Hepatozoonosis in a Dog with Skeletal and Joint Involvement: A Case Report and Review of the Literature

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ABSTRACT

A 4-5 month-old dog was presented with a one week history of inappetence, lethargy and pain, mostly evident when walking. The dog had been found infested with ticks in a field three weeks prior to presentation. Physical examination findings included poor body condition score, tachycardia, pale mucous membranes, weakness and unwillingness to stand, extreme pain on palpation of all limbs, mild lymphadenomegaly and multiple swollen joints. The dog suffered from moderate anemia and mild thrombocytopenia. Blood smear evaluation revealed that 12% of the neutrophils and monocytes were parasitized by *Hepatozoon* gamonts confirmed as *Hepatozoon canis* by PCR and sequencing. Serum biochemistry abnormalities included hypoalbuminemia, hyperglobulinemia, elevated alkaline phosphatase activity and mild hyponatremia. PCR for *Ehrlichia canis* was negative. Survey radiographs showed evidence of polyostotic involvement of bone cortices with thickening and marked diffuse continuous periosteal proliferation of the humerus, ulna, radius, femur and tibia. Joint fluid from the tarsal joints showed marked increase in WBC, predominantly of neutrophils, some of which parasitized by *H. canis*. Bacterial cultures, including specific culture for *Mycoplasma* spp. were negative. The pup was treated with doxycycline, amoxicillin-clavulanic acid and multiple doses of imidocarb dipropionate. During a period of four months the pup's clinical signs were resolved and its hematological and radiographic parameters improved substantially. Despite that, the *H.canis* parasite load increased. This is an unusual case of canine hepatozoonosis with concurrent periosteal reaction and polyarthiritis.

Key words: Dog, *Hepatozoon canis*, infection, periosteal reaction, polyarthritis

INTRODUCTION

Hepatozoonosis is an arthropod-borne infection caused by apicomplexan protozoa from the family Hepatozoidea (1). *Hepatozoon* species have a basic life cycle that includes asexual development with merogony followed by gamontogony in a vertebrate intermediate host such as the dog and sexual development leading to sporogony in a hematophagous invertebrate definitive host such as the tick. *Hepatozoon* transmission takes place by ingestion of the arthropod definitive host by the intermediate vertebrate host (1).

Hepatozoon canis infection (HCI) in dogs was first described from India in 1905, and until 1997 it was presumed that canine hepatozoonosis was caused by a single species. However, further research has led to the identification of two distinct species that use domestic dogs as intermediate host – *H. canis* and *Hepatozoon americanum* (1, 2). HCI has been reported from Asia, Europe, Africa, South and North America (1). The prevalence of HCI in different regions ranges considerably. A seroepidemiologic study in Israel, using indirect fluorescent antibody (IFA) testing, detecting the presence of

anti- *H. canis* antibodies revealed that 33% of the surveyed dogs have been exposed to *H. canis* (1, 3). As found also for other tick-borne diseases, such as canine ehrlichiosis and babesiosis, the exposure rate for HCI in endemic areas is often much greater than the prevalence of clinical disease (1, 3).

The primary vector of *H. canis* is the brown dog tick, *Rhipicephalaus sanguineus*. *H. canis* is transmitted transtadially from the nymph to the adult stage in *R. sanguineus* (1, 2). In addition to infection by ingesting ticks that contain mature oocysts, it has also been shown to be transmitted transplacentally from the dam to its offspring (4).

When the dog ingests the vector tick or tick parts, *H. canis* sporozoites invade mononuclear cells and disseminate hematogenously or via the lymph to hemolymphatic organs such as the bone marrow, spleen and lymph nodes and to other internal organs such as the liver, kidney and lung (1). The pathogenesis of HCI is influenced by immunodeficient conditions such as an immature immune system in young pups, a congenital defect or concurrent infectious agent. Concurrent infections with *Babesia*, *Ehrlichia*, *Toxoplasma*, *Leishmania*, *Anaplasma* species, parvovirus and canine distemper virus are common and predispose to clinical illness (1). A variety of clinical presentations are associated with HCI, ranging from an incidental hematologic finding in an apparently healthy dog to a debilitating and life threatening illness. A low level of *H. canis* parasitemia with gamonts found in less than 5% of neutrophils is the most common presentation and it is usually associated with an asymptomatic to mild disease. A high rate of parasitemia, sometimes approaching 100% of the neutrophils with leukocytosis, is usually associated with a severe disease (1).

Anemia, usually normocytic normochromic, is the most common hematologic abnormality in HCI. The leukocytes count is usually normal in low parasitemia and may reach 100,000 cell/µl blood in cases with high parasitemia (1, 2). Thrombocytopenia occurs in approximately one third of dogs with HCI and may be associated in some cases with concurrent canine ehrlichiosis. Serum chemistry abnormalities include elevated creatine kinase and alkaline phosphatase activities, hypoglycemia and hyperproteinemia with hyperglobulinemia and hypoalbuminemia (1, 2).

Microscopic detection of gamonts within white blood cells in stained blood smears is the most common diagnostic technique for HCI (1, 2). The gamonts are large (approximately 11 by 4 µm) and have an ellipsoidal shape (1). PCR can be used for the detection of *Hepatozoon* infection in the blood or other tissues and together with sequencing it is used for determining the species of *Hepatozoon* responsible for infection (2).

The current treatment protocol for HCI is with imidocarb dipropionate at 5-6 mg/kg subcutaneously or intramuscularly, every 14 days, until gamonts are no longer present in blood smears (1). Pathology to skeletal tissues is common in *H. americanum* infection, however, it has rarely been reported in HCI. The present case describes HCI in a pup with concurrent skeletal and joint pathology.

CASE HISTORY

A 4-5 month-old, intact female, mixed breed dog was presented to the Hebrew University Veterinary Teaching Hospital (HUVTH) with a one week history of inappetence, lethargy and pain, mostly evident when walking. The pup was unwilling to flex its hind limb joints. A previously consulted practitioner measured elevated rectal body temperature (40.2^0 C), performed a complete blood count (CBC) (results unavailable) and injected imidocarb dipropionate (Imizol, Schering-Plough Animal Health, New Jersey USA) two days prior to arrival based on the assumption that the dog had babesiosis. The owner was advised to treat the pup with doxycycline and amoxicillin-clavulanic acid. These treatments were initiated and later discontinued by the owner.

The dog had been found, along with two male littermates, in a field three weeks prior to arrival. The puppies were infested with ticks and were housed in different foster homes. None of the littermates was vaccinated.

Upon physical examination (PE), the pup was quiet, alert and responsive, had a body condition score (BCS) of 3/9, 5% dehydration, tachycardia (200 heart beats/minute), and pale mucous membranes. It was weak and unwilling to stand, and extremely painful on palpation of all limbs. Both tarsal and carpal joints, stifles and elbows were swollen and a moderate enlargement of the popliteal lymph nodes was noted.

Abnormalities in the CBC (Table 1) included: moderate to severe normocytic-normochromic anemia with mild anisocytosis, relative leucopenia, and thrombocytopenia with megathrombocytes. Blood smear evaluation revealed moderate left shift with mild toxicity in the neutrophils, some of the monocytes showed reactive changes. The rate of parasitemia with *Hepatozoon* gamonts was calculated by light microscopy

Table 1: Complete blood count measures from a pup with *Hepatozoon canis* infection at presentation, eleven days post-presentation and four months thereafter. Reference intervals include adult as well as young dogs.

Parameter	Day 1	Day 11	4 months	Reference interval
Red blood cells ($\times 10^6$/μL)	4.4	4.42	7.31	5.7-8.8
Hemoglobin (g/dL)	8.6	8.7	15.2	12.9-18.4
Hematocrit (%)	28	26	43.7	37.1-57
MCV (fL)	60.8	58	60	58.8-71.2
MCH (pg)	19.6	19.7	20.9	20.5-24.2
MCHC (g/dL)	32.2	33.9	34.9	31-36.2
RDW (%)	15.3	16.8	19.4	11.9-14.5
Platelets ($\times 10^5$/μL)	127	92	104	143.3-400
MPV (fL)	17.9	12.8	10.3	7-11
White Blood Cell ($\times 10^3$/μL)	9.54	17.79	12.2	5.2-13.9
Neutrophils ($\times 10^3$/μL)	6.00	7.42	N/A	3.9-8
Lymphocytes ($\times 10^3$/μL)	2.47	6.63	N/A	1.3-4.1
Monocytes ($\times 10^3$/μL)	0.72	1.59	N/A	0.2-1.1
Eosinophils ($\times 10^3$/μL)	0.08	0.14	N/A	0-0.6
Basophils ($\times 10^3$/μL)	0.03	0.18	N/A	0-0.1

MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration RDW: red blood cell distribution width, MPV: mean platelet volume, N/A: not available

Table 2: Serum biochemistry measures from a pup with *Hepatozoon cnais* infection at presentation, eleven days post-presentation and four months thereafter.

Parameter	Day 1	Day 11	4 months	Reference interval
Albumin (g/dl)	2.5		2.72	3-4.4
Alkaline phosphatase (U/L)	214		93.9	21-170
Alanine-aminotransferase (U/L)	54		31.3	19-67
Amylase (U/L)	1172		1452.9	103-1510
Aspartate aminotransferase (U/L)	38		28.3	19-42
β-Hydroxybutyric acid (mmol/L)	0.58			0.3-0.75
Calcium-ionized (mmol/L)	1.33	1.523		0.9-1.35
Calcium, total (mg/dL)	8.52		11.2	9.7-11.5
Chloride (mmol/L)	110.4	109.4	110.6	108-118
Cholesterol (mg/dL)	354		207.76	135-361
Creatine kinase (U/L)	301		124	51-399
Creatinine (mg/dL)	0.24	0.33	0.64	0.3-1.2
γ-Glutamyl-transferase (U/L)	0		0.3	0-6
Glucose (mg/dL)	86		127.56	64-123
Potassium (mmol/L)	4.47	4.82	4.36	3.6-5.3
Phosphorus (mg/dL)	5.2		6.66	3-6.2
Sodium (mmol/L)	139.2	140.8	143.4	145-154
Total bilirubin (mg/dL)	0		0.1	0-0.2
Total protein (g/dL)	6.9		7.2	5.4-7.6
Triglycerides (mg/dL)	93		71.89	19-123
Urea (mg/dL)	25.9		34.27	10.7-53.5
Globulins (g/dl)	4.4		4.48	1.8-3.9

observation of the number of parasitized neutrophils and monocytes, and by dividing this number by the total number of 200 neutrophils and monocytes observed at the magnification of 1000. The parasitemia rate was expressed as percent by multiplication by 100. Twelve percent of the neutrophils and monocytes were parasitized by *Hepatozoon* sp. gamonts that were morphologically consistent with *H. canis* (Figure 1).

Serum biochemistry (Table 2) abnormalities included hypoalbuminemia, hyperglobulinemia, elevated alkaline phosphatase activity and mild hyponatremia. Urinalysis results were unremarkable.

PCR for the detection of *Ehrlichia canis* performed at the Hebrew University as previously described (5) was negative. PCR for *Hepatozoon* spp. followed by sequencing as previously described (6) confirmed that the pup was infected with *H. canis*.

Survey radiographs of the chest, abdomen, spine and the appendicular skeleton showed evidence of polyostotic involvement of bone cortices with thickening and marked diffuse continuous periosteal proliferation of the humerus, ulna, radius, femur and tibia (Figure 2). The periosteal reactions were observed mainly at the diaphysis, but focal metaphysis and epiphysis involvement were also observed. The spectrum of the periosteal reactions varied from a smooth and solid type at the diaphyses and metaphyses to a palisading type at the distal epiphysis of the femur.

The pup was treated with an injection of imidocarb dipropionate, 6 mg/kg SC at the HUVTH and discharged with instructions for treatment with doxycycline (Doxylin, Dexon, Hadera, Israel) at 10 mg/kg PO, SID for 21 days.

Two weeks later, the pup was presented for follow up and its owner reported mild improvement in appetite despite the persistence of severe pain. Treatment with doxycycline was not administered by the owner due to his concern about discoloration of the pup's teeth. Instead, treatment was start-

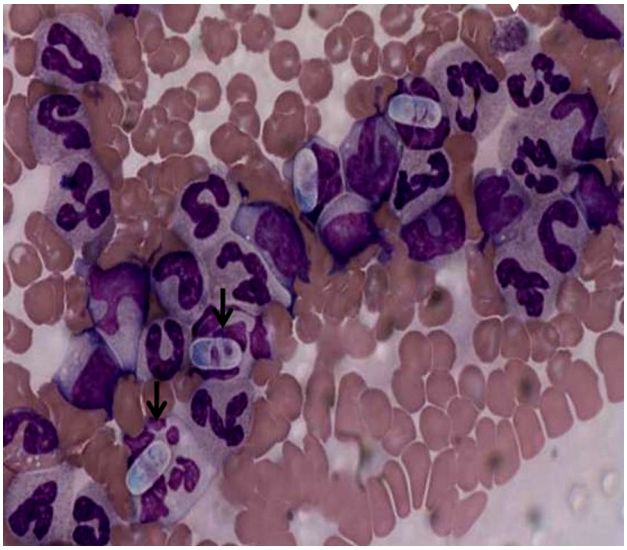

Figure 1: Blood smear from the pup's first admission showing large platelet (white arrow) and neutrophils parasitized with *H. canis* gamonts (black arrows). The parasitemia level was 12 % of neutrophils and monocytes (Giemsa stain, magnification X 1000).

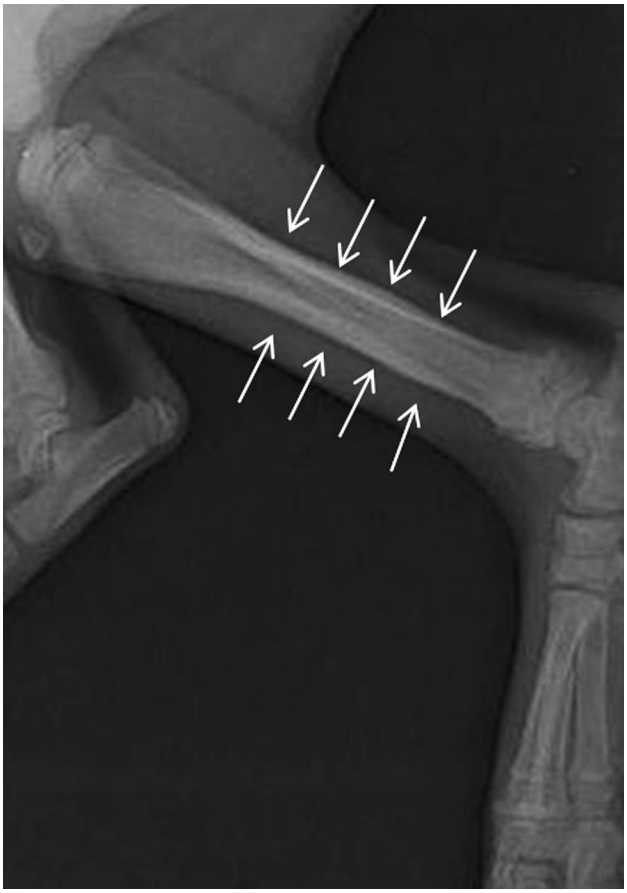

Figure 2: Polyostotic involvement of bone cortices with thickening and marked diffuse continuous periosteal proliferation of the tibia (white arrows).

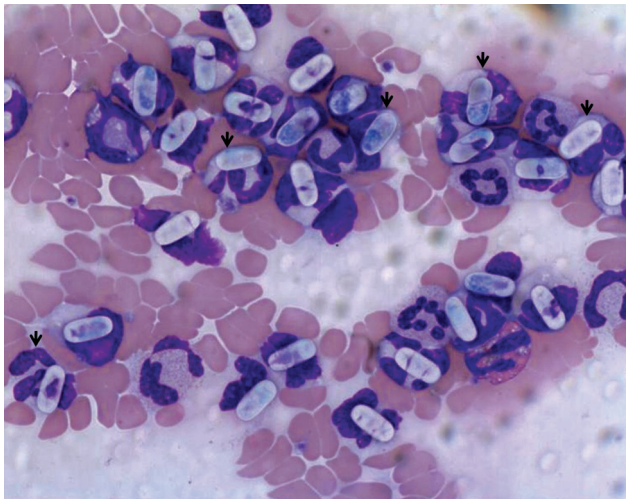

Figure 3: Blood smear from the pup, 41 days after its first admission showing a heavy load of *H. canis* gamonts parasitizing neutrophils (arrows). The parasitemia level was 82 % of the neutrophils and monocytes (Giemsa stain, magnification X 1000).

ed with amoxicillin-clavulanic acid (Synulox, Pfizer, Latina, Italy) at 4 mg/kg PO once daily for 10 days. These extra-label low dose and long dosing interval were given independently by the owner. The PE findings on that visit were similar to those reported in the pup's first admission.

Abnormalities in blood tests (CBC, creatinine and electrolytes levels, table 1 and 2, respectively) included mild leukocytosis with absolute lymphocytosis and monocytosis, normocytic normohromic anemia with anisocytosis, thrombocytopenia with megaplatelets and hypercalcaemia. Blood smear evaluation revealed moderate to severe left shift with moderate toxicity in neutrophils. Some of the monocytes showed reactive changes. Forty eight percent of the neutrophils and monocytes were parasitized by *Hepatozoon* spp. gamonts. Joint fluid was aseptically sampled from both tarsal joints under anesthesia for cytological evaluation and for culture and sensitivity. Cytology of the fluid from both joints showed marked increase in white blood cells (WBC), predominantly of neutrophils. Only a few of the joint fluid neutrophils were parasitized by *H. canis* gamonts. Bacterial cultures of joint fluid, including specific culture for *Mycoplasma* spp., a potential cause of arthritis in dogs and other animals (7, 8), were negative.

The pup was treated with a third dose of imidocarb dipropionate at 6 mg/kg SC and discharged on the same day with instructions to continue the treatment with amoxicillin-clavulanic acid at 15 mg/kg PO every 12 hours for 14 days,

and doxycycline at 10 mg/kg PO, once daily for 21 days. The owner reported by telephone two weeks later that the pup was treated according to the instructions received upon release from the hospital and mentioned that there was some improvement in its appetite and level of activity.

One month after its last visit to the HUVTH, it was presented again for an examination. The owner reported normal behavior and appetite. PE findings included normal vital signs, very mildly swollen elbow joints and moderately enlarged popliteal lymph nodes. There was no pain or abnormalities upon palpation of the long bones. Blood smear evaluation revealed mild left shift with mild to moderate toxicity in neutrophils. A small number of monocytes showed reactive changes and 82% of the neutrophils and monocytes were parasitized by *H. canis* gamonts (Figure 3). The pup was treated with another imidocarb dipropionate injection at the previous dose and discharged on the same day.

The last follow-up was preformed two and a half months later, four months after the first presentation. The owner reported that that the dog had normal behavior and appetite, without any evidence of pain or lameness. PE findings were unremarkable except for mildly enlarged popliteal lymph nodes. There was an improvement in the BCS (5/9) compared to the first admission. Abnormalities in the CBC (Table 1) included mild anisocytosis, and thrombocytopenia. Blood smear evaluation revealed mature non-toxic neutrophils and 36 % of the neutrophils and monocytes were parasitized by *H.* canis gamonts. Serum biochemistry (Table 2) abnormalities included hypoalbuminemia, hyperglobulinemia, and mild hyperphosphatemia.

Survey radiographs of the appendicular skeleton showed no evidence for the previously reported lesions. The pup was treated with a fourth imidocarb dipropionate injection at the previous dose and discharged. Eight months later, the owner reported by telephone that the dog was apparently normal and healthy.

DISCUSSION

Skeletal lesions, such as periosteal proliferation, are considered a major finding in dogs infected with *H. americanum* but have been reported only rarely in *H. canis* infection (9-11). In contrast to *H. canis*, the primary tissue sites for development of meronts in dogs with *H. americanum* infection are skeletal and cardiac muscles (12). In the target tissue, a cystic structure is formed around the infected cell. Clinical signs occur when the cyst ruptures inducing severe pyogranulomatous myositis. When such inflammation occurs in muscles adjacent to bones it may stimulate a marked periosteal reaction along bone surfaces. For dogs infected with *H. americanum*, radiographic findings may range from marked periosteal reaction to no evident changes. Periosteal bone proliferation has been associated with the attachment of muscle on most bones of the body except the skull (12).

Skeletal pain and sub-acute periostitis have been described in an experimental *H. canis* infection of dogs (13). However, this reaction was associated with the presence of *H. canis* meronts that were clearly visible in the periosteum by histopathology. Although skeletal muscle specimens were examined, parasites were not detected (13). No visible parasites have been reported in the periosteum of *H. americanum* infected dogs (14-16). The different radiographic characteristics of the periosteal reaction, mainly in terms of location of the lesions, combined with evidence of direct periosteal association with *H. canis* merogony leaves questions regarding the comparative pathogenesis of periosteal pathology in canine hepatozoonosis caused by *H. canis* and *H. americanum*.

Due to the fact that the pup was found infested with ticks, it is most likely that it was infected orally by ingestion of the vector tick or tick parts, containing *H. canis* sporozoites. Intrauterine transmission can also be considered in this case, as the pup was young, however, it can't be proven since the pup had obvious exposure to ticks.

A sub-clinical infection to mild disease is the most common presentation of HCI and it is usually associated with a low level of parasitemia (1–5%) (1). In the present case, when first presented 12 % of the neutrophils and monocytes were found to be infected which therefore represents a high level of parasitemia. During checkups, the percentage of neutrophils and monocytes infected became elevated to as high as 82 %. A level of >800 *H. canis* gamonts/μL was considered the cut-off level for a high *H. canis* parasitemia in a retrospective study (17). A significant difference was found when comparing clinical and hematological findings between the groups of dogs with high and low *H. canis* parasitemia. The number of gamonts was calculated in this retrospective study by multiplying the neutrophils number as determined by CBC, by the percentage of parasitized neutrophils found in the peripheral blood smear. The calculated number of *H. canis* gamonts/μL in the present case was not determined for

neutrophils alone, yet the level of 48 % observed in its second visit represent 4,325 *H. canis* gamonts/μL, well above this cut off and therefore, corresponds to a high level of parasitemia. Tissue meronts produce merozoites that eventually invade leukocytes and transform to gamonts. High *H. canis* parasitemia and tissue parasite load take their toll by demanding nutrients and exerting tissue injury. This explains the weight loss leading to cachexia and the profound lethargy observed in this subgroup of infected dogs (1, 2), and would explain the severity of clinical signs described in this case, e.g. the lethargy, fever, and poor body condition.

The hematologic and biochemical abnormalities reported in this pup included anemia, thrombocytopenia, hypoalbuminemia, hyperglobulinemia, elevated alkaline phosphatase activity and hypercalcemia. Anemia is the most common hematological abnormality in *H. canis* infection (1, 9, 17). This may be due to chronic inflammation, reduced erythropoiesis due to bone marrow suppression, or blood loss due to massive infestation with ticks, or to combination of several factors. Thrombocytopenia may result from a similar interference with production at the bone marrow but it may also be attributed to co-infection with *Ehrlichia* or *Anaplasma* species. In fact, such co-infections are considered common (1, 17, 18) and treatment for such potential infection in the case was the rational behind therapy with doxycycline even in light of the negative PCR results for *E. canis*. PCR is a sensitive method for the detection of *E. canis* DNA (19) and the p-30 based PCR assay used here was found to be 100 fold more sensitive compared to another commonly used assay, the 16S rDNA-based PCR (5). The PCR result may be falsely negative as a consequence of the earlier treatment with doxycycline and imidocarb dipropionate administered by the practitioner who referred the dog, or due to failure in detection of a minute amount of the pathogen's DNA in the sample.

In a study comparing simultaneous splenic sample PCR with Blood PCR for the detection of *E. canis*, negative blood samples were obtained from 5 experimentally infected dogs 9 days after treatment initiation. Samples obtained from 3 of these dogs' spleens were positive for *E. canis* (20). This finding may insinuate that treatment may first eliminate or reduce the parasite load in the blood before it is completely obliterated from the patient.

Hypoalbuminemia with hyperglobulinemia is yet another commonly reported finding in *H. canis* infection (1) and may be a consequence of decreased albumin production secondary to inflammation. Another possible explanation for the lowered albumin, loss through the kidney, was ruled out by the lack of proteinuria on urine analysis. Increased serum alkaline phosphatase activity is observed in all young dogs and it is up to 10-fold greater in normal puppies in comparison to adult dogs (21). It's unknown whether the alkaline phosphatase increase in this pup was the result of a pathologic or physiologic increase in osteoblastic activity. A similar debate is relevant for the mild hypercalcemia.

The pup's swollen joints and the joint's fluid analysis support the diagnosis of polyarthritis. The neutrophils infected with *H. canis* gamonts which were present in the fluid are most likely to have arisen from the blood. Tissue meronts were not seen in the smear and to the best of the author's knowledge, no reports implicate joints as a favorable site for *Hepatozoon* merogony. However, in one report (13), periosteal involvement with merogony was demonstrated in an experimental *H. canis* infection of dogs. Due to the proximity of the periosteum to the joint, the possibility of joint tissue merogony or a local inflammation secondary to the parasite's presence cannot be ruled out. Although the lack of success in culturing bacteria from the joint fluid and the inability to demonstrate bacteria by cytology cannot rule out bacterial polyarthritis, these negative results decrease the likelihood that such a process was responsible for the polyarthritis in this case. Additional mechanism that could explain polyarthritis would be an immune mediated process. To the best of our knowledge such a process has not been described previously in conjugation with HCI. In contrast, dogs with ehrlichiosis may develop lameness with a stiff gait secondary to polyarthropathy. Such joint disease may occur from hemarthosis or immune complex deposition with resultant arthritis and neutrophilic effusion into the joint (22). As previously mentioned a concurrent infection with *E. canis* could not be ruled out.

The pup's clinical signs were resolved and its hematological and radiographic parameters improved substantially with medical treatment. Despite that, *H. canis* gamonts were not eliminated from the blood. In fact, the *H. canis* gamont load increased over time. This finding is not surprising as it is reported (1) that achieving this goal in cases of heavy parasitemia requires four consecutive treatments or more, in two weeks interval. In this case, consecutive timely treatments were not done due to poor owner compliance. Whether this persistent infection will have clinical relevance in the fu-

ture is unclear. Clinical hepatozoonosis has been reported to be associated with young age and immunodeficient conditions (1). The fact that the pup matured during the follow up period and according to its owner's report was seemingly healthy supports the assumption that infection was brought under control of the immune system and although it was not eliminated, it was not responsible for clinical signs at that stage of the dog's life.

In conclusion, we report a case of canine hepatozoonosis, with profound concurrent periosteal reaction and polyarthritis. Further research is required to determine the pathogenesis of this unusual presentation and to determine the possible consequences of such skeletal involvement and persistent infection on the future well being of the infected dog..

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