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Hepatozoon canis in three imported dogs: a new tick-borne disease reaching the United Kingdom

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ABSTRACT

An increasing number of non-endemic vector-borne pathogens have been described in dogs imported to the UK in the past two decades. Recently, an outbreak of canine babesiosis in south-east England has raised veterinary awareness with regard to the impact of such diseases on the UK canine population. Canine hepatozoonosis, caused by *Hepatozoon canis* and transmitted by the ingestion of *Rhipicephalus sanguineus* ticks, is widespread in the Mediterranean basin. Herein we describe the first three molecularly confirmed clinical cases of canine hepatozoonosis in dogs imported into the UK. Veterinarians in the UK should be aware of *H. canis* as a potential infection in imported dogs, especially in the face of the expanding distribution of *R. sanguineus* ticks in Europe.

Keywords: hepatozoonosis, *Hepatozoon canis*, dog, canine tick-borne pathogens, imported disease, UK

Introduction

Hepatozoon canis (Apicomplexa, Adeleorina, Hepatozooidae) is a tick-borne pathogen that belongs to a diverse group of parasites which includes approximately 340 species that infect a wide range of vertebrates, such as mammals, birds, and reptiles (1). Canine hepatozoonosis was first described in India by a British medical officer in 1905 (2) and since then has been identified worldwide, with *H. canis* and *Hepatozoon americanum*, being of clinical importance for dogs (3). These two species differ in geographical distribution, pathogenicity and definitive invertebrate host (4). *Hepatozoon americanum*, is found in the Southern USA and causes severe, and often fatal, disease whereas *H. canis* is present in tropical and sub-tropical areas globally (5).

The life cycle for *H. canis* begins with the ingestion of infected ticks, containing sporulated oocysts, by the canine host. Sporozoites are released in the gut, penetrate the intestinal epithelium, and disseminate via lymphatics or blood vessels to the haemolymphatic tissues (including bone marrow, spleen, and lymph nodes) where they undergo merogony. Merozoites are subsequently released and invade leukocytes (neutrophils and monocytes) forming gamonts. Gamonts are ingested by ticks during blood feeding, undergo a sexual stage, and form oocysts (4, 5). While *Rhipicephalus sanguineus* (brown dog tick) is considered to be the main vector of *H. canis*, other tick species have been confirmed as definitive vectors for this parasite including *Amblyomma ovale* and *Rhipicephalus turanicus* (6, 7). Transplacental infections of *H. canis* have also been reported (8), and a recent case-control study, using structural equation modelling, found that younger dogs are more likely to be infected with *H. canis* compared to adult dogs (9). Interestingly, *H. americanum* may additionally be spread via ingestion of prey containing the cystozoite stages of the parasite. However this mode of transmission has not been evaluated for *H. canis* (4).

Clinical signs of *H. canis* relate to the severity of the parasite burden. It frequently causes a chronic sub-clinical infection. Dogs commonly may have a low parasite burden (<1%

of neutrophils containing gamonts) and be asymptomatic or show only mild clinical signs, whereas more severe clinical signs including fever, lethargy and emaciation are noted with high parasite burdens (4, 10, 11). In published case reports of dogs suffering from clinical signs of *H. canis*, the percentage of neutrophils containing gamonts varied from 21% to 90% (12-14). The commonly reported periostitis caused by *H. americanum* has also occasionally been reported with *H. canis*, and can be associated with skeletal and muscle pain (8, 14, 15).

The most common haematological abnormalities associated with *H. canis* infection include mild anaemia and neutrophilia, while rare extreme leukocytosis (up to 150 $x10^9$/L leukocytes) can occur with high parasitaemia (12-14, 16, 17). Serum biochemistry abnormalities typically include hyperproteinaemia with hyperglobulinaemia, hypoalbuminaemia, and increased activities of creatine kinase and alkaline phosphatase (4, 17).

Infection of dogs with *H. canis* has been recognised in Asia (13), Europe (18), the Mediterranean basin (19-21), the Middle East (17, 22), South America (23), and in the southern states of the USA in North America (24). Most recently, *H. canis* was unexpectedly identified for the first time in Queensland, Australia, in an *Ixodes holocyclus* Neumann tick collected from a dog, and the Australian biosecurity authorities are investigating the potential sources of this infection (25). The first known case of canine hepatozoonosis in the UK was presented in 2011 at the European Society of Veterinary Clinical Pathology congress in a dog imported from Ireland (26). Here we further evaluate this case using phylogenetic analysis, and we report two additional clinical cases of this infection imported from Cyprus.

Case 1

A 12-year-old, entire male, Beagle, was presented in September 2010 to a veterinary practice in London, UK, having been acquired from a rescue centre in Ireland. There was no clinical history available from prior to the Irish rescue centre and no microchip or tattoo was present.

92 The dog was presented on the 14th of September 2010 (Day 1), was thin but bright and
93 alert. Significant clinical findings included pale mucous membranes, a slightly enlarged
94 prostate (presumed to be benign prostatic hyperplasia), occasional cough, slight nasal
95 discharge and positive tracheal pinch. Haematology results are shown in Table 1. On Day 1,
96 the dog had a mild to moderate, normocytic, normochromic, non-regenerative anaemia. On
97 blood smear examination moderate numbers of neutrophils contained intracytoplasmic
98 elliptical structures (~9-11µm long, ~4-5µm wide) which were clear to lightly basophilic in
99 colour and interpreted as *Hepatozoon* gamonts (Figures 1 and 2). *Hepatozoon* gamonts were
100 noted in approximately 33% neutrophils. Testing for vector borne diseases (VBD; see
101 molecular investigation) revealed infection with *H. canis*. Serum biochemistry revealed only
102 a mild hyperglobulinaemia and mild hypoalbuminaemia. Due to the moderate parasitaemia
103 and mild clinical signs, a diagnosis of hepatozoonosis was made. Treatment was initiated with
104 imidocarb dipropionate (Imizol® Schering-Plough Animal Health, Darmstadt, Germany;
105 6.6mg/kg, by subcutaneous injection, every 14 days) and doxycycline (Ronaxan, Merial,
106 Lyon, France; 10mg/kg orally once daily for 28 days).
107 Haematology on Day 30 revealed an improvement in the anaemia and a borderline
108 monocytosis. Although *Hepatozoon* gamonts were still present in neutrophils (approximately
109 5%), there was reduction in the peripheral parasite burden. Further injections of imidocarb
110 dipropionate were administered (total of four injections). At this time, the dog was castrated
111 for management of the prostatomegaly. Haematology on Day 44 revealed resolution of the
112 anaemia and a mild, novel, neutropenia. No *Hepatozoon* gamonts were encountered during
113 the blood smear examination.
114 Two months later (Day 112) haematology demonstrated recurrence of the borderline
115 anaemia. Very rare *Hepatozoon* gamonts were present in neutrophils (<1%). A final course
116 of two injections of imidocarb dipropionate (6.6mg/kg, subcutaneously 14 days apart) were
117 administered. A final haematology on Day 154 demonstrated continued borderline anaemia

with slight regeneration and a mild leukopenia. No *Hepatozoon* gamonts were encountered on examination of peripheral blood smears and on buffy coat preparations. This finding was supported by conventional PCR analysis for *Hepatozoon* spp. which was negative. Monthly ectoparasitic prevention was recommended for the dog. The dog was doing clinically well until the end of 2011 after which time clinical follow up was unavailable.

Case 2

A five-month-old, entire male, cross-breed, clinically healthy dog was imported into the UK from a rescue centre in Paphos, Cyprus (Day 0); the day before travelling it had been treated with fipronil and (S)-methoprene spot-on (FrontlineCombo®, Merial, Lyon, France). The dog presented to a veterinary practice in Leicester, UK on the 7th of September 2014 (Day 1) due to lethargy and presence of tick infestation. Fipronil spray (Frontline® Spray 0.25% w/v Cutaneous Spray Solution, Merial) was applied, visible ticks were manually removed and disposed of without any further identification. EDTA blood was collected for VBD testing, which revealed infection with *H. canis*.

The dog's lethargy resolved spontaneously on Day 2. Due to financial limitations, the foster owner declined further investigations and treatment. On Day 22, automated haematology and serum biochemistry parameters were unremarkable. However, blood smear and buffy coat examinations revealed the presence of low numbers *Hepatozoon* gamonts in neutrophils (approximately 8%) (Table 2). Imidocarb dipropionate (6.6 mg/kg, by subcutaneous injection, 14 days apart) was administered on Days 22 and 36. On Day 36, the dog remained well but low numbers of *Hepatozoon* gamonts were still visible on blood smear examination (<1%) and PCR was positive. Another six injections of imidocarb dipropionate (6.6 mg/kg, subcutaneously) were administered weekly. On Day 85 the parasitaemia was not apparent on blood smear examination, but PCR remained positive. Monthly ectoparasitic

prevention was recommended. One and three years following treatment completion, the dog was described as healthy by the owner via telephone communication.

Case 3

An adult, neutered female, Poodle cross, clinically healthy dog was imported into the UK from a rescue centre in Paphos, Cyprus (Day 0); the day before travelling it had been treated with fipronil and (S)-methoprene spot-on. The dog presented to a veterinary practice in the Midlands, UK on the 10th of August 2015 (Day 1) due to anorexia, lethargy and presence of ticks which were manually removed and disposed of without any further identification. EDTA blood was collected for blood smear examination and VBD testing. On Day 1, the dog had a mild neutrophilia and on blood smear examination, moderate numbers of neutrophils (approximately 40%) contained *Hepatozoon* gamonts. Testing for VBD revealed infection with *H. canis* (Table 3). Due to the moderate parasitaemia and mild clinical signs, a diagnosis of hepatozoonosis was made. Treatment was initiated with imidocarb dipropionate (6.6 mg/kg, by subcutaneous injection, 14 days apart, for 8 weeks) and doxycycline (10 mg/kg, orally once daily, for 28 days).

On Day 60 the dog was reported to be clinically healthy by the veterinarian and no *Hepatozoon* gamonts were noted on blood smear examination; however, the dog remained PCR positive for *H. canis*. It was subsequently lost to follow-up and no further clinical information was available for this case.

Travel history

All cases reported here were dogs imported to the UK. The dogs in Cases 2 and 3 were imported from Cyprus, a European Union (EU) member island state situated in the eastern Mediterranean basin (35°10'N and 33°22'E) with a temperate climate. The predominant tick species found in Cyprus is *R. sanguineus* (27, 28) and a recent study has found that clinically

169 healthy dogs from the area of Paphos have a PCR prevalence of 45% for *H. canis*, 20% for
170 *Mycoplasma haemocanis*, 3% for *Anaplasma platys* and 1% for *Ehrlichia canis* (9).
171 According to the Ministry of Agriculture, Rural Development and Environment of the
172 Republic of Cyprus 8244 dogs travelled from Cyprus to the UK in the years 2015, 2016 and
173 2017 with the numbers increasing each year
174 (http://www.philenews.com/koinonia/eidiseis/article/536613/steilame-10-850-adespotoys-
175 skyloys-sto-exoteriko-pinakas).

176 Both Cases 2 and 3, fulfilled all the requirements set by UK's pet travel scheme
177 (PETS) for entering the country, that includes microchip identification, rabies vaccination 21-
178 days prior to arrival into the UK, and tapeworm treatment administration by a certified vet
179 between 5-days and 24-hours prior to arrival into the UK (https://www.gov.uk/take-pet-
180 abroad). Despite not being a requirement since January 2012, both dogs received acaricide
181 treatment 24-hours prior to for entry into the UK, and yet attached ticks were noted upon
182 arrival.

183 Case 1 did not have a microchip or a tattoo, making it difficult to trace its movements
184 and determine where it became infected with *H. canis*. Both Ireland and UK were
185 considered unlikely countries for acquiring *H. canis* infection as it has not previously been
186 documented in either of these countries and the main vector, *R. sanguineus*, does not appear
187 to be endemic in Ireland or the UK (29, 30). The most common tick encountered in both
188 Ireland and the UK is *Ixodes ricinus*, which has not been shown to be a vector for *H. canis*
189 (29-31). It was considered most likely that Case 1 became chronically infected with *H.*
190 *canis* in an endemic area, most likely in Southern Europe, possibly in Cyprus (9), France
191 (32), Greece (33), Italy (34), Portugal (35) or Spain (36) and then entered Ireland, either
192 prior to the introduction of PETS or illegally (37). Another possibility, considered less
193 likely, was infection following ingestion of a tick in Ireland that had previously fed on a
194 dog infected with *H. canis*.

Molecular investigation, sequencing and phylogenetic analysis

For all three cases DNA was extracted from 100 μL of EDTA-blood using a commercial kit (NucleoSpin® Blood, Machery-Nagel, Germany) according to the manufacturer's instructions. For the VBD testing, previously described conventional PCR assays , were used to detect infection with *Ehrlichia*/*Anaplasma* spp. (38) and *Hepatozoon* spp. (39), and quantitative PCR assays were used to detect infection with *Leishmania* spp. (40), *Babesia* spp.(41), "*Candidatus* Mycoplasma haematoparvum" and *M. haemocanis* (42). For each PCR assay, appropriate positive and negative controls were included.

Hepatozoon spp. PCR amplicons were purified using a commercial kit (ExoSAP-IT, Affymetrix, USB, Cleveland, Ohio, USA) according to the manufacturer's instructions, and the DNA sequenced using forward and reverse primers. The derived sequences were assembled using MacVector v15.5.4 (MacVector Inc, Cambridge, UK). DNA sequences were deposited in the European Nucleotide Archive. The derived sequence from Case 1 (LS453286) yielded 100% identity to an existing 18s rRNA gene for *H. canis* (AF418558) over 625 bp. The derived sequences from Cases 2 and 3 (LS453287 and LS453288) yielded 99% identities to an existing 18s rRNA gene for *H. canis* (KX818220) over 625 bp and 577 bp respectively. Sequences obtained in this study were aligned using ClustalW to selected 18S rRNA gene sequences from *Hepatozoon* spp. found in GenBank and a phylogenetic tree was subsequently generated (Figure 3). All *H. canis* sequences compared clustered into two clades, separate from *H. felis*, with Cases 2 and 3 separate from Case 1. It was not possible to predict the origin of Case 1's *H. canis* using available sequence data.

Discussion

These three cases highlight the risk of introducing non-endemic diseases, such as *H. canis* infection, into the UK through dogs being imported from, or having a travel history to,

countries where *H. canis* is endemic. Furthermore, they illustrate the spectrum of clinicopathological changes which *H. canis* infected dogs present with, as well as the diagnostic and treatment options available.

All cases had mild clinical signs that developed shortly after arrival, thus potentially the transportation stress may have aided the development of clinical hepatozoonosis from a prior sub-clinical infection (43). Only Case 1 displayed mild abnormalities on its haematology and biochemistry. Despite the high parasite burden (approximately 33%) a neutrophilia was not observed. Indeed, a transient neutropenia was present on Day 44. It is unknown if this was related to therapy resulting in the removal of parasitized neutrophils, or whether there was underlying inflammation resulting in neutrophil consumption. Dogs with a high parasite burden may be at an increased risk of secondary infections. Immune compromise can occur for multiple reasons. Neutrophils which contain gamonts have reduced myeloperoxidase activity (44), and have been reported to be deficient in oxidative bactericidal capacity (45). The mild non-regenerative anaemia noted in this case was attributed to anaemia of inflammatory disease, despite the lack of an inflammatory leukogram. The anaemia did improve with treatment; however, a borderline anaemia still remained on the final haematology. Also, in Case 1 there was a mild hyperglobulinaemia and hypoalbuminaemia, as with other reported cases of canine hepatozoonosis due to *H. canis* (4, 17). The hypoalbuminaemia most likely was due to an acute phase protein response or developed in compensation to the hyperglobulinaemia, and the hyperglobulinaemia likely reflected chronic inflammation. The timing of clinical presentation of all 3 dogs would suggest that they became infected during summer when *R. sanguineus* is most abundant and there is increased risk of pathogen transmission (46). Therefore, veterinarians should be aware that dogs imported to UK, or having a travel history to, countries where *H. canis* is endemic during summer or early autumn are more likely to have acquired this pathogen compared to dogs

246 imported during the winter or spring. Still, given the existence of chronic subclinical infection
247 with *H. canis*, it is possible that dogs imported all year round could develop clinical signs.
248 Blood smear examination was the most important diagnostic step in order to identify
249 the *Hepatozoon* gamonts and establish the infection in these three cases. The morphology of
250 the gamonts alone cannot distinguish infecting species and given the different prognosis and
251 treatment recommendations, PCR and sequencing were performed (4). Interestingly, none of
252 the three cases presented here were found to be co-infected with other vector-borne pathogens
253 that have frequently been reported in *H. canis*-infected dogs, such as *A. platys*, *E. canis*, or *L.*
254 *infantum* (21). These other vector-borne pathogens are common in the canine population of
255 Cyprus (9, 19, 47) and for Cases 1 and 3 there were clinical concerns initially for *E. canis* co-
256 infection, thus doxycycline was administrated. Interestingly, the highest PCR prevalence
257 (37.9%) recorded for *Hepatozoon felis* in cats has been reported in Cyprus, and *H. felis*
258 infected cats are 12 times more likely to be co-infected with *Leishmania infantum* compared
259 to the cats that are PCR negative for *H. felis* (48, 49).
260 Imidocarb dipropionate has been described as the drug of choice for treatment of
261 hepatozoonosis caused by *H. canis* (4). However, as in Cases 2 and 3, imidocarb dipropionate
262 has been described as being ineffective in eliminating *H. canis* infection, despite repeated
263 administration over a period of eight months to three naturally infected dogs (34). In all of
264 our three cases, treatment resulted in a decrease in the peripheral parasite burden, and eventual
265 absence of *Hepatozoon* gamonts on blood smear examination, and a negative *Hepatozoon*
266 spp. PCR result on blood in Case 1. As PCR was not performed on haemolymphatic tissues,
267 complete elimination of the infection could not be confirmed for Case 1. Complete
268 elimination of the parasitaemia is difficult to determine on examination of peripheral blood
269 smears alone. This is also supported by a published case report of a dog in Japan described as
270 having a positive blood PCR for *H. canis* 242 days after diagnosis, despite an absence of
271 gamonts on peripheral blood smear examination (13). In the absence of a more effective

treatment, imidocarb dipropionate currently remains the drug of choice (6.6 mg/kg, subcutaneously 14 days apart) to manage clinical hepatozoonosis due to *H. canis*, and the prognosis is considered good (4).

We recommend that *H. canis* positive dogs receive regular and effective ectoparasitic prevention to prevent onward transmission and to minimise the risk of acquiring co-infections with other vector-borne pathogens, and that they are not used as blood donors. Repeat blood smear and buffy coat examinations, as well as PCR's would be advised every 6-months to monitor for parasitaemia, and treatment initiated if clinically warranted (e.g. lethargy, weight loss, pyrexia) alongside a positive PCR result or blood smear examination. Administration of immunosuppressive or chemotherapeutic agents should be avoided if possible, but if necessary, more frequent monitoring of parasitaemia can be performed.

Hepatozoon species have been previously reported in the UK from pine martens (*Martes martes*) in Scotland (50), wild red squirrels (*Sciurus vulgaris*) in the Isle of Wight (51) and most recently in ticks infesting cats from south-east England for *H. felis* and from Wales for *Hepatozoon silvestris* (52). Additionally, a letter to Veterinary Record by Skeldon et al. described a case of *H. canis* infection in a dog imported into the UK from Cyprus (53). Due to clinical deterioration that dog was euthanised and no further diagnostic tests were performed.

At the moment the risk of *H. canis* becoming an endemic infection in the canine population of UK is low since the current climate does not favour the survival of the main vector *R. sanguineus* (54). However, if climate changes progress to establishing suitable conditions for these ticks, then *H. canis* could potentially become endemic in UK especially in the face of the expanding distribution of *R. sanguineus* ticks in northern Europe (55). The recent outbreak of canine babesiosis in UK (56) has raised awareness of the risks associated with dog importation and the Public Health England's Tick Surveillance Scheme's (https://www.gov.uk/guidance/tick-surveillance-scheme) data analysis revealed that dogs

298 travelling form Cyprus and Spain may result in *R. sanguineus* tick importation (57).
299 *Rhipicephalus sanguineus* ticks can survive and establish populations within houses in the
300 UK where canine hosts are present, and could transmit *H. canis* to other canine hosts within
301 such environments (57). Additionally, other potential vector ticks that have not yet been
302 investigated may transmit *H. canis*. In south Hungary, an area considered free from *R.*
303 *sanguineus* ticks, canine hepatozoonosis has been reported, so *Dermacentor marginatus* and
304 *Dermacentor reticulatus* ticks that are present there have been considered as possible *H. canis*
305 vectors, although this has not been confirmed (58). *Dermacentor reticulatus* ticks are present
306 in parts of the UK such as western Wales and south-west England, but in small numbers (29)
307 so, the overall risk of *H. canis* transmission in the UK is thought to be very limited.

308 These findings, alongside the identification of various non-UK endemic infectious
309 pathogens in imported dogs has sparked discussion of altering the current PETS following the
310 Brexit referendum (59). Possible reintroduction of a requirement for acaricide treatment of
311 dogs by a veterinarian 24-hours prior to entry into the UK has been considered as a measure
312 for reducing the risk of tick importation in the UK. Still, it is questionable whether it would
313 be effective as demonstrated by Cases 2 and 3 that, despite receiving acaricides prior to
314 travelling, both dogs were still found to be infested with ticks upon arrival. A modification of
315 this scheme for acaricide treatment of dogs 48-72 hours, followed by examination by a
316 veterinarian 24 hours, prior to entry into the UK, to document an apparent absence of ticks
317 could also be discussed. Implementing stricter requirements, for example a 10-day quarantine
318 facility stay and extensive infectious agent screening such as those in existence in Australia
319 (http://www.agriculture.gov.au/cats-dogs/step-by-step-guides/category-3-step-by-step-
320 guide-for-dogs), could also be explored.

321 In the era of increased canine international travel, UK veterinary surgeons and
322 diagnosticians should be aware of *H. canis* infection. Dogs with a travel history from endemic

countries, especially from Southern Europe, are advised to be molecularly tested for *Hepatozoon* spp. alongside other VBD and blood smear evaluation.

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Figure Legends

Figure 1. Case 1, Day 1 blood smear: Neutrophil containing a *Hepatozoon canis* gamont in the cytoplasm. 100x oil; Modified Wright's stain.

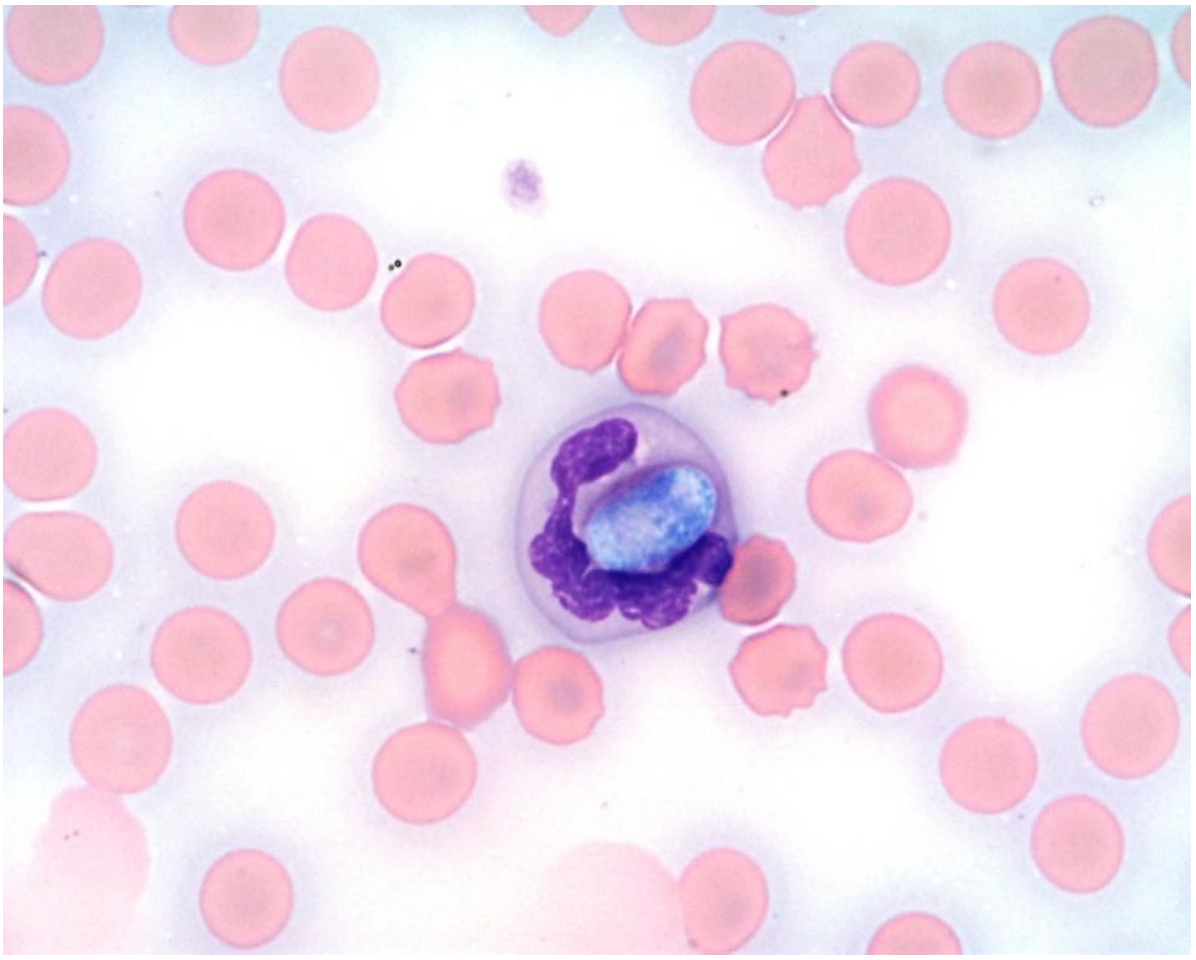

Figure 2. Case 1, Day 1 blood smear: Neutrophils on the feathered edge containing numerous *Hepatozoon canis* gamonts in the cytoplasm. 100x oil; Modified Wright's stain.

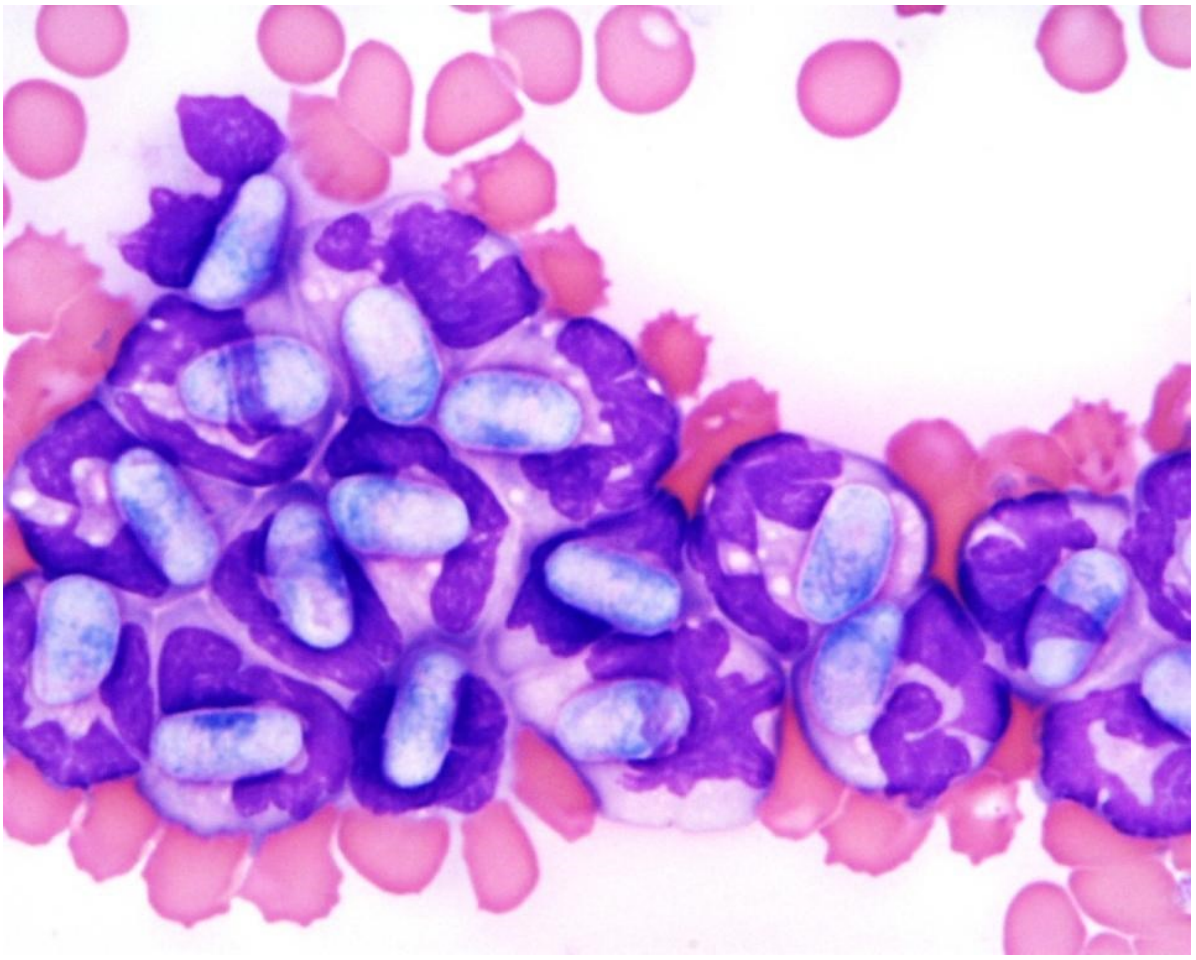

Figure 3. Phylogenetic tree constructed using the neighbour-joining program, corrected for nucleotide substitutions by the Kimura-2 parameter model, in MacVector. The data set was resampled 1000 times to generate bootstrap percentages. The country of origin is indicated in bold letters for *H. canis* sequences.

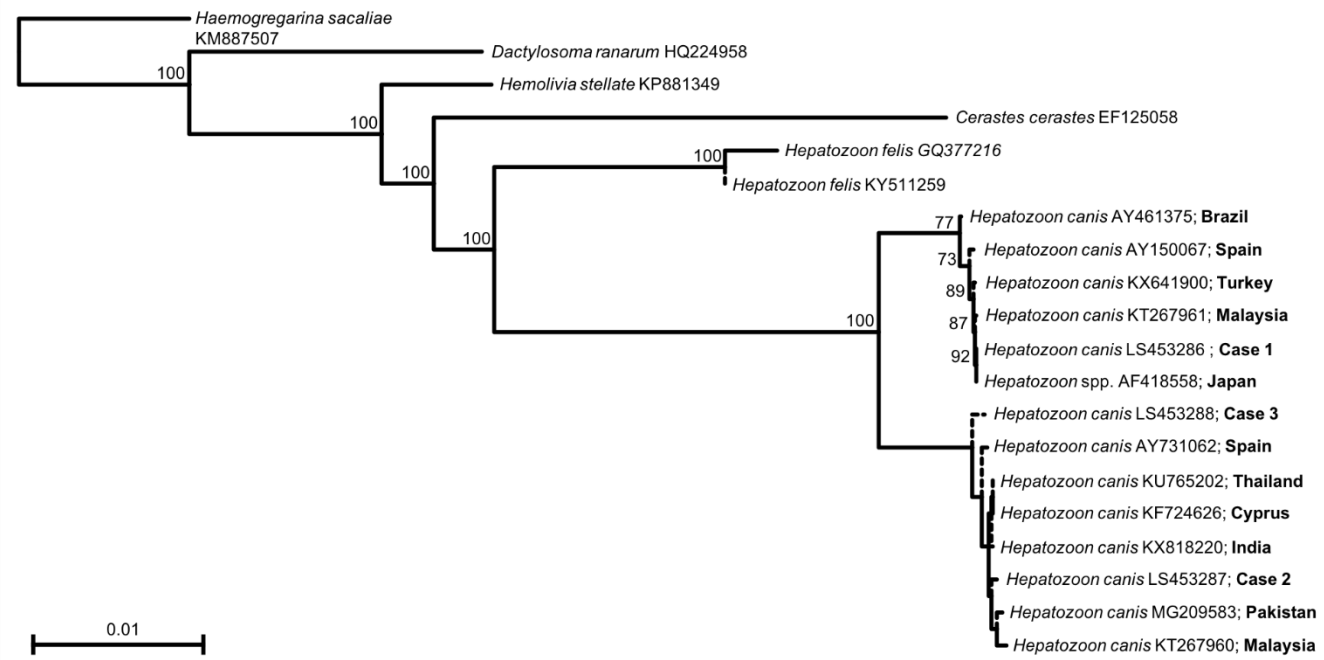

Table 1 Serial haematology and molecular results from Case 1 (days from initial diagnosis)

Parameter	Day 1	Day 30	Day 44	Day 112	Day 154	Reference Interval	Units
RBC	**4.2**	**5.0**	**5.2**	**4.4**	**4.7**	5.5 – 8.5	$x10^{12}$/L
HGB	**9.8**	**11.8**	12.4	**10.6**	**11.2**	12.0 – 18.0	g/dL
HCT	**30.0**	**35.0**	37.0	**35.0**	38.0	37.0 – 55.0	na
MCV	70.8	69.7	70.6	**80.1***	**81.5***	60.0 – 77.0	f/L
MCH	23.2	23.7	23.9	24.1	23.9	19.5 – 24.5	p/g
MCHC	32.7	34.0	33.8	**30.1**	**29.4**	31.0 – 37.0	g/dL
WBC	8.0	7.4	8.0	7.3	**4.9**	6.0 – 17.1	$x10^9$/L
Neutrophils	5.5	3.3	**2.6**	4.3	3.0	3.0 – 11.5	$x10^9$/L
Lymphocytes	1.3	1.9	2.9	2.0	1.6	1.0 – 4.8	$x10^9$/L
Monocytes	0.8	**1.8**	1.4	0.7	0.2	0.2 -1.5	$x10^9$/L
Eosinophils	0.4	0.4	1.1	0.4	0.2	0.0 – 1.3	$x10^9$/L
Polychromasia	Abs.	Mild	Mild	Abs.	Mild	na	na
Platelets	**114****	249	282	**111****	187	150 - 900	$x10^9$/L
Hepatozoon spp. PCR	**Pos.**	na	na	na	Neg.	na	na
Hepatozoon gamonts on blood smear+	**~33%**	**~5%**	Neg.	**<1%**	Neg.	na	na

Haematology analyses were performed with Cell-DYN 3500 Haematology Analyser (Abbott, Chicago, Illinois, United States).

Abnormal findings are denoted by bold font.
+: % of neutrophils containing *H. canis* gamonts on the monolayer
*: *In vitro* swelling
**: Moderate platelet clumping, platelet numbers adequate on blood smear examination.

Abbreviations: *RBC* red blood cells; *HGB* haemoglobin; *HCT* haematocrit; *MCV* mean corpuscular volume; *MCH* mean cell haemoglobin; *MCHC* mean corpuscular haemoglobin concentration; *WBC* white blood cell; *Abs*. absent; *Neg.* negative; *na* not applicable; *pos.* positive

Table 2 Serial blood smear and molecular results from Case 2 (days from initial presentation)

Parameter	Day 1	Day 22	Day 36	Day 85
Hepatozoon spp PCR	na	na	**Pos.**	**Pos.**
Hepatozoon gamonts on blood smear+	na	**~8%**	**<1%**	Neg.

Abnormal findings are denoted by bold font.
+: % of neutrophils containing *H. canis* gamonts on the monolayer

Abbreviations: *na* not applicable; *Pos.* positive; N*eg.* negative

Table 3 Serial blood smear and molecular results from Case 3 (days from initial diagnosis)

Parameter	Day 1	Day 60
Hepatozoon spp PCR	**Pos.**	**Pos.**
Hepatozoon gamonts on blood smear[+]	**~40%**	Neg.

Abnormal findings are denoted by bold font.

+: % of neutrophils containing *H. canis* gamonts on the monolayer

Abbreviations: *Pos.* positive; N*eg.* negative