

An unexpected guest in the joints of a puppy

Contributors

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Specimen

EDTA Synovial fluid/ Synovial fluid smear / synovial fluid analysis

Signalment

4-month-old mixed-breed (Griffon x Border Collie) male puppy

History

The puppy was referred for hyperthermia, lameness and joint pain, progressing for the past ten days. Three out of five puppies from the same litter exhibited similar clinical signs. Limited clinical information was available for the other affected puppies, as they were examined by veterinarians at different clinics.

Initial investigations by the referring veterinarian included a complete blood count (CBC), a serum biochemistry profile, and a SNAP 4Dx® test which yielded negative results. The CBC showed mild normocytic normochromic anemia, mild leukopenia due to mild neutropenia, and mild thrombocytopenia. No blood smear was performed to confirm these findings. The biochemistry profile revealed mild hyperglycemia, increased alkaline phosphatase (ALP) activity, mild hypocreatinemia, and elevated C-reactive protein concentration (CRP), compared with reference intervals in adults (Table 1).

The puppy was initially treated with oral doxycycline (10 mg/kg) and meloxicam (0.1 mg/kg). In the absence of significant clinical improvement, the puppy was then hospitalized for two days, placed on intravenous fluids, and treated with imidocarb. Doxycycline treatment was continued. PCR assays performed on blood samples for *Anaplasma*, *Babesia*, and *Ehrlichia* spp. were all negative. Serological testing for *Anaplasma phagocytophilum* (IgG ELISA), *Borrelia burgdorferi* (ELISA), and *Ehrlichia canis* (ELISA) was also negative. An abdominal ultrasound examination was performed, with no abnormalities detected. The puppy was then referred for joint taps and further management.

	Result	Reference Interval in adults
Glucose (mmol/L)	6.1	4.1 - 7.0
Urea (mmol/L)	4.0	3.2 - 10
Creatinine (μmol/L)	18.6	35.4 - 124
Total proteins (g/L)	77	50 - 72
ALT (U/L)	18	17 - 78
ALP (U/L)	121	15 - 85

Table 1: Results of blood biochemistry (FUJI DRI-CHEM NX500)

Clinical findings

Clinical examination in the referral clinic showed similar abnormalities already described. A computed tomography scan was performed and was suggestive of non-erosive polyarthritis. Synovial fluid examination of knees, shoulders, and elbows was performed (Figure 1,2 and Table 2). Serum capillary zone electrophoresis revealed moderate hypoalbuminemia and hyper- α -globulinemia (Figure 3 and Table 3).

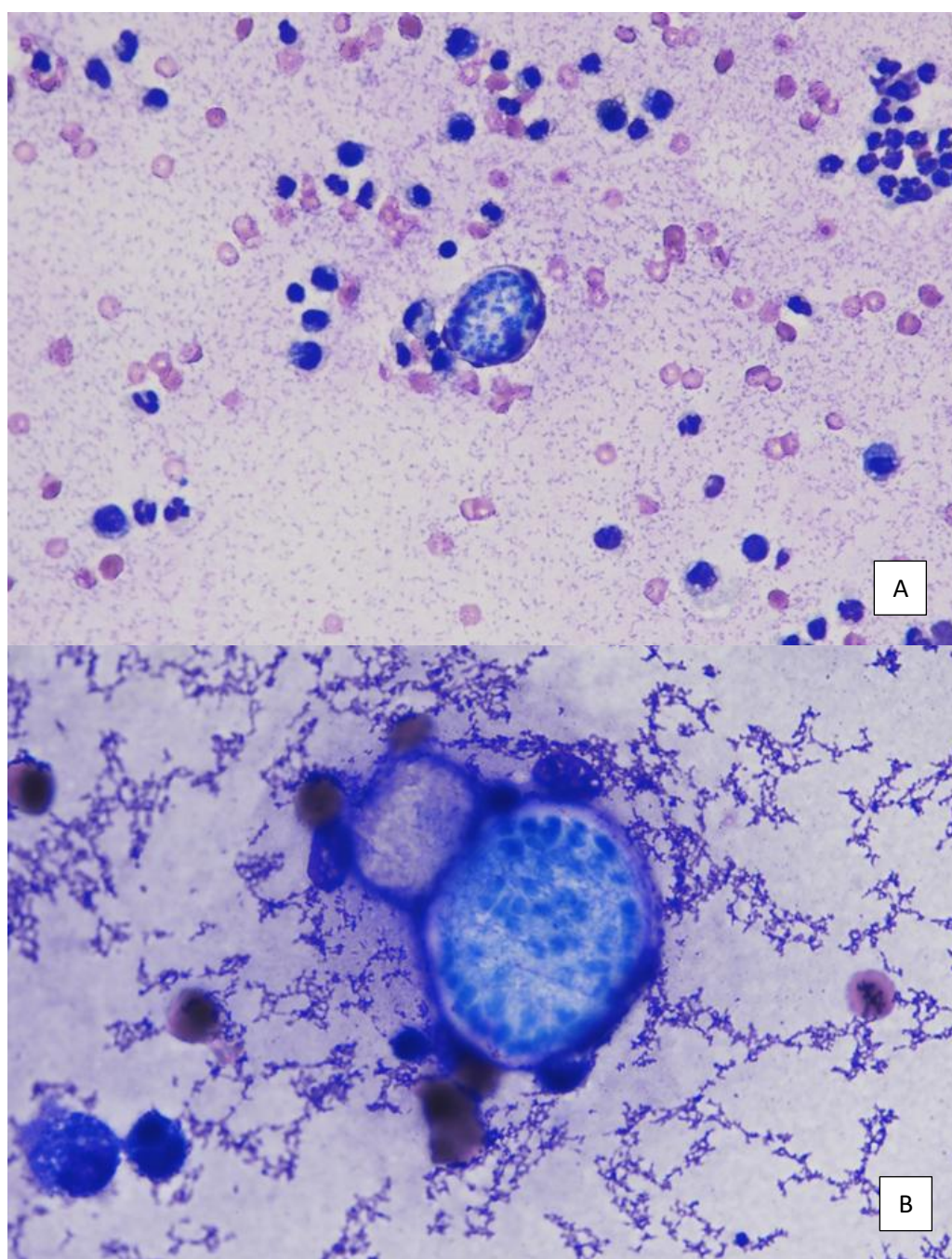


Figure 1 -A, B: Photomicrographs of synovial fluid smears of the puppy, May-Grunwald-Giemsa stain, x500 (A), x1000, oil (B)

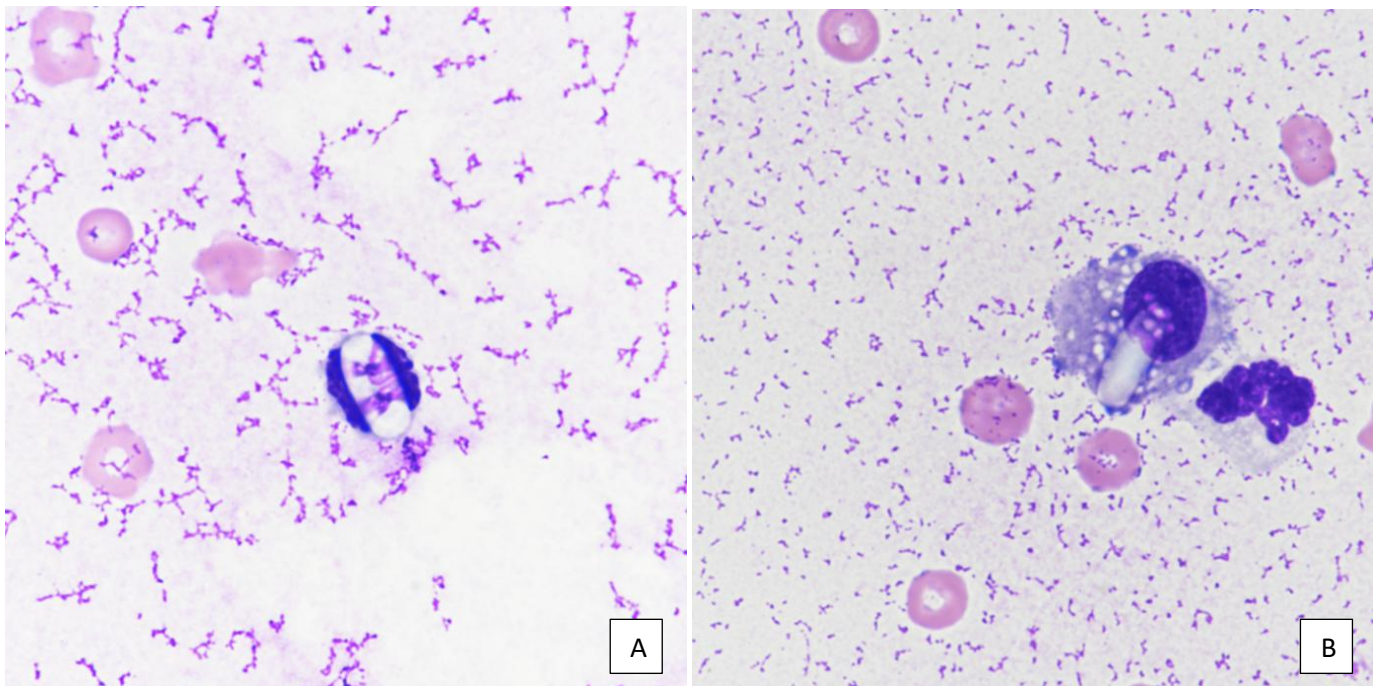


Figure 2 -A, B: Photomicrographs of synovial fluid smears of the puppy, May-Grunwald-Giemsa stain, x1000, oil

	Result	Reference Interval in adult
Proteins concentration (g/L)	62	< 30
Nucleated cell count ($10^9/L$)	48.30	< 3.00
Neutrophils (%)	51	<5
Macrophages (%)	49	>95
Red blood cell count ($10^{12}/L$)	0.20	< 0.01

Table 2: Results of synovial fluid composition. Nucleated cell count was measured using a Sysmex XN-1000V analyzer, and protein concentration was determined by refractometry.

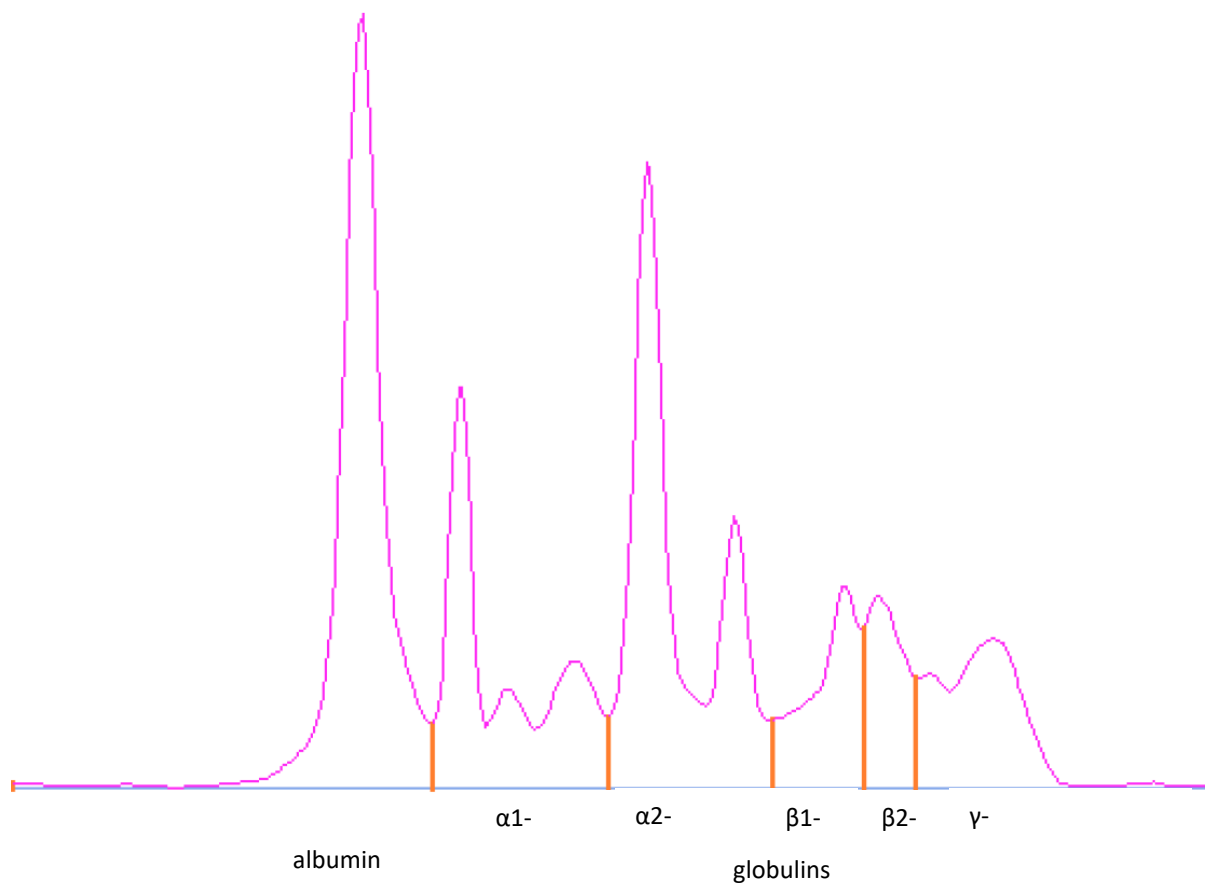


Figure 3: Serum capillary zone electrophoresis profile (Sebia CAPILLARYS)

	Result	Reference interval in adult
Albumin (g/dL)	1.56	2.86-4.36
α -globulins (g/dL)	2.34	0.63-1.21
$\alpha 1$ -globulins (g/dL)	0.92	0.25-0.47
$\alpha 2$ -globulins (g/dL)	1.42	0.33-0.83
β globulins (g/dL)	0.79	0.75-1.52
$\beta 1$ -globulins (g/dL)	0.45	0.31-0.92
$\beta 2$ -globulins (g/dL)	0.34	0.34-0.81
γ -globulins (g/dL)	0.60	0.3-1.2

Table 3: Quantitative composition of serum proteins fractions by serum capillary zone electrophoresis (Sebia CAPILLARYS)

Questions

- 1) What is your description of the cytological specimens?
- 2) Based on the clinical and pathological findings, what is your main diagnosis?
- 3) What additional tests would you use for confirming a diagnosis and further characterize it?

Interpretation/Diagnosis

Answer to question 1 (“What is your description of the cytological specimens?”): On a slightly to moderately hemorrhagic background, with an azurophilic granular matrix, there was an abundant leukocyte population. This population consisted of approximately equal proportions of segmented neutrophils and mononuclear cells. Most mononuclear cells were macrophages, often microvacuolated and occasionally phagocytic of tingible bodies. Rare cylindrical elements, approximately 10 x 5 µm in size, ranging from pale to transparent, phagocytosed by neutrophils and macrophages, were observed. These were highly suggestive of *Hepatozoon* spp. gamonts (Figure 2). Some giant cells, probably macrophagic cells, are phagocytizing large ovoid structures, approximately 50 µm in diameter, composed of multiples round, dark basophilic elements separated by optically empty vacuoles with more or less distinct boundaries, suggestive of *Hepatozoon* spp. meronts (Figure 1).

Answer to question 2 (“Based on the clinical and pathological findings, what is your main diagnosis?”): Based on synovial fluid examination, suppurative arthritis secondary to *Hepatozoon* spp. infection was diagnosed.

Additional information

Answer to question 3 (“What additional tests would you use for confirming a diagnosis and further characterize it?”): PCR analysis of the synovial effusion was positive for *Hepatozoon* spp. PCR analysis of blood was negative for Canine Distemper Virus, Canine Parvovirus, and *Leishmania* spp.

Another puppy was also tested positive for *Hepatozoon* spp. by PCR on a blood sample, but no information was available for the other puppies.

Follow up and clinical outcome

The puppy was treated with imidocarb (unknown dose, as the puppy was sent back to the primary veterinarian) every two weeks, for a total of six administrations, in addition to the doxycycline (10mg/kg) which had been started at the onset of clinical signs. The puppy showed clinical improvement and gained weight.

While waiting for PCR results, the puppy received prednisolone (0.8 mg/kg) that was progressively reduced after the detection of *Hepatozoon* spp.

Discussion

To the best of our knowledge, this is the first report of cytologically identified *Hepatozoon* meronts, with concomitant identification of gamonts, in synovial fluid from a puppy with suppurative polyarthritis. The morphology of the intraleukocytic structures is characteristic of *Hepatozoon* spp., which are typically observed in canine blood smears. Blood contamination is an unlikely source of the observed gamonts, given their presence on minimally hemorrhagic slides. Moreover, meronts have not been described in peripheral blood but have been described in bone marrow aspirates (Baneth et al., 2007). *Hepatozoon* spp. gamonts have previously been reported within leukocytes in the synovial fluid of a wild canid (*Chrysocyon brachyurus*) in Brazil (Perles et al., 2019). That animal presented with joint swelling after one month of hospitalization following limb amputation due to musculoskeletal trauma. In that case, no gamonts were detected in leukocytes on blood smear or buffy coat examination, whereas PCR testing of both blood and synovial fluid was positive for *Hepatozoon* spp.

Genomic analysis identified the organism as *Hepatozoon canis*. Another case, more similar to ours, has been described and involved a puppy that presented with hyperthermia, pale mucous membranes, tick infestation, and severe polyarthralgia. *Hepatozoon canis* gamonts were observed in the blood but not in the synovial fluid. Analysis of the latter was consistent with aseptic polyarthritis. Despite treatment, the puppy ultimately had to be euthanized because of clinical deterioration. Meronts were then identified on histopathology in the spleen, lymph nodes, liver, and joint capsules (Iveli et al., 2015).

The large structures observed within macrophagic cells are suggestive of *Hepatozoon* spp. meronts, although cystic stages of other infectious agents such as *Toxoplasma gondii*, *Neospora caninum*, or *Sarcocystis* spp. cannot be completely excluded. PCR was not performed on these three infectious agents. However, in this case, it is reasonable to hypothesize that these meronts are of the same origin as the gamonts identified. Furthermore, the non-cystic stages of the aforementioned organisms are morphologically distinct from *Hepatozoon* spp. gamonts, and none were observed in this case. Tissue cysts or meronts of *Toxoplasma gondii*, *Neospora caninum*, and *Sarcocystis* spp. are typically found in tissues (central nervous system, muscles...) and have not been described in canine synovial fluid. Finally, clinical remission would not have been expected with the treatment administered in cases of toxoplasmosis or neosporosis. Regarding *Sarcocystis* spp., there is currently no consensus on an effective treatment (Sykes, 2022).

The diagnosis of hepatozoonosis can be established by identifying gamonts on blood smears or buffy coat preparations; however, these methods have limited sensitivity for *Hepatozoon canis* (18.4% for blood smears and 69.4% for buffy coat) (Otranto et al., 2011). These examinations were not performed in the puppy described in this study. PCR testing on blood or buffy coat remains the most sensitive diagnostic method (85.7%) (Otranto et al., 2011). In this case, PCR enabled the definitive diagnosis of hepatozoonosis in the affected puppy, as well as in another puppy from the same litter presenting with similar clinical signs. While PCR allows detection of *Hepatozoon* spp., it does not differentiate between *Hepatozoon americanum* and *Hepatozoon canis*, the two species known to infect dogs. Species differentiation is usually performed with sequencing methods, which were not performed in this case. Furthermore, the gold standard for the diagnosis of american hepatozoonosis is muscle biopsy, which was not carried out in this study. Although sequencing was not performed, *Hepatozoon canis* is strongly suspected in the puppy described in this study. *Hepatozoon americanum* is primarily found in North America and has also been reported in domestic dogs in Brazil, whereas *H. canis* has a worldwide distribution (Allen et al., 2011; Gomes et al., 2016). In histopathological samples, meronts of *H. americanum* typically exhibit an “onion-skin” appearance, while those of *H. canis* display a “wheel-spoke” pattern. Although histopathological examination was not performed in this case, some micromerozoites within the observed meronts were arranged peripherally, strongly suggesting a “wheel-spoke” morphology and therefore supporting *H. canis* infection (Figure 1).

Dogs infected with *Hepatozoon canis* typically exhibit low parasitemia and few or no clinical signs (Otranto et al., 2011). In such cases, the observation of gamonts on a blood smear may be an incidental finding. However, some dogs- particularly those that are immunocompromised, may develop more severe clinical signs, including weight loss, fever, lethargy, and organ involvement such as hepatitis, glomerulonephritis, or pneumonia. These manifestations are associated with the presence of meronts and the accompanying immune response are often linked to higher levels of parasitemia. Hematological and biochemical abnormalities may include anemia (regenerative or non-regenerative), polyclonal gammopathy, and hypoalbuminemia (Allen et al., 2011; Baneth & Weigler, 1997; Sakuma et al., 2009). In our case, the puppy had hyperalphaglobulinemia involving both the α 1- and α 2-globulin fractions, hyperthermia, lameness and joint pain with no other organic abnormalities detected. In contrast, infection with *H. americanum* results in a severe disease that can affect even immunocompetent dogs. Common clinical signs include fever, lameness, and pain. The prognosis is guarded, with affected dogs often dying within one to two years without appropriate treatment.

Parasitemia is typically very low, although marked neutrophilic leukocytosis can be observed. Periosteal bone proliferation is also a common finding.

In dogs, which act as intermediate hosts, infection occurs through ingestion of a tick, the definitive host, harboring mature oocysts of the parasite. The tick vector species are *Amblyomma maculatum* for *H. americanum* and *Rhipicephalus sanguineus* for *H. canis*. Alternative routes of transmission have also been described, including ingestion of monozytic cysts from an infected intermediate host and vertical transmission (Schäfer et al., 2022). In the present case, the route of infection remains undetermined and may involve either vertical transmission or ingestion of infected ticks. Following ingestion, oocysts release sporozoites that disseminate and localize within macrophages in tissues such as the spleen, liver, lymph nodes, and bone marrow. Asexual replication (merogony) then occurs, leading to the formation of macromeronts containing macromerozoites or meronts containing micromerozoites. After rupture of macromeronts, macromerozoites give rise to new meronts, whereas micromerozoites released from meronts infect neutrophils and monocytes, where they develop into gamonts. Ticks subsequently become infected by ingesting leukocytes containing gamonts. In experimental infections, gamonts can typically be detected in peripheral blood approximately four weeks post-infection (Allen et al., 2011; Sykes, 2022).

This case has several limitations. A more comprehensive investigation for infectious agents in the synovial fluid, such as those discussed above, would have been valuable, particularly as *Hepatozoon* spp. infections can occur in association with other pathogens. However, some of these agents were ruled out by PCR testing on blood samples. Leishmaniasis, for which PCR testing was performed only on blood and returned negative, appears unlikely given the favorable response to antiparasitic treatment. In addition, performing quantitative PCR for *Hepatozoon* spp. on blood, along with a blood smear and potentially a bone marrow examination, would have been valuable to assess the level of parasitemia, as high parasite burdens are often associated with more severe forms of the disease.

In conclusion, this is an unusual case describing canine hepatozoonosis, an infection typically considered of low pathogenicity, identified in the synovial fluid of a puppy and likely affecting the entire litter.

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