucoccidiorida: Adeleorina: Hepatozoidae) are unicellular apicomplexan parasites that primarily affect organs of the haemolymphatic system of vertebrates (Smith, 1996). In mammals, they develop with asexual multiplication in the spleen, bone marrow, liver, lymph nodes, and muscles, until reaching the presexual gamont form in white blood cells (Dubey and Baneth, 2025). The gamont can only develop further if ingested by a suitable blood-sucking arthropod vector, as exemplified by fleas, mosquitoes, mites, or ticks, depending on the *Hepatozoon* species (Smith, 1996; Uiterwijk et al., 2023).

Among domestic animals, clinical hepatozoonosis occurs in dogs and cats, and these hosts are susceptible to a few species of the genus, including most often *Hepatozoon canis, Hepatozoon americanum* in canids and *Hepatozoon felis, Hepatozoon silvestris* in felids (Baneth, 2011; Carbonara et al., 2023; Simonato et al., 2022). The biological vectors of *Hepatozoon* species affecting dogs and cats are ixodid ticks. Importantly, when sexual development is completed in these vectors, the oocysts remain intact in the body cavity of the tick, and thus infectious forms, the sporozoites will not reach the salivary gland and cannot be inoculated during the next blood meal. Instead, *Hepatozoon* species can only induce infection in the next susceptible host if the vector tick is ingested, e.g. during grooming activity (Baneth, 2011). In addition, transplacental infection and transmission of monozoic cysts via carnivorism were also

* Corresponding author.
E-mail address: kurtos.gergo@vetmedlabor.hu (G. Kürtös).

https://doi.org/10.1016/j.ttbdis.2025.102556
Received 13 June 2025; Received in revised form 12 September 2025; Accepted 26 September 2025
Available online 10 October 2025

reported in some *Hepatozoon* species of dogs and cats (Baneth, 2011).

In Europe, until the beginning of the 21st century, the diagnosis of canine hepatozoonosis caused by *H. canis* was restricted to the Mediterranean region, where the main vector of this protozoon, the brown dog tick (*Rhipicephalus sanguineus*), is indigenous (Baneth et al., 2003). More recently, this infection started to emerge north of the Mediterranean Basin in the southern part of Central Europe, in Hungary, with high prevalence among dogs (Hornok et al., 2013). Similarly, feline hepatozoonosis caused by *H. felis* in domestic cats used to be endemic only to Mediterranean countries of Europe (Carbonara et al., 2023; Pereira et al., 2019), and cases reported in most parts of Central Europe, e.g. Germany, were imported from the southern part of the continent (Schäfer et al., 2022). However, recently, a single infection was diagnosed in a domestic cat in Austria (Basso et al., 2019), and multiple infections among wild cats in Hungary (Hornok et al., 2022) and in Germany (Unterköfler et al., 2022). In and close to the relevant endemic region of Hungary, domestic cats were also screened, but at that time, only a single asymptomatic infection was reported (Tuska-Szalay et al., 2023).

In the era of molecular methods, the emerging character of most infections may be difficult to assess based on prevalence alone, because previously no similar, highly sensitive diagnostic tools were available. On the other hand, the appearance and increasing prevalence of hepatozoonosis is more certainly recognized, since in blood smears the characteristic gamonts are easy to recognize during routine haematological examination (Baneth et al., 2013; Smith et al., 2025). In this study, we report three clinical cases of feline hepatozoonosis among domestic cats in a region of southern Central Europe previously thought to be nonendemic, together with molecular-phylogenetic analysis of the causative agent.

2. Materials and methods

2.1. Origin of cases and sampling

Hepatozoon-infections were reported at the beginning of 2025, involving cats presented at two small animal clinics in Budapest and Budakalász, Hungary. These were middle-aged to senior domestic shorthair cats, with outdoor access, no known history of travel, and a known (case #1), or probable (cases #2 and #3) history of tick infestations. In the anamnesis notable factors were a confirmed FeLV infection for case #2, and a successfully treated infection with feline infectious peritonitis for cases #2 and #3.

Medical imaging was performed by the attending veterinarians in all cases, and blood samples were collected from all three cats during veterinary care. In cases #1 and #2, sampling was done twice, 3 and 25 days apart, respectively. In cases #2 and #3, collection was performed under anaesthesia. Fine-needle aspiration for cytologic evaluation was carried out from the spleen in case #1, and from a mandibular lymph node in case #2, furthermore, peritoneal effusion was sampled and submitted for laboratory analysis in case #2. Retroviral status was tested in cases #1 and #3 using point-of-care serology. No ethical permission was needed, because sampling was performed during veterinary care. Pathological examination was done in case #3, for which consent was provided by the owner of the relevant cat. A more detailed history is provided in Additional file 1.

2.2. Haematological, biochemical and serological analyses

The haematological and biochemical analyses were performed at a commercial veterinary diagnostic laboratory (Vet-Med-Labor, Budapest, Hungary). The haematological parameters were determined using Sysmex XN-1500 V (Sysmex Corporation, Kobe, Japan). Blood smear examinations were assisted by Sysmex DI-60 (Sysmex Corporation, Kobe, Japan), the leukocyte differential counts and photographs provided by the digital imaging analyser were used to calculate the levels of parasitaemia, and the software ImageView (BestScope, Beijing, China) to measure the size of gamonts ($n = 11$). Biochemical measurements were carried out with Advia 1800 Chemistry System (Siemens AG, Munich, Germany).

Testing for FIV and FeLV in cases #1 and #3 was performed with FIV Ab/FeLV Ag Test Kit (Alvetra & Werfft GmbH, Neufeld, Austria).

2.3. Pathological examination and histology

In case #3, the carcass was submitted after approximately 4 h of cooling at −4 °C. Gross postmortem examination was performed in dorsal recumbency. Samples were taken from the spleen, liver, kidneys, pancreas, mesenteric lymph node, lungs, heart, mandibular lymph node, thyroid glands, skeletal muscle, eyes, brain, and tissue sections were prepared from the stomach, small and large bowels. Histopathologic samples were fixed in 10 % neutral-buffered formalin for 24 h at room temperature. Formalin-fixed and paraffin-embedded sections were stained with hematoxylin and eosin and subjected to routine histological examination. Special staining methods were used to detect collagenous connective tissue (Masson's trichrome) or siderophages (Perls').

2.4. DNA extraction and PCR analyses

From 200 µl of three EDTA-containing blood samples and pleural effusion (case #2), the DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA extracts were screened for *Hepatozoon* spp. by amplifying an approx. 650-bp-long part of the 18S rRNA gene with the primers HepF (5′-ATA CAT GAG CAA AAT CTC AAC-3′) and HepR (5′-CTT ATT ATT CCA TGC TGC AG-3′) (Inokuma et al., 2002). In this reaction, 2.5 µl of extracted DNA was added to 22.5 µl of reaction mixture containing 1.0 U HotStar Taq Plus DNA Polymerase (5 U/µL) (QIAGEN, Hilden, Germany), 0.5 µl dNTP Mix (10 mM), 0.2 µl of each primer (50 µM), 2.5 µl of 10 × Coral Load PCR buffer (15 mM $MgCl_2$ included), 1 µl extra $MgCl_2$ (25 mM) and 17.9 µl distilled water. Cycling parameters of this PCR included an initial denaturation at 95 °C for 5 min; then 35 cycles of denaturation at 95 °C for 40 s, annealing at 57 °C for 40 s and extension at 72 °C for 60 s. Final extension was performed at 72 °C for 7 min. Consequently, a longer, approx. 1700-bp-long part of the 18S rRNA gene was also targeted for sequencing and phylogenetic analysis (Criado-Fornelio et al., 2006; Hodžić et al., 2017). This PCR was run with the primers HAM-1F (5′-GCC AGT AGT CAT ATG CTT GTC-3′) and HPF-2R (5′-GAC TTC TCC TTC GTC TAA G-3′), using 0.5 µl of each primer (50 µM), 2.5 µl of 10 × Coral Load PCR buffer (15 mM $MgCl_2$ included), and 15.8 µl distilled water. Cycling parameters of this PCR included an initial denaturation at 95 °C for 5 min; then 40 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 1.5 min. Final extension was performed at 72 °C for 5 min. Sequence-verified positive controls (Hornok et al., 2022) were included in PCR analyses.

2.5. Sequencing and phylogenetic analysis

Purification and sequencing of the PCR products were performed by the EuroFins Biomi Ltd. (Gödöllő, Hungary). The newly generated sequence was submitted to GenBank under accession number PPV569832. For the phylogenetic analysis, sequences with 91–100 % coverage were used, and 1000 bootstrap replicates were performed. The trees were generated with the Neighbor-Joining method and p-distance model by the program MEGA version 11.0 (Tamura et al., 2021).

3. Results

3.1. Clinical and laboratory findings

The main complaints before presentation to the clinics were anorexia

(cases #1 and #3), weight loss and abdominal distension (case #2), and dyspnoea (case #3). Findings on physical examination were the following: Case #1 had pale mucous membranes and a palpable mid-abdominal mass. In case #2, a generalised lymphadenopathy and abdominal effusion were noted. Case #3 showed mild dehydration, slight pallor, dyspnoea and increased respiratory sounds.

In case #1, abdominal ultrasound revealed the palpable mass to be splenomegaly, along with a small amount of peritoneal effusion. In case #2, ultrasound found splenomegaly with diffuse effacement of normal architecture, multiple hepatic nodules, and a moderate amount of free abdominal fluid. For case #3, thoracic radiographs showed no overt abnormalities.

Haematological and biochemical parameters are shown in Table 1. Notable abnormalities were anaemia (all three cats), thrombocytopenia (case #1), neutropenia and lymphopenia (case #2), leucocytosis (case #3), hyperbilirubinemia (case #1), hyperproteinemia (cases #1 and #3), hypoalbuminemia (cases #1 and #3), and elevated creatine kinase and lactate dehydrogenase activities (case #1). Cats in cases #1 and #3 tested negative for both FIV and FeLV. Blood smear examination revealed that up to 0.3 % of neutrophil granulocytes contained *Hepatozoon* spp. gamonts in all cases.

In case #1, the blood smear detected atypical intermediate-to-large lymphoid cells, and cytology of the splenic aspirate was consistent with malignant round cell neoplasia, with splenic lymphoma considered highly probable. In case #2, cytology of the lymph node was interpreted as reactive lymphoid hyperplasia, and the abdominal fluid was classified as chylous effusion.

In all three cases, the owners elected euthanasia due to worsening clinical conditions, which in case #2 included the additional onset of dyspnoea. In case #3, the carcass was submitted for necropsy. A more detailed clinical description, and the treatments received by the cats are included in Additional file 1.

3.2. Post-mortem findings (case #3)

No macroscopic pathologies were detected in the abdominal cavity. Examination of the thorax revealed fluid-filled lungs and a thick mucoid discharge in the distal part of the trachea and lower airways. The heart's

Table 1
Haematological and biochemical parameters of the cats. Compared to the reference ranges, elevated parameters are marked in bold and with a "+", decreased values are marked in italics and with a "-". The dates below the case labels indicate the days of sampling. The empty cells in the table indicate that the given parameter was not measured at that time. Decreased platelet counts marked with a "*" are cases of pseudothrombocytopenia.

Parameter	Unit	Reference range	Value				
			Case 1		Case 2		Case 3
			21.03.2025.	24.03.2025.	14.03.2025	07.04.2025.	29.04.2025.
Leukocyte count	G/l	6.0–12.0	6.9	7.1	*1.4* -	*5.9* -	**14.7** +
Neutrophil granulocyte count	G/l	3.0–11.5	3.4	5.1	*1* -	4.9	11.3
Lymphocyte count	G/l	1.0–4.8	1.3	1	*0.3* -	*0.5* -	2.2
Monocyte count	G/l	< 1.4	**2.0** +	1	0.1	0.4	0.6
Eosinophil granulocyte count	G/l	0.1	0	0	0	0	0.6
Basophil granulocyte count	G/l	0.1	0	0	0	0	0
Red blood cell count	T/l	5.5–10.5	*3.0* -	*2.7* -	5.6	*4.2* -	7.2
Haemoglobin concentration	g/l	85–155	*54* -	*51* -	87	*74* -	103
Haematocrit	%	30–47	*16* -	*15* -	*28* -	*25* -	*26* -
MCV	fl	40–53	**55** +	**56** +	50	**59** +	*35* -
MCH	pg	13–18	**18.3** +	**18.8** +	15.5	17.7	14.3
MCHC	g/l	300–370	331	338	307	301	**404** +
Nucleated red blood cell count	G/l		0.02	0.04	0.01	0.05	0.01
Reticulocyte count	G/l	9–61		11		**65** +	22
Ret-He	pg	> 12		22		19	15
Platelet count	G/l	200–800	*3* -	*12* -	*12* -*	*111* -*	632
Aspartate aminotransferase	IU/l	0–50	**108** +		20		14
Alanine transaminase	IU/l	0–60	37		56		36
Alkaline phosphatase	IU/l	1–190	109		25		54
Gamma-glutamyl transferase	IU/l	< 10	1		< 1		< 1
Glutamate dehydrogenase	IU/l	< 10	8				
Total bilirubin	μmol/l	< 17	**17.7** +		< 10		< 10
Direct bilirubin	μmol/l	< 7	**12.4** +		< 5		< 5
Total protein	g/l	60–80	**82.3** +		65		**84.3** +
Albumin	g/l	25–45	*19* -		25.3		*23.2* -
Albumin/globulin ratio		0.5–1.5	*0.3* -		0.6		*0.4* -
Glucose	mmol/l	3.8–6.0	5.9		**6.4** +		4.7
Fructosamine	μmol/l	116–365	**373** +		186		188
Amylase	IU/l	200–1660	**3886** +				**2638** +
Lipase	IU/l	< 26					9
Cholesterol	mmol/l	2.0–6.5	6		*1.9* -		2.8
Triglycerides	mmol/l	0.21–1.65	0.81		0.33		0.39
Urea	mmol/l	5.0–12.5	8.4		9.2		6
Creatinine	μmol/l	40–190	96		121		129
Phosphorus	mmol/l	1.2–2.4	*0.9* -		1.2		*1.1* -
Sodium	mmol/l	135–155	147.3		**158.2** +		151.1
Potassium	mmol/l	3.6–5.2	4.3		4		4.6
Total calcium	mmol/l	2.0–3.0	2.1		2.4		2.4
Iron	μmol/l	6–40	**57.3** +		16.5		6.3
Creatine kinase	IU/l	20–290	**1252** +		88		260
Lactate dehydrogenase	IU/l	20–450	**1081** +		233		**506** +
hemolysis		< 45	13		14		14
lipaemia		< 120	13		6		5
icterus		< 2	1		0		0

Abbreviations: MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration.

right ventricle and atrium were severely dilated, and the ventricle wall was attenuated.

Microscopic examination of the lungs identified moderate alveolar edema and interstitial hyperemia in the caudal lobes, with mild multifocal interstitial fibrosis. Bronchi and bronchioli were occasionally filled with homogenous eosinophilic fluid. In the heart, there were signs of multifocal myocardial degeneration, both in the left and right ventricles. Edema, interstitial fibrosis, and disarray formation were mild to moderate. There was no sign of inflammation or malignancy. These microscopic lesions were compatible with chronic hypertrophic cardiomyopathy and related respiratory pathology.

3.3. Morphological and phylogenetic analyses

The mean size of gamonts in neutrophils was 12.3 ± 0.66 µm length, 6 ± 0.75 µm in width. The nucleus was elongated and polymorphic. The cytoplasm contained eosinophilic or basophilic granules (Fig. 1). Sequences from all cases were 100 % (1663/1663 bp) identical in the amplified part of the 18S rRNA gene. These sequences showed only 98.3 % (1634/1663 bp) and 97.7 % (1622/1661 bp) sequence identities with *H. felis* (GenBank: AY628681) and *H. silvestris* (GenBank: KX757032), respectively. Phylogenetically, this isolate clustered separately, with moderately high (87 %) support, from the classical (type) *H. felis* represented in the analysis by the Spanish genotype (AY628681) and was more closely related (as a sister species) to *H. silvestris* (Fig. 2). In a shorter part of the 18S rRNA gene, the *Hepatozoon* species in this study had 99.6 % (557/559 bp) sequence identity to *Hepatozoon luiperdjie*, and they clustered together phylogenetically, although with low (56 %) support (Fig. 3).

4. Discussion

This study reports for the first time, multiple cases of feline hepatozoonosis among domestic cats in a country that is situated north of the Mediterranean region. In Europe, to our knowledge, the concurrent presence of both genogroups of *H. felis* in the felid population was reported for the first time in Hungary (Hornok et al., 2022), underlining the epidemiological importance (hot-spot nature) of southern Central Europe in this context. Interestingly, compared to Hungary, *H. felis* was reported with lower prevalence among wildcats in Germany, but in the latter country, unlike in Hungary, *H. silvestris* was also detected in these hosts with high prevalence (Unterköfler et al., 2022).

The biological (ixodid tick) vector of *H. felis* is not known (Basso et al., 2019). Nevertheless, this parasite was molecularly detected in several tick species, as exemplified by *R. sanguineus* (Maia et al., 2014) and *Ixodes ricinus* (Aktas, 2014; Hornok et al., 2022). Both of these tick species are indigenous to Hungary (Hornok et al., 2020). However, in this study, no ticks were found on the cats with hepatozoonosis, although they were repeatedly exposed to tick bites as observed by the owner (case #1) or had outdoor access (in cases #2 and #3).

Hepatozoon felis was redescribed based on sequences reported from Israel and Spain (Baneth et al., 2013). Importantly, based on the phylogenetic analysis of the long 18S rRNA sequences in this study, the species emerging in domestic cats in Hungary clustered separately (with 87 % support) from the Spanish isolate representing *H. felis*. The morphological differences and phylogenetic clustering support that the species reported herein is distinct from *H. felis*. In particular, based on the degree of 18S rRNA sequence identity and the results of phylogenetic analyses, the protozoan parasite in the present study is most likely *H. luiperdjie*. This is supported by the morphology of the gamont, including its size (this study: 12.3 ± 0.66 µm, *H. luiperdjie*: 9.9–12.6 µm)

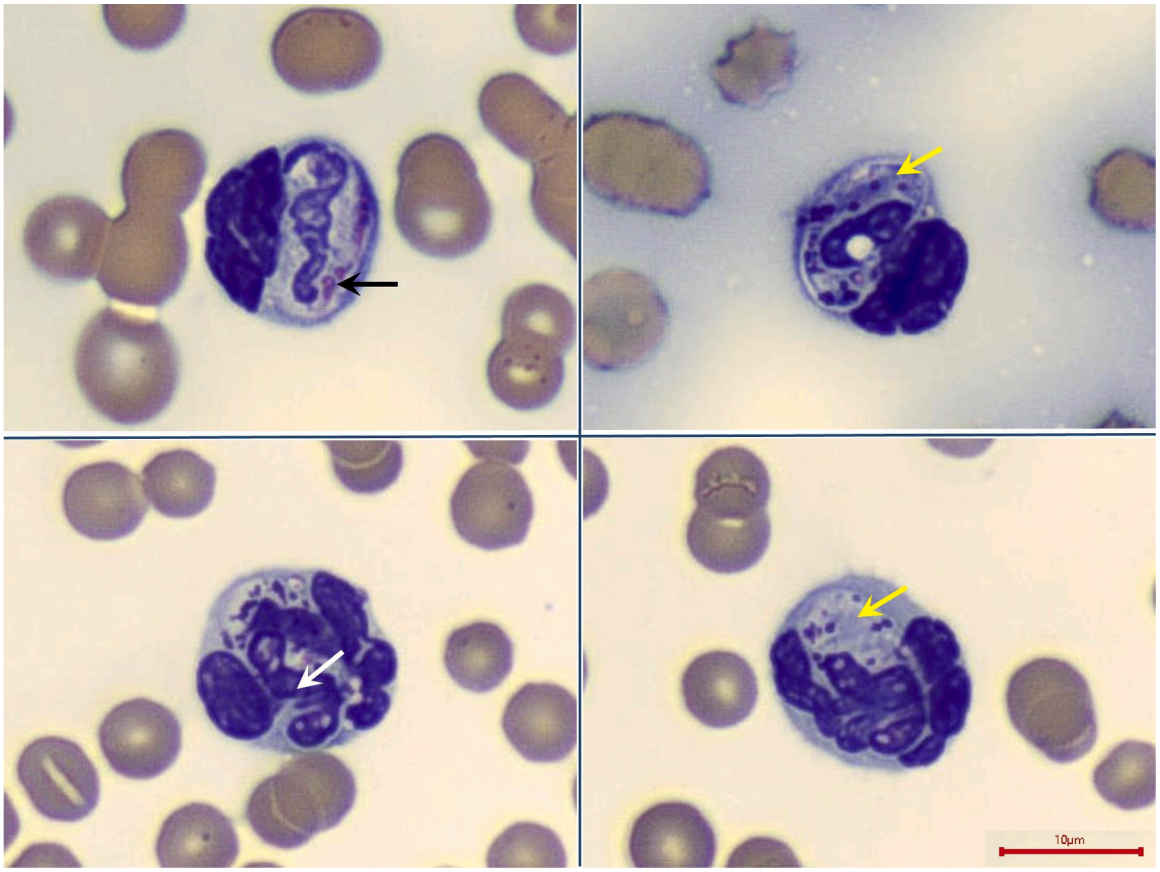

Fig. 1. Morphology of *Hepatozoon* gamonts (yellow arrows) in blood smear, stained with May-Grünwald Giemsa. Within the gamonts, white arrow indicates the nucleus, and black arrow marks eosinophilic granules.

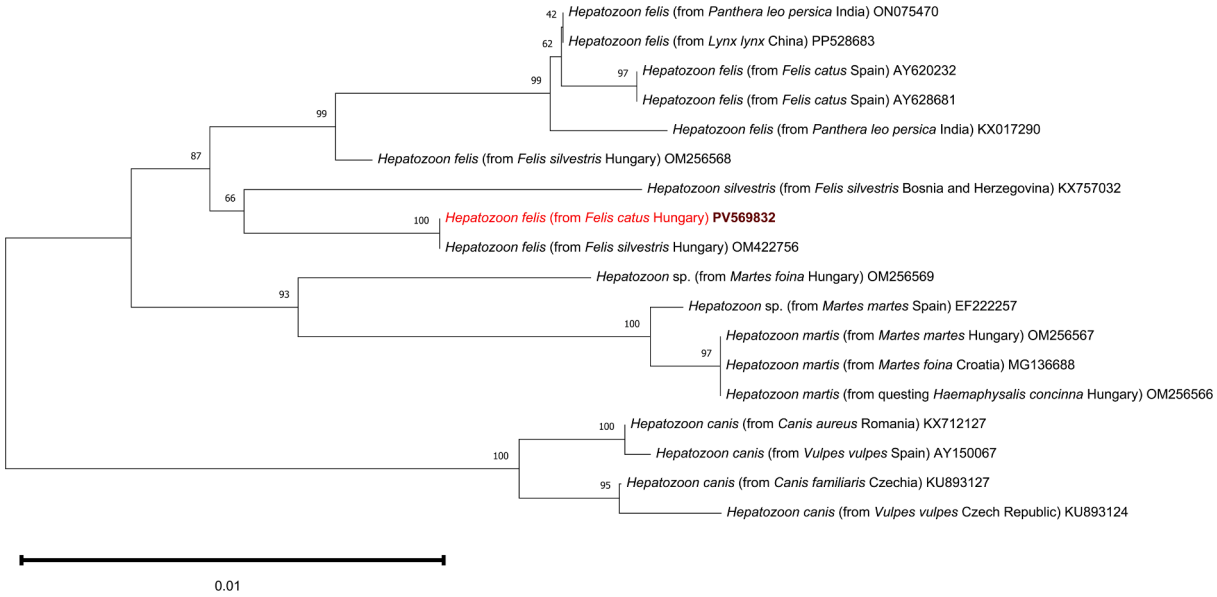

Fig. 2. Phylogenetic tree based on the long fragment of the 18S rRNA gene. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches. The evolutionary distances were computed using the p-distance model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 19 nucleotide sequences. There were a total of 1653 positions in the final dataset. Four sequences of *Hepatozoon canis* were used as outgroup.

and contents of eosinophilic and basophilic granules (van As et al., 2020).

This is in line with our previous data, discovering *H. felis* repeatedly from both of its genogroups among wild cats in Hungary (Hornok et al., 2022; Tuska-Szalay et al., 2023). Importantly, however, here in this study and previously (Tuska-Szalay et al., 2023), only one species representing one of the two genogroups was found in domestic cats. In summary, it was formerly suggested that the high genetic diversity characteristic of *H. felis* as reported in a broader context may in part also be explained by its taxonomic status, being a complex of at least two species (Cui et al., 2024; Hornok et al., 2022; Unterköfler et al., 2022).

Infection with *H. felis* is usually subclinical, without significant local inflammatory response in affected organs, and clinicopathological consequences tend to be more likely observed in immunocompromised cats (Baneth et al., 2013). Such a state could also result from neoplasia, as verified in case #1 and suspected in case #2. This might have also played a role in promoting detectable levels of parasitaemia. Similarly, while anaemia in case #1 may have been caused by the *Hepatozoon* species (see below), it could also result from neoplastic infiltration of the bone marrow (which is supported by presence of neoplastic cells in the peripheral blood and concurrently detected thrombocytopenia), or associative immune-mediated hemolytic anaemia (supported by hyperbilirubinemia demonstrated in this cat) (Garden et al., 2019). Although mild anaemia was also detected in blood samples from cats #2 and #3, these were all taken under anaesthesia, which might result in spuriously decreased hematocrit values in these animals (Dhumeaux et al., 2012).

Nevertheless, clinical signs and alterations in haematological-biochemical parameters as observed among cats involved in the current study may, at least in part, be attributable to *Hepatozoon*-infection, as reflected by literature data on *H. felis* and/or feline hepatozoonosis. These pathophysiological changes include: (1) anaemia (Baneth and Allen, 2022; Díaz-Regañón et al., 2017; Jones et al., 2024); (2) leukopenia (Basso et al., 2019); (3) enlargement and involvement of organs of haemolymphatic tissues, such as the spleen and the liver (Baneth, 2011; Baneth et al., 2013); and (4) elevated creatine kinase activity indicating muscle damage, i.e. infection of heart and skeletal muscles (Baneth et al., 1998; Baneth and Allen, 2022; Lloret et al., 2015). In addition, (5) the non-regenerative nature of anaemia in case #1 might also be explained by bone marrow involvement, known to be caused by *H. felis* (Díaz-Regañón et al., 2017; Lloret et al., 2015), and (6) lung involvement may explain dyspnoea in case #2 of this study, similarly to what has been reported (Baneth et al., 2013). Finally, (7) in case #3, *Hepatozoon*-infection might have played a role in cardiomyopathy, as reported in feline hepatozoonosis caused by *H. silvestris* (Kegler et al., 2018; Smith et al., 2025). However, in the present study, extravascular *Hepatozoon* developmental stages were not found.

In conclusion, based on sequencing a long portion of the 18S rRNA gene, and as reflected by morphological characteristics, this study revealed that the protozoan parasite emerging among domestic cats in the southern part of Central Europe is a species different from *H. felis*. Although in the present cases *Hepatozoon*-infection might have contributed to the pathophysiological changes, the extent of this is not known, because the role of other factors (e.g., neoplasia) cannot be ruled out. The emergence of this protozoan parasite in the region warrants further attention, in particular monitoring its presence in diagnostic samples of domestic cats.

Funding

Molecular work was supported by the Office for Supported Research Groups, Hungarian Research Network (HUN-REN), Hungary (Project No 1500107).

CRediT authorship contribution statement

Gergő Kürtös: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nóra Rácz:** Investigation, Data curation. **Viktória Kassay:** Investigation, Data curation. **Krisztián Olaszy:** Investigation, Data curation. **Zsuzsanna Vizi:** Validation, Methodology. **Anna Szilasi:** Investigation, Data curation. **Nóra Takács:** Methodology, Data curation. **Gergő Keve:** Methodology, Data curation. **Barbara Tuska-Szalay:** Methodology, Formal analysis. **Sándor Hornok:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Conceptualization.

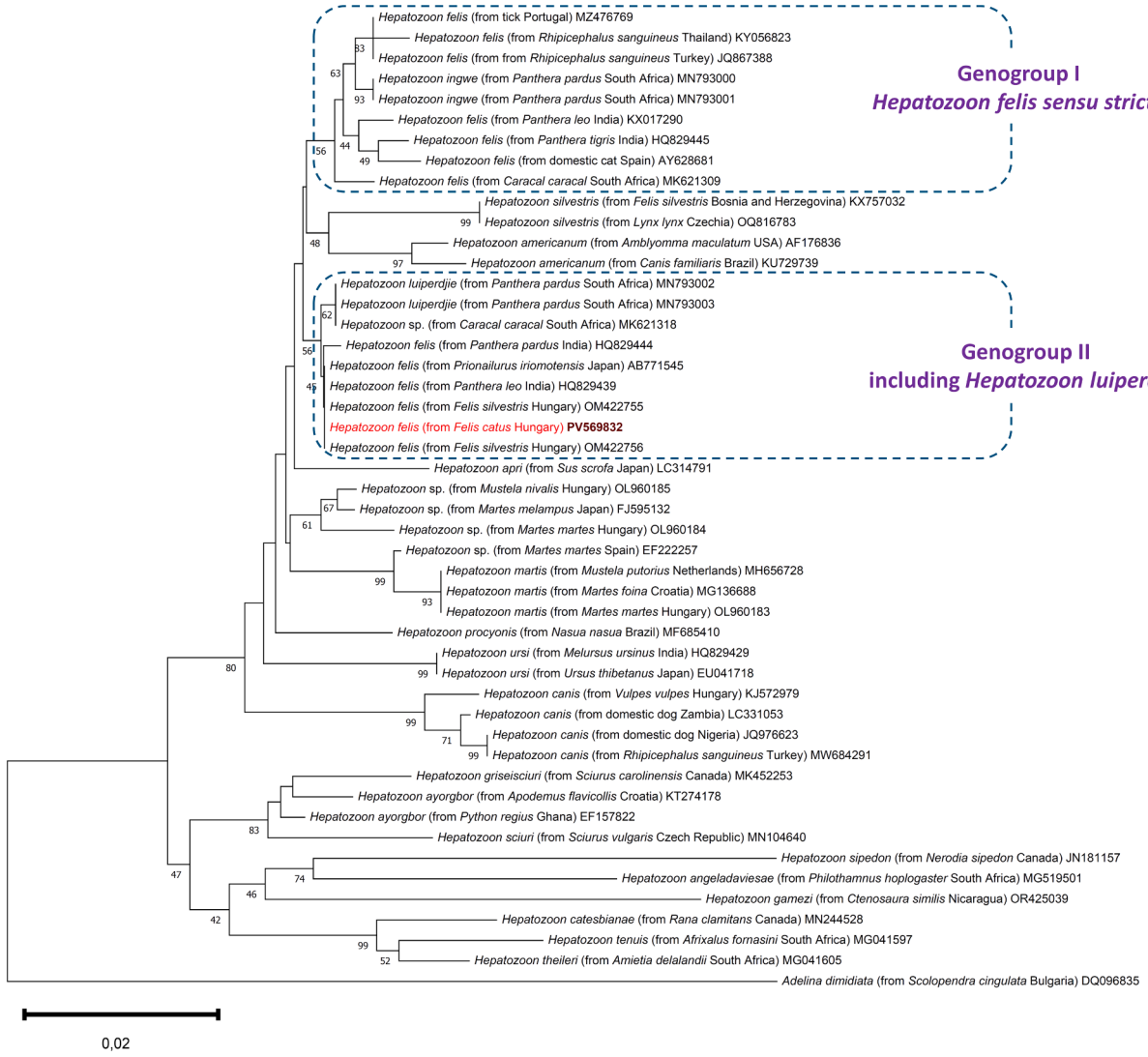

Fig. 3. Phylogenetic tree based on the shorter fragment of the 18S rRNA gene. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches. The evolutionary distances were computed using the p-distance model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 48 nucleotide sequences. There were a total of 567 positions in the final dataset. *Adelina dimidiata* was used as outgroup.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgements

The authors are grateful to Dr. Hegedűs György-Tamás for ensuring facilities for hematological and biochemical analyses.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2025.102556.

Data availability

All three sequences obtained during this study were identical, deposited in GenBank under the accession number PPV569832. All other relevant data are included in the manuscript and the supplementary material or are available upon request by the corresponding author.

References

Aktas, M., 2014. A survey of ixodid tick species and molecular identification of tick-borne pathogens. Vet. Parasitol. 200, 276–283. https://doi.org/10.1016/j.vetpar.2013.12.008.

Baneth, G., Aroch, I., Tal, N., Harrus, S., 1998. *Hepatozoon* species infection in domestic cats: a retrospective study. Vet. Parasitol. 79, 123–133. https://doi.org/10.1016/s0304-4017(98)00160-5.

Baneth, G., Mathew, J.S., Shkap, V., Macintire, D.K., Barta, J.R., Ewing, S.A., 2003. Canine hepatozoonosis: two disease syndromes caused by separate *Hepatozoon* spp. Trends Parasitol. 19, 27–31. https://doi.org/10.1016/s1471-4922(02)00016-8.

Baneth, G., 2011. Perspectives on canine and feline hepatozoonosis. Vet. Parasitol. 181, 3–11. https://doi.org/10.1016/j.vetpar.2011.04.015.

Baneth, G., Sheiner, A., Eyal, O., Hahn, S., Beaufils, J.P., Anug, Y., Talmi-Frank, D., 2013. Redescription of *Hepatozoon felis* (Apicomplexa: Hepatozoidae) based on phylogenetic analysis, tissue and blood form morphology, and possible transplacental transmission. Parasit. Vect. 6, 102. https://doi.org/10.1186/1756-3305-6-102.

Baneth, G., Allen, K., 2022. Hepatozoonosis of dogs and cats. Vet. Clin. North Am. Small Anim. Pract. 52, 1341–1358. https://doi.org/10.1016/j.cvsm.2022.06.011.

Basso, W., Görner, D., Globokar, M., Keidel, A., Pantchev, N., 2019. First autochthonous case of clinical *Hepatozoon felis* infection in a domestic cat in Central Europe. Parasitol. Int. 72, 101945. https://doi.org/10.1016/j.parint.2019.101945.

Carbonara, M., Iatta, R., Sgroi, G., Papadopoulos, E., Lima, C., Bouhsira, E., Miró, G., Nachum-Biala, Y., Baneth, G., Otranto, D., 2023. *Hepatozoon* species infecting domestic cats from countries of the Mediterranean basin. Ticks Tick Borne Dis 14, 102192. https://doi.org/10.1016/j.ttbdis.2023.102192.

Criado-Fornelio, A., Ruas, J.L., Casado, N., Farias, N.A., Soares, M.P., Müller, G., Brumt, J.G., Berne, M.E., Buling-Saraña, A., Barba-Carretero, J.C., 2006. New molecular data on mammalian *Hepatozoon* species (Apicomplexa: Adeleorina) from Brazil and Spain. J. Parasitol. 92, 93–99. https://doi.org/10.1645/GE-464R.1.

Cui, N., Su, L., Wang, Z., Hornok, S., Tang, L., Yang, M., Zhang, Y., Zhao, G., Wang, Y., 2024. First identification of *Cytauxzoon manul* in Eurasian lynx (*Lynx lynx*) in northwestern China. Parasit. Vect. 17, 249. https://doi.org/10.1186/s13071-024-06326-1.

Dhumeaux, M.P., Snead, E.C., Epp, T.Y., Taylor, S.M., Carr, A.P., Dickinson, R.M., Leis, M.L., 2012. Effects of a standardized anesthetic protocol on hematologic variables in healthy cats. J. Feline Med. Surg. 14, 701–705. https://doi.org/10.1177/1098612X12448588.

Díaz-Regañón, D., Villaescusa, A., Ayllón, T., Rodríguez-Franco, F., Baneth, G., Calleja-Bueno, L., García-Sancho, M., Agulla, B., Sainz, Á., 2017. Molecular detection of *Hepatozoon* spp. and *Cytauxzoon* sp. in domestic and stray cats from Madrid, Spain. Parasit. Vect. 10, 112. https://doi.org/10.1186/s13071-017-2056-1.

Dubey, J.P., Baneth, G., 2025. *Hepatozoon* infections in dogs and cats in USA: history, life cycle, and suggestions for uniform terminology for hepatozoonosis in endotherms and ectotherms. Vet. Parasitol. 334, 110408. https://doi.org/10.1016/j.vetpar.2025.110408.

Garden, O.A., Kidd, L., Mexas, A.M., Chang, Y.M., Jeffery, U., Blois, S.L., Fogle, J.E., MacNeill, A.L., Lubas, G., Birkenheuer, A., Buoncompagni, S., Dandrieux, J.R.S., Di Loria, A., Fellman, C.L., Glanemann, B., Goggs, R., Granick, J.L., LeVine, D.N., Sharp, C.R., Smith-Carr, S., Swann, J.W., Szladovits, B., 2019. ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats. J. Vet. Intern. Med. 33, 313–334. https://doi.org/10.1111/jvim.15441.

Hodžić, A., Alić, A., Prašović, S., Otranto, D., Baneth, G., Duscher, G.G., 2017. *Hepatozoon silvestris* sp. nov.: morphological and molecular characterization of a new species of *Hepatozoon* (Adeleorina: Hepatozoidae) from the European wild cat (*Felis silvestris silvestris*). Parasitology 144, 650–661. https://doi.org/10.1017/S0031182016002316.

Hornok, S., Tánczos, B., Fernández de Mera, I.G., de la Fuente, J., Hofmann-Lehmann, R., Farkas, R., 2013. High prevalence of *Hepatozoon*-infection among shepherd dogs in a region considered to be free of *Rhipicephalus sanguineus*. Vet. Parasitol. 196, 189–193. https://doi.org/10.1016/j.vetpar.2013.02.009.

Hornok, S., Kováts, D., Horváth, G., Kontschán, J., Farkas, R., 2020. Checklist of the hard tick (Acari: Ixodidae) fauna of Hungary with emphasis on host-associations and the emergence of *Rhipicephalus sanguineus*. Exp. Appl. Acarol. 80, 311–328. https://doi.org/10.1007/s10493-019-00461-6.

Hornok, S., Boldogh, S.A., Takács, N., Kontschán, J., Szekeres, S., Sós, E., Sándor, A.D., Wang, Y., Tuska-Szalay, B., 2022. Molecular epidemiological study on ticks and tick-borne protozoan parasites (Apicomplexa: *Cytauxzoon* and *Hepatozoon* spp.) from wild cats (*Felis silvestris*), Mustelidae and red squirrels (*Sciurus vulgaris*) in central Europe, Hungary. Parasit. Vect. 15, 174. https://doi.org/10.1186/s13071-022-05271-1.

Inokuma, H., Okuda, M., Ohno, K., Shimoda, K., Onishi, T., 2002. Analysis of the 18S rRNA gene sequence of a *Hepatozoon* detected in two Japanese dogs. Vet. Parasitol. 106, 265–271. https://doi.org/10.1016/s0304-4017(02)00065-1.

Jones, L., Lara, A.S., Peschard, L., McGregor, O., Skelly, B.J., 2024. Polyarthritis associated with clinical *Hepatozoon felis* infection in a domestic cat. Vet. Rec. Case Rep. 12, e914. https://doi.org/10.1002/vrc2.914.

Kegler, K., Nufer, U., Alic, A., Posthaus, H., Olias, P., Basso, W., 2018. Fatal infection with emerging apicomplexan parasite *Hepatozoon silvestris* in a domestic cat. Parasites Vectors 11, 428. https://doi.org/10.1186/s13071-018-2992-4.

Lloret, A., Addie, D.D., Boucraut-Baralon, C., Egberink, H., Frymus, T., Gruffydd-Jones, T., Hartmann, K., Horzinek, M.C., Hosie, M.J., Lutz, H., Marsilio, F., Pennisi, M.G., Radford, A.D., Thiry, E., Truyen, U., Möstl, K., 2015. European Advisory Board on Cat Diseases. Hepatozoonosis in cats: ABCD guidelines on prevention and management. J. Feline. Med. Surg. 17, 642–644. https://doi.org/10.1177/1098612X15589879.

Maia, C., Ferreira, A., Nunes, M., Vieira, M.L., Campino, L., Cardoso, L., 2014. Molecular detection of bacterial and parasitic pathogens in hard ticks from Portugal. Ticks Tick Borne Dis 5, 409–414. https://doi.org/10.1016/j.ttbdis.2014.01.009.

Pereira, C., Maia, J.P., Marcos, R., Luzzago, C., Puente-Payo, P., Dall'Ara, P., Faustino, A., Lauzi, S., 2019. Molecular detection of *Hepatozoon felis* in cats from Maio Island, Republic of Cape Verde and global distribution of feline hepatozoonosis. Parasit. Vect. 12, 294. https://doi.org/10.1186/s13071-019-3551-3.

Schäfer, I., Kohn, B., Nijhof, A.M., Müller, E., 2022. Molecular detection of *Hepatozoon* species infections in domestic cats living in Germany. J. Feline Med. Surg. 24, 994–1000. https://doi.org/10.1177/1098612X211055680.

Simonato, G., Franco, V., Salvatore, G., Manzocchi, S., Dotto, G., Morelli, S., Grillini, M., Cavicchioli, L., Gelain, M.E., Zini, E., 2022. First autochthonous clinical case of *Hepatozoon silvestris* in a domestic cat in Italy with unusual presentation. Parasit. Vect. 15, 440. https://doi.org/10.1186/s13071-022-05534-x.

Smith, T.G., 1996. The genus *Hepatozoon* (Apicomplexa: Adeleina). J. Parasitol. 82, 565–585. https://doi.org/10.2307/3283781.

Smith, R.C., Nolan, S., Harshbarger, A., Meinkoth, J., Scimeca, R.C., 2025. First report of *Hepatozoon silvestris* in a domestic cat (*Felis catus*) from New York. Vet. Parasitol. Reg. Stud. Reports 61, 101270. https://doi.org/10.1016/j.vprsr.2025.101270.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular Evolutionary Genetics Analysis Version 11. Mol. Biol. Evol. 38, 3022–3027. https://doi.org/10.1093/molbev/msab120.

Tuska-Szalay, B., Boldogh, S.A., Farkas, R., Rompos, L., Takács, N., Beresnyák, V., Izsó, Á., Kontschán, J., Lanszki, J., Hornok, S., 2023. Screening of domestic cats from North-Eastern Hungary for *Hepatozoon felis* and *Cytauxzoon europaeus* that cause infections in local wildcat populations. Pathogens 12, 656. https://doi.org/10.3390/pathogens12050656.

Uiterwijk, M., Vojta, L., Šprem, N., Beck, A., Jurković, D., Kik, M., Duscher, G.G., Hodžić, A., Reljić, S., Sprong, H., Beck, R., 2023. Diversity of *Hepatozoon* species in wild mammals and ticks in Europe. Parasit. Vect. 16, 27. https://doi.org/10.1186/s13071-022-05626-8.

Unterköfler, M.S., Harl, J., Barogh, B.S., Spergser, J., Hrazdilová, K., Müller, F., Jeschke, D., Anders, O., Steinbach, P., Ansorge, H., Fuehrer, H.P., Heddergott, M., 2022. Molecular analysis of blood-associated pathogens in European wildcats (*Felis silvestris silvestris*) from Germany. Int. J. Parasitol. Parasites Wildl. 19, 128–137. https://doi.org/10.1016/j.ijppaw.2022.08.012.

van As, M., Netherlands, E.C., Smit, N.J., 2020. Molecular characterisation and morphological description of two new species of *Hepatozoon* Miller, 1908 (Apicomplexa: Adeleorina: Hepatozoidae) infecting leukocytes of African leopards *Panthera pardus pardus* (L.). Parasit. Vect. 13, 222. https://doi.org/10.1186/s13071-020-3933-6.