Hepatozoon canis in a Beagle dog living in Ireland

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Case Presentation:

A 12 year old, male entire Beagle, presented in September 2010 to the Dogs Trust Rehoming Centre in Harefield, West London, having been acquired from a rescue centre in Ireland. There was no clinical history available prior to the Irish rescue centre and no microchip or tattoo was present.

On admission to the Dogs Trust on 14th September 2010 (Day 1), it was thin but bright and alert. Significant clinical findings included pale mucous membranes, a slightly enlarged prostate, occasional cough, slight nasal discharge and positive tracheal pinch. Mild generalised seborrhoea was also noted.

Haematology and biochemistry results are shown in Tables 1 and 2. On Day 1, it had mild to moderate, normocytic, normochromic, non-regenerative anaemia. Serum biochemistry findings included a mild hypoalbuminaemia, mild to moderate hyperglobulinaemia, slight hyperkalaemia and a mild increase in serum amylase. Total T_4 was within normal reference intervals, however TSH concentrations were increased. On blood smear examination moderate numbers of neutrophils contained intracytoplasmic elliptical structures (~9-11µm in length, ~4-5µm in width) which were clear to lightly basophilic in colour, and interpreted as *Hepatozoon* gamonts (Figures 1 and 2). *Hepatozoon* gamonts were noted in about 33% of all neutrophils. However, numbers varied depending upon the region of blood smear examined, with the feathered edge containing the majority of gamonts.

Due to the moderate parasitaemia and mild clinical signs, a presumptive diagnosis of *Hepatozoon canis* (*H. canis*) infection was made. Treatment was initiated with two injections

of imidocarb dipropionate (6.6mg/kg, subcutaneously 14 days apart) and doxcycline (10mg/Kg SID for 28 days per os).

To further characterise the species of *Hepatozoon* involved, DNA was extracted from whole EDTA blood and conventional PCR and sequencing from products of both the reverse and forward primers were performed at the Hebrew University of Jerusalem using previously reported methods[1]. Primers used were HEP-F and HEP-R for the detection of a fragment of the *Hepatozoon* 18s ribosomal RNA gene[2]. Sequences were evaluated with ChromasPro software version 1.33 and compared to sequence data available from GenBank® using the BLAST 2.2.9 program (http://www.ncbi.nlm.nih.gov/BLAST/) [1]. The partial 18s rRNA gene DNA sequence (642bp) obtained from *Hepatozoon* positive PCR was 99% identical to the *H. canis* sequence in GenBank® (*H.canis* isolate Spain-1: accession number: AY150067.2)

Due to the evidence of mild cutaneous lesions and to rule out co-infections, a serum sample was submitted for detection of IgG antibodies against *Leishmania infantum* by immunofluorescence assay(IFA) (Testapet, Liverpool School of Tropical Medicine, Liverpool University), and IFA antibody titre was 1:40 and was interpreted as a weak positive. An in-house quantitative ELISA for detection of IgG against *L. infantum* using previously reported methods[3] and real time-PCR analysis on EDTA blood for *L. infantum* as previously described[4] were subsequently performed, and both were negative.

Repeat haematology on Day 30 revealed an improvement in RBC concentration with slight evidence of polychromasia. A mild monocytosis was present. Although there was reduction in the peripheral parasite burden based on the blood smear examination, *Hepatozoon* gamonts were still present in neutrophils, albeit in reduced numbers (about 5%). Repeat injection of imidocarb dipropionate was advised. At this time, it was castrated to reduce prostatic enlargement.

Haematology on Day 44 revealed further improvement in RBC concentration, with mild evidence of regeneration. A slight neutropaenia was reported. No *H. canis* gamonts were encountered during the blood smear examination.

Two months later (Day 112) repeat haematology demonstrated a slight decrease in RBC concentration. Very rare *H. canis* gamonts were present in neutrophils (<1%). A final course of two injections of imidocarb dipropionate, 14 days apart was administered.

A final haematology on Day 154 demonstrated continued mild anaemia with mild evidence of regeneration and a mild leukopaenia. No *H. canis* gamonts were encountered on examination of peripheral blood smears and on buffy coat preparations. This finding was supported by conventional PCR analysis for *Hepatozoon*[1] which was negative at this point.

Throughout its stay at the Dog Trust Rehoming Centre, it remained bright and gained weight. It was re-homed and is clinically doing well at present.

Table 1. Haematology results.

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	0.30	0.35	0.37	0.35	0.38	0.37 - 0.55	
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	neg	mild	mild	neg	mild		
Platelets	114**	249	282	111**	187	150 - 900	$x10^{9}$/L
***H. canis* blood smear**[+]	~33%	~5%	neg	<1%	neg		
PCR analysis	pos				neg		

[+] % of neutrophils containing *H. canis* gamonts
* In vitro swelling
** Moderate platelet clumping, platelet numbers adequate

Table 2. Biochemistry results.

Parameter	Day 1	Reference Interval	Units
Total protein	75.1	49.0-71.0	g/L
Albumin	27.7	28.0-39.0	g/L
Globulin	47.4	21.0-41.0	g/L
Sodium	150	140-153	mmol/L
Potassium	5.4	4.1-5.3	mmol/L
Chloride	109	107-115	mmol/L
Calcium	2.51	2.13-2.70	mmol/L
Glucose	5.3	3.0-6.0	mmol/L
Inorganic phosphorus	1.8	0.8-2.0	mmol/L
Urea	7.5	3.0-9.1	mmol/L
Creatinine	87	20-150	µmol/L
Cholesterol	5.2	3.3-8.9	mmol/L
Total Bilirubin	0.9	0.0-2.4	µmol/L
Amylase	1524	176-1245	U/L
Lipase	123	72-1115	U/L
ALT	27	13-88	U/L
CK	370	61-394	U/L
ALP	27	19-285	U/L
Thyroxine (T4)	17	13-52	nmol/L
TSH	0.97	<0.41	ng/ml

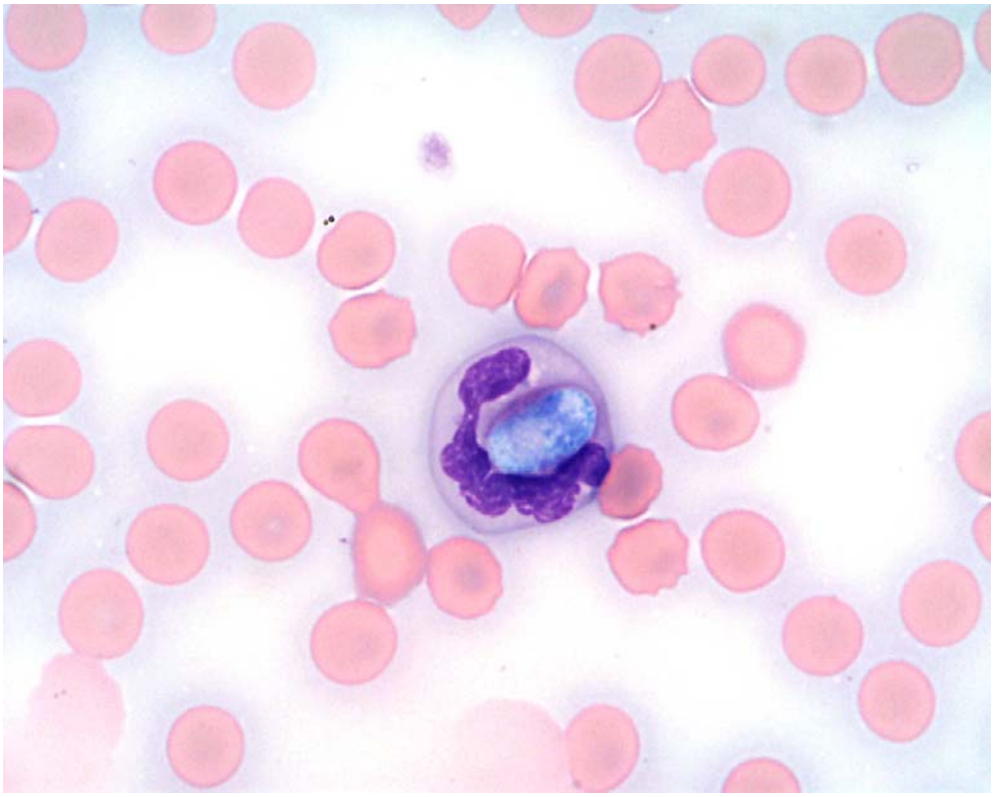

Figure 1. Day 1 blood smear: Neutrophil containing a *H. canis* gamont in the cytoplasm. 100x oil; Modified Wright's stain.

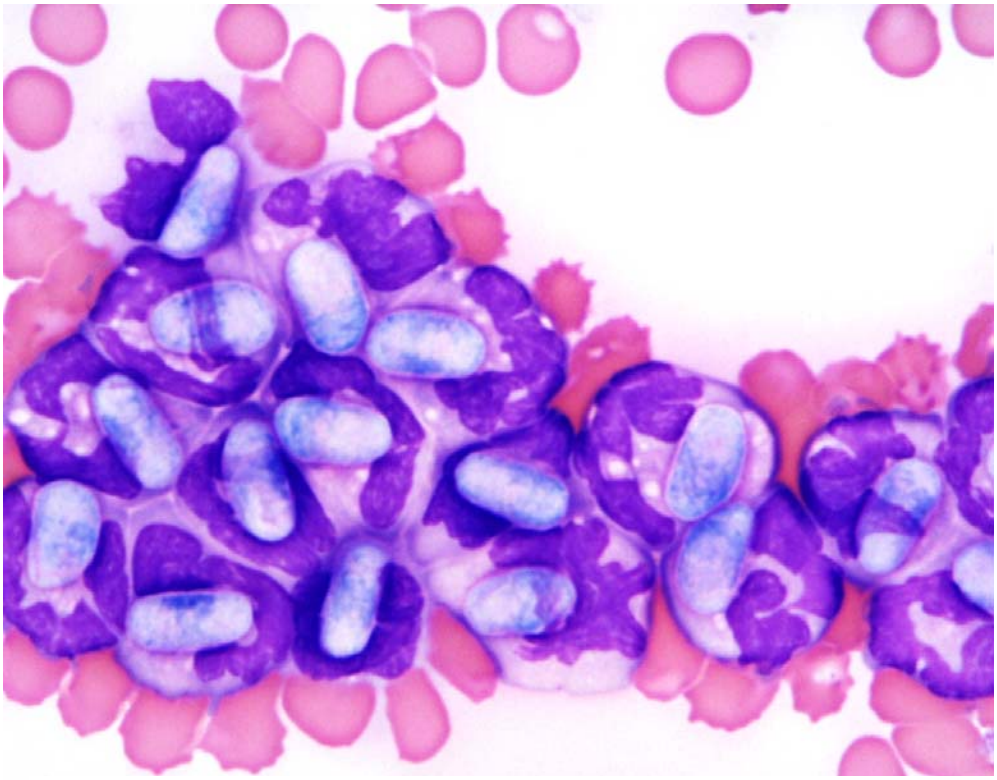

Figure 2. Day 1 blood smear: Neutrophils on the feathered edge containing numerous *H.canis* gamonts in the cytoplasm. 100x oil; Modified Wright's stain

Discussion:

This report describes a 12 year old male Beagle dog, from Ireland, infected with *Hepatozoon canis*. Diagnosis was reached based on haematological findings, clinical signs and PCR analysis.

Canine hepatozoonosis is a tick transmitted protozoal infection. Two species of *Hepatozoon* are reported to infect dogs, *H. canis* and *H. americanum*. These species differ in geographical location, pathogenicity and definitive host[5].

The life cycle for *H. canis* begins with ingestion of infected ticks containing sporulated oocysts. After ingestion, sporozoites are released and penetrate the intestinal epithelium, where they disseminate to the hemolymphatic tissues via lymphatics or blood vessels[6]. Sporozoites undergo merogony in the bone marrow, spleen, lymph nodes and other tissues[6]. Merozoites are released and invade leukocytes (neutrophils and monocytes) forming gamonts[6], which are then ingested by ticks, undergo a sexual stage, and form oocysts.

While *Rhipicephalus sanguineus* (brown dog tick) is considered to be the main vector of *H. canis* other tick species have been shown to be alternative or potential vectors, such as *Amblyomma ovale* in South America[7]. Transplacental infections of *H canis* have also been reported[8]. Interestingly, *H. americanum* may spread via ingestion of prey containing the cystozoite stages of the parasite; however this mode of transmission has not been evaluated for *H. canis*[5].

H. canis has been recognised in dogs in Asia[9], the Mediterranean basin[1], the Middle East[10] and South America[11], and recently in the southern states of the USA in North America[12]. However to the best of the author's knowledge *H. canis* has never previously been reported in Ireland or the United Kingdom.

As part of the Pet Passport Scheme (PETS) dogs are required to be microchipped. The dog in our case report did not have a microchip or a tattoo, making it difficult to trace its movements and determine where it became infected with *H. canis*. Both Ireland and England were considered unlikely countries for acquiring *H. canis* infection as it has not previously been documented in the UK and the vector, *Rhipicephalus sanguineus* does not appear to be endemic in the British Isles[13]. The most common tick encountered in the UK is *Ixodes ricinus*[14]. It was considered most likely that it became chronically infected with *H. canis* in an endemic area such as Southern Europe including Italy[1], Spain[15] and Portugal[16], and, then entered Ireland, either prior to the introduction of PETS or illegally. Another much less likely possibility was contact with a dog in Ireland carrying a tick infected with *H. canis* and ingestion of the same tick. Even with the requirement to administer acaricides before a dog enters the British Isles, there are reports of female *R. sanguineus* on dogs returning to the UK from Cyprus via PETS[13]. This may reflect resistance to, or improper administration of acaricides.

It was speculated by the re-homing centre that it may have originated from the United States of America, although this is difficult to substantiate. If it did originate in the United States it

would add further confirmation of the widespread presence of *H. canis* in North America. Historically, all cases of canine hepatozoonosis in America were erroneously believed to be due to strains of *H. canis*. However, in 1997 the novel species of *H. americanum* was identified[17] and it is only in recent times that published material has demonstrated the presence of *H. canis* in the south eastern United States as well as *H. americanum*[12]. Others have also confirmed the presence of *H. canis* in North America with the vast majority of cases identified in the southern states of Mississippi and Alabama and further north, in states such as Virginia[18].

Clinical signs of *H. canis* relate to the severity of the parasite burden[19]. Dogs with a low parasite burden (<1% of neutrophils containing gamonts) may be normal or show only mild clinical signs[20], whereas more severe clinical signs including fever, lethargy and emaciation are noted with high parasite burdens. In the few published case reports of dogs suffering clinical signs of *H. canis*, the percentage of neutrophils containing gamonts varied from 21% [19] to 48% [9] to 90%[21]. The dog in this case report had a parasite burden of about 33% of neutrophils containing gamonts. Despite this high parasite burden, it remained bright throughout its stay and only displayed mild changes on his haematology and biochemistry.

Common haematology abnormalities include mild anaemia and neutrophilia[19], however extreme leukocytosis may occur (20-200 $x10^9$/L leukocytes)[22]. In contrast, in cases of *H. americanum* extreme leukocytosis is often reported, with a mature neutrophilia and occasionally a left shift [22]. In our case, despite the high parasitaemia, a neutrophilia was never observed. Indeed neutropaenia was present on Day 44. It is unknown if this was related to therapy resulting in the removal of parasitised neutrophils, or whether there was underlying inflammation resulting in the mild neutropaenia. 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 a reduced myeloperoxidase activity [23], and have been reported to be deficient in oxidative bactericidal capacity[24]. 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 to mild anaemia still remained on the final haematology.

As with other reported cases of *H. canis*, there was a mild hypoalbuminaenia[19, 9, 21] and hyperglobulinaemia [9, 10]. Hypoalbuminaemia might be due to an acute phase protein response, decreased synthesis due to increased globulin production or decreased protein intake. The hyperglobulinaemia may reflect chronic inflammation.

The normal T_4 with a single high TSH was of uncertain significance. It may indicate compensatory hypothyroidism whereby the T_4 concentration is maintained within reference intervals only by increased TSH production or possibly may occur after non-thyroidal illness[25]. While it does not rule out hypothyroidism, there was no elevation in serum cholesterol. Without further endocrine monitoring, and measurement of thyroglobulin autoantibodies, a possible underlying hypothyroidism was not ruled out completely.

The seborrhoea present on the initial clinical exam resolved with therapy. Previous case reports have documented skin and hair coat abnormalities, however they were considered unrelated to *H. canis* infection[19].

Cases of co-infection with other infectious organisms including *Leishmania*[26], or *Ehrlichia canis*[27] are reported. Doxycycline was administered at the recommended dose, to treat for any possible *Ehrlichia*/*Anaplasma* co-infections. *L. infantum* infection was unlikely based on negative serological and PCR results.

Imidocarb dipropionate has previously been reported as the drug of choice for treatment of *H.canis*[20], and the prognosis has been reported as good[22]. However, in a recent study imidocarb dipropionate was not found to be effective in eliminating *H. canis* in three naturally infected dogs treated repeatedly over 8 months[1]. In our case report treatment did result in a decrease in the peripheral parasite burden, an eventual absence of *H. canis* gamonts on blood smear examination and a negative result with conventional PCR analysis. As PCR was not performed on bone marrow or other tissue sites, complete elimination of the parasite could not be determined. Complete elimination of the parasite is difficult to determine on examination of peripheral blood smears alone. A case report of a dog in Japan reported positive PCR analysis for *H. canis* 242 days after diagnosis, despite an absence of gamonts on peripheral blood smear examination[9]. Repeat blood smear examinations/PCR's would be advised every 6 months to monitor for blood parasitaemia and treatment initiated again if clinically warranted. In the absence of a more effective treatment, imidocarb dipropionate currently remains the drug of choice.

In conclusion this is the first time *H. canis* has been documented in a dog in the British Isles. With the prevalence of *H. canis* reported to be 21.1% in certain European countries (e.g. Portugal)[16] and the increased mobility of pets, veterinary surgeons and diagnosticians within the British Isles may come into contact with *H. canis* more frequently.

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