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Molecular detection of *Hepatozoon* species infections in domestic cats living in Germany

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Abstract

Objectives Three species of protozoal *Hepatozoon* species (*H felis*, *H canis* and *H silvestris*) are known to infect cats in Europe. The objective of this study was to determine the prevalence of *Hepatozoon* species in samples from cats living in Germany that were submitted to a veterinary laboratory.

Methods The study included cats tested for *Hepatozoon* species by PCR between 2007 and 2020 by the Laboklin laboratory. Travel history and haematological results were documented for cats with positive test results. From 2018 onwards, a partial 18S rRNA *Hepatozoon* gene fragment was sequenced from cats with positive PCR results.

Results Sixty-four of 931 cats (7%) tested positive for *Hepatozoon* species. Sex and age did not have a statistically significant impact. Sequencing was carried out for 16 samples and revealed *H felis* in all cases. All cats with positive test results and a relevant travel history had been imported from the Mediterranean or south-eastern Europe. There were no autochthonous infections with *Hepatozoon* species. Leukocytosis, haemoconcentration and anaemia were the most common haematological abnormalities.

Conclusions and relevance Although infections with *Hepatozoon* species in cats are usually subclinical, it may be useful to screen cats imported from the Mediterranean and south-eastern Europe for these pathogens to prevent local transmission cycles. There was no evidence of autochthonous infections in Germany; however, further investigations regarding a possible transmission of *Hepatozoon* species from infected cats to blood-feeding arthropods in Germany may be of interest. To avoid potential spread of the pathogens, ectoparasite prophylaxis is advisable.

Keywords: Vector-borne infection; tick-borne infection; hepatozoonosis; PCR

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Introduction

Hepatozoon species are protozoal, apicomplexan vector-borne infectious pathogens transmitted by blood-feeding arthropods to mammals, birds, reptiles and amphibians.[1–4] In dogs, this pathogen is transmitted by ingestion of the hard tick *Rhipicephalus sanguineus* sensu lato (s.l.) infected with oocysts.[3] Neither vectors nor pathogenesis have been established in cats.[5] Therefore, other modes of *Hepatozoon* species transmission that do not involve vectors, such as transplacental infections of *Hepatozoon canis* and *Hepatozoon felis* and meat consumption, cannot be excluded.[1,6]

In domestic cats, infection with *Hepatozoon* species was first reported in India in 1908.[7] There have been numerous reports worldwide since then. In Europe, the main pathogen in domestic cats is *H felis*,[1,8–12] followed by *H canis* and *Hepatozoon silvestris*.[1,13,14] These pathogens are found mainly in cats in the Mediterranean and south-eastern Europe and can be detected by PCR (Table 1). Examples include *H felis* infections in cats from Italy,[13,15] Spain,[12,21,22] Portugal[9,10] and Cyprus,[8,11] and *H canis* infections in cats from Italy,[13] Spain[12,26] and France.[24] *Hepatozoon*

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Table 1 Reported prevalence of *Hepatozoon* species infections as determined by PCR in European cats

Country	Region	Number of tested cats	Time period	Number of positive cats (%)	Species differentiation	Publication
Italy	North-eastern Italy	103 client-owned cats and 55 stray cats	–	26/158 (16.5)	*H silvestris* (n = 16), *H felis* (n = 10)	Grillini et al (2021)[15]
	Northern Italy (Bologna and Rimini)	85 stray cats	February 2018 to October 2019	0/85	–	Ebani et al (2020)[16]
	Italy	958 client-owned cats	–	0/958	–	Latrofa et al (2020)[17]
	Southern Italy (Sicily and Calabria)	197 client-owned cats (134 outdoor cats without ectoparasitic treatment, 63 indoor cats with ectoparasitic treatment)	March 2012 to March 2013	0/197	–	Persichetti et al (2018)[18]
	Aeolian Islands	330 client-owned outdoor cats	January 2015 to June 2016	1/330 (0.3)	*H felis*	Otranto et al (2017)[19]
	Southern Italy (Bari, Lecce and Matera)	196 cats	–	10/196 (5.1)	*H canis* (n = 8), *H felis* (n = 1), *H canis* (n = 1)	Giannelli et al (2017)[13]
	Southern Italy (Sicily and Calabria)	42 cats with tick/flea infestation (22 ill cats, 20 apparently healthy cats)	March 2012 to January 2013	0/42	–	Persichetti et al (2016)[20]
Spain	Madrid area	644 cats (506 client-owned cats, 138 stray cats)	September 2005 to December 2008	10/644 (1.6)	*H felis* (n = 9), *H canis* (n = 1)	Diaz-Reganon et al (2017)[12]
	Barcelona area	100 client-owned cats (52 ill cats, 48 apparently healthy cats)	January 2006 to December 2006	4/100 (4)	*H felis* (n = 4)	Tabar et al (2008)[21]
	Barcelona	25 stray cats	–	4/25 (16)	*H felis* (n = 4)	Ortuno et al (2008)[22]
Portugal	Southern Portugal (Lisbon, Setubal and Faro)	649 cats (320 client-owned cats, 329 stray cats)	January 2012 to August 2013	56/649 (8.6)	*H felis* (n = 13)	Maia et al (2014)[10]
	Northern and central Portugal	320 client-owned cats	–	50/320 (15.6)	*H felis* (n = 4)	Vilhena et al (2013)[9]
Greece	–	Client-owned cats and stray cats	–	72/282 (25.5)	*H felis* (n = 35)	Morelli et al (2021)[23]
France	–	116 ill cats suspicious for vector-borne disease	December 2006 to July 2007	2/116 (1.7)	*H canis* (n = 2)	Criado-Fornelio et al (2009)[24]
Cyprus	–	174 cats (138 client-owned cats, 36 shelter feral cats; 131 ill cats, 43 apparently healthy cats)	March 2014 to September 2014	66/174 (37.9)	*H felis* (n = 12)	Attipa et al (2017)[11]
Bosnia and Herzegovina	–	18 adult wild felids (*Felis silvestris silvestris*), tissue samples	2011–2016	6/9 (67)	*H silvestris, H felis, Hepatozoon* species	Hodzic et al (2018)[25]

silvestris was reported in a feral cat (*Felis silvestris silvestris*) in Bosnia and Herzegovina,[2] and in cats from Italy.[13,15] First reports of autochthonous infections in central Europe were recently published, including infections with *H silvestris* in Switzerland[14] and *H felis* in Austria.[27]

In Germany, *Hepatozoon* species were detected in 53/618 cats (9%) tested by PCR in the context of a 'feline travel profile' conducted in the Laboklin laboratory (Bad Kissingen, Germany).[28] In addition to *Hepatozoon* species (PCR), this diagnostic panel tests for *Dirofilaria* species (PCR), *Leishmania* species (immunofluorescence antibody test [IFAT]), *Ehrlichia* species (IFAT) and – as of July 2015 – *Rickettsia* species (IFAT). Foxes may act as potential reservoirs for *H canis* in Germany. In the federal state Berlin-Brandenburg, *Hepatozoon* species were detected by PCR in the splenic tissue of 176/201 foxes (87.6%). Sequencing revealed *H canis* in 56/176 positive tested samples, of which some were identical to the *H canis* genotypes found in dogs.[29]

Knowledge about feline vector-borne infectious agents is limited compared with dogs, possibly due to a lack of awareness and the fact that infections are mostly subclinical in cats.[1,13] The aims of this study were to assess the frequency of positive test results for *Hepatozoon* species in cats living in Germany, to gain knowledge about the *Hepatozoon* species involved and to examine the aetiology of feline *Hepatozoon* species infections.

Materials and methods

The Laboklin database was searched for PCR test results (TaqMan real-time PCR, target: 18S rRNA) for *Hepatozoon* species between January 2007 and December 2020 (Table 2). Additional haematological data from a complete blood count (CBC) was collected for cats with positive test results (Laboklin; ADVIA 2120i [Siemens Healthineers]; Sysmex XT 2000iv [Sysmex Deutschland]), as well as antigen testing for feline leukaemia virus (FeLV; NovaTec VetLine Feline Leukemia Virus Antigen ELISA [NovaTec Immundiagnostica]) and antibody-testing for feline immunodeficiency virus (FIV; NovaTec VetLine Feline Immunodeficiency Virus ELISA [NovaTec Immundiagnostica]).

Age groups were defined as 'kitten' (≤6 months), 'junior' (>6 months to 3 years), 'adult' (>3–7 years), 'mature' (>7–11 years), 'senior' (>11–15 years) and 'geriatric' (>15 years) (Table 3).

In cats that tested positive for *Hepatozoon* species between 2018 and 2020, an additional *Hepatozoon* species PCR targeting a ~562 base pair fragment of the 18S rRNA gene was performed. This PCR was performed in a 25 µl reaction volume consisting of 0.5 U Fusion S7 polymerase (Mobidiag), 5 µl 5 × HF buffer, 200 µM of each dNTP, 10 pmol primer HepF (5'-TAC ATG AGC AAA ATC TCA AC-3') and primer HepR (5'-CTT ATT ATT CCA TGC TGC AG-3'), and 2.5 µl template DNA. PCR cycle parameters included an initial denaturation at 98°C for 30 s, followed by 40 cycles of 98°C for 7 s, 57°C for 10 s, 72°C for 15 s and a final extension at 72°C for 5 mins. PCRs were performed in a C1000 thermal cycler (Bio-Rad Laboratories). Samples with positive PCR results were subsequently sequenced by LGC Genomics. Veterinarians were contacted about travel history of cats that tested positive. The statistical analysis was performed using SPPS for Windows (version 27.0; IBM). Data were checked for normal distribution by Kolmogorov–Smirnov test. Mann–Whitney U-test was used to calculate statistical significance ($P < 0.05$) in non-normally distributed data.

Table 2 Results of the *Hepatozoon* species PCR in cats at Laboklin (Bad Kissingen, Germany) from 2007 to 2020

Year	No. of tested cats (n = 931)	No. of negative cats (n = 867)	No. of positive cats (n = 64)
2007	6 (0.6)	6 (100)	0 (0)
2008	9 (1.0)	8 (88.9)	1 (11.1)
2009	31 (3.3)	31 (100)	0 (0)
2010	16 (1.7)	16 (100)	0 (0)
2011	18 (1.9)	18 (100)	0 (0)
2012	42 (4.5)	39 (92.9)	3 (7.1)
2013	42 (4.5)	37 (88.1)	5 (11.9)
2014	67 (7.2)	62 (92.5)	5 (7.5)
2015	59 (6.3)	53 (89.8)	6 (10.2)
2016	103 (11.1)	96 (93.2)	7 (6.8)
2017	104 (11.2)	96 (92.3)	8 (7.7)
2018	104 (11.2)	94 (90.4)	10 (9.6)
2019	155 (16.6)	143 (92.3)	12 (7.7)
2020	175 (18.8)	168 (96.0)	7 (4.0)

Data are n (%). TaqMan real-time PCR, target: 18S rRNA. In 16 positive tested cats from 2018 to 2020, species differentiation revealed *H felis* infections

Table 3 Age distribution in cats tested by *Hepatozoon* species PCR at Laboklin from 2007 to 2020

Age	No. of tested cats (n = 931)	No. of negative cats (n = 867)	No. of positive cats (n = 64)
Kitten (<6 months)	34 (4.3)	27 (79.4)	7 (20.6)
Junior (>6 months to 3 years)	450 (57.3)	418 (92.9)	32 (7.1)
Adult (>3–7 years)	140 (17.8)	128 (91.4)	12 (8.6)
Mature (>7–10 years)	90 (11.5)	85 (94.4)	5 (5.6)
Senior (>11–14 years)	54 (6.9)	52 (96.3)	2 (3.7)
Geriatric (>15 years)	18 (2.3)	18 (100)	0 (0)
Unknown	145 (15.6)	139 (95.9)	6 (4.1)

Data are n (%)

Results

A total of 931 cats were tested by *Hepatozoon* species PCR from 2007 to 2020. The breed was known in 901/931 cats (96.8%). The majority were European Shorthair cats (n = 708 [78.6%]), followed by mixed breeds (n = 99 [11%]), Siamese cats (n = 22 [2.4%]) and Maine Coone (n = 15 [1.7%]). The sex was known in 856/931 cats (91.9%), of which 456 were male (53.3%) and 400 were female (46.7%). Age was known for 786/931 cats (84.4%), with a mean of 3.94 ± 3.94 years (median 2, range 0.2–22). Data regarding years of sample submission, sex and age groups were not normally distributed in PCR-positive and PCR-negative tested groups ($P < 0.01$ each). There were no statistically significant differences regarding *Hepatozoon* species PCR test results and years ($U = 27018.00$, $Z = -0.353$, $P = 0.724$), sex ($U = 27501.00$, $Z = -0.123$, $P = 0.902$) or age group ($U = 18200.00$, $Z = -1.951$, $P = 0.051$) using Mann–Whitney U test. Cats aged <3 years tested most frequently positive (39/64 positive tested cats [60.9%]; Table 3).

Sixty-four of 931 cats (6.9%) had positive PCR results for *Hepatozoon* species (Table 2). A travel history was available in 47/64 positive tested cats (73.4%). These cats were imported from Spain (n = 22), Greece (n = 12), Romania, Cyprus, Turkey and Morocco (n = 2 each), and Malta, Portugal, Bulgaria, Israel or Dubai (n = 1 each). In total, 618/931 cats (66.4%) were tested as part of the 'feline travel profile'. Fifty-two of these 618 cats tested positive for *Hepatozoon* species. Of these 52 cats, 10 (19.2%) also tested positive for additional pathogens (*Leishmania* species IFAT, n = 4; *Ehrlichia* species IFAT, n = 3; *Rickettsia* species IFAT, n = 2; *Leishmania* species and *Rickettsia* species IFAT, n = 1). The remaining 315/931 cats (33.8%) were not screened for possible coinfections. Eighteen of 64 cats (28.1%) with positive PCR results for *Hepatozoon* species were tested for FeLV antigen; all tested negative. Seventeen of 64 cats (26.6%) were tested for FIV antibodies, one of which (5.9%) was positive.

DNA was available from 26 cats with positive PCR results between 2018 and 2020. Sequencing was successful in 16 of these samples. The resulting sequences were 98.7–99.7% identical to the 18S rRNA gene of *H felis* from Spain (GenBank Accession Number AY620232). Thirteen of these 16 cats had a travel history and were imported to Germany from different countries (Spain, n = 7; Romania, n = 2; Greece, n = 2; Israel, n = 1; Morocco, n = 1). These data were not available for the remaining three cats.

In total, haematology was available for 12/64 cats with positive PCR results for *Hepatozoon* species. *Hepatozoon felis* was identified by sequencing in 6/12 cats. The 'feline travel profile' had been conducted in 11/12 cats, with negative results for *Dirofilaria* species (PCR), *Leishmania* species (IFAT), *Ehrlichia* species (IFAT) and *Rickettsia* species (IFAT). CBC was unremarkable in six cats (four of which were infected with *H felis*) and showed haemoconcentration and mild anaemia (haematocrit [Hct] 29%, n = 2; 26%, n = 1; 23%, n = 1) for four cats each. One cat tested positive for *H felis*, as well as *Leishmania* species (IFAT) and was severely anaemic (Hct 9%). Leukocytosis was detected in five cats (mildly elevated white blood cell count [WBC], n = 3 [12.6–13 × 10^9/l]; moderately elevated WBC, n = 2 [26.5 × 10^9/l and 32.4 × 10^9/l, respectively]), while one cat was mildly leukopenic (WBC 5.5 × 10^9/l). In two cats, a mild thrombocytopenia was detected (142 × 10^9/l and 101 × 10^9/l, respectively).

Discussion

Sixty four of 931 cats (6.9%) tested positive for *Hepatozoon* species by PCR and *H felis* was detected by sequencing in 16 cats. A background travel history was available for 13 of these cats; all of them had been imported to Germany from Mediterranean countries. There are reports of autochthonous infections with *Hepatozoon* species in central Europe, namely with *H felis* in one cat from Austria[27] and with *H silvestris* in one cat from Switzerland.[14] The apparent lack of autochthonous infections with *Hepatozoon* species in cats in Germany is therefore interesting, as is the fact that only *H felis* was documented. Autochthonous infections with *Hepatozoon* species in Germany may be relatively unlikely in the cats tested by a 'feline travel profile' in this study, as the choice of the diagnostic panel implies the presence of stays outside of Germany in the majority of tested cats. Nevertheless, vector contact and infections with *Hepatozoon* species in Germany cannot be excluded in these individuals. While there are reports that foxes in Germany tested positive for *H canis* with genotypes typically found in dogs,[29] there do not seem to be records of infections with *Hepatozoon* species commonly found in cats, such as *H felis* and/or *H silvestris*. Foxes in Germany may therefore not currently represent an important reservoir for *H felis* and *H silvestris*. According to the results of our study, *H felis* and *H silvestris* do not currently appear to be endemic or cause autochthonous feline infections in Germany, but this should be investigated further.

Hepatozoon felis has previously been detected in *R sanguineus* s.l.,[10] *R turanicusi*,[30] *Ixodes ricinus*,[31] *Ixodes hexagonus*,[32] *Haemaphysalis erinacei*[33] and *Haemaphysalis sulcata*.[31] Of these ticks, *I ricinus* and *I hexagonus* are widespread in Germany, while the occurrence of *R sanguineus* is mainly linked to importation from other countries and is currently not considered endemic in Germany.[34] It remains unclear whether these tick species contribute to the spread of the pathogen in Germany, especially considering that transmission routes and the pathogenesis of *Hepatozoon* species in cats are as yet unknown.

The incidence of infected cats appears to be increasing since 2012. This finding may be linked to the introduction of a so-called 'feline travel profile' as a screening test for travelling and imported cats offered by LABOKLIN from April 2012 onwards. Other possible explanations are a rising awareness of feline vector-borne pathogens as potential differential diagnoses by owners and veterinarians or an increase in the number of imported cats.[28] Spain and Greece were the most prominent countries of origin

in cats imported to Germany. Prevalences of 1.6–16% (Spain) and 25.5% (Greece) were reported, with *H felis* being the species most frequently documented (Table 1). This is consistent with the findings of this study, in which only *H felis* was detected.

Sex and age were not found to be significantly associated with the presence of *Hepatozoon* species in cats in Israel.[1] This study has similar results, although particularly young cats (<3 years of age) tested positive. A relatively high number of positive tested cats (21%) were younger than 6 months of age (Table 3), which may suggest potential vertical transmission from queens to litters.[1] Of the seven affected kittens, three were imported from Greece and two from Spain. The country of origin for the other two kittens could not be identified. The majority of infected cats in a study from Greece were from an isolated colony in Mykonos, where tick infestations were not observed at the time of the study, suggesting that transplacental transmission might be the primary route of transmission in these cats.[23] It should also be noted that the exact age of imported cats is often unknown and thus estimated by veterinarians or owners.

Studies have observed a correlation between *Hepatozoon* species infections and living outdoors, which may suggest ectoparasite contact as a predisposing factor.[1,5,27] The removal of ticks as part of the grooming behaviour in cats may be responsible for vector ingestion and influence the transmission of *Hepatozoon* species in cats.[23] Most cats imported to Germany from the Mediterranean are likely to be stray cats without previous veterinary care or parasite prophylaxis. It is therefore highly recommended to screen such cats for vector-borne pathogens, not only for the sake of the individual animal, but also to take precautions to prevent the possible local spread of these pathogens.

Coinfections were detected in 10/52 cats tested by the 'feline travel profile'. In the majority of cats with coinfections, antibodies for *Leishmania* species were detected by IFAT.[28] This highlights the importance of screening cats infected with *Hepatozoon* species for coinfections with other vector-borne pathogens. Reports on the impact of FeLV and FIV infections in cats infected with *Hepatozoon* species differ. One study showed no association regarding FIV infections,[1] whereas other studies have reported an association with FeLV and FIV.[22,35,36] In this study, all 18 cats tested for FeLV were negative. Seventeen cats were tested for FIV, of which one tested positive.

Haematological results were available for 12/64 cats that tested positive for *Hepatozoon* species. CBC was unremarkable in 6/12 cats. This is consistent with the literature, which reports mostly subclinical infections with *H felis*.[1,5,13] The first case report to describe an autochthonous infection with *H felis* in a cat in central Europe (Austria) noted haematological abnormalities (pancytopenia) with concurrent kidney and liver disease. No coninfections and other causes for immunosuppression were detected, suggesting some significance of *H felis* as a role as primary pathogen in cats.[27] In this study, there were no cases of pancytopenia; however, anaemia, leukopenia and thrombocytopenia occurred separately. In a domestic cat infected with *H silvestris* in Switzerland, the only haematological abnormality was a mild thrombocytopenia.[14] Thrombocytopenia in cats is to be interpreted with caution owing to the influence of impedance measurements by platelet aggregation, the size of feline platelets and therefore inadequate separation of erythrocytic and thrombocytic cells, all of which can lead to falsely low values.

The limitations of the study are associated with its retrospective study design. Owing to limited data availability, it was not possible to consider reasons for PCR testing (eg, screening or clinical signs). Additionally, haematological data were only available for some infected cats and biochemistry was not included at all.

Conclusions

The pathogenesis of feline hepatozoonosis is not yet clear. Owing to a positive association between an outdoor lifestyle and *Hepatozoon* infection, blood-sucking arthropods are suspected vectors. In addition, transplacental infections may also play an important role. The epidemiology of *Hepatozoon* species infections in cats in central Europe requires further monitoring. Owing to the relatively high number of cats infected with *Hepatozoon* species in some regions, imported cats from endemic countries should be screened for vector-borne pathogens such as *Hepatozoon* species. Ectoparasite prophylaxis is recommended in cats, not only to protect the individual animal, but also to avoid the possible establishment of a local transmission route by arthropod vectors in non-endemic countries such as Germany.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval This work involved the use of non-experimental animals only (including owned or unowned animals and data from prospective or retrospective studies). Established internationally recognised high standards ('best practice') of individual veterinary clinical patient care were followed. Ethical approval from a committee was therefore not specifically required for publication in *JFMS*.

Informed consent This work did not involve the use of animals (including cadavers) and therefore informed consent was not required. No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

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