UNINVITED GUESTS: BLOOD SMEAR FROM A DOG

Ioannis L. Oikonomidis¹*, Theodora K. Tsouloufi¹, Mathios E. Mylonakis², Dimitra Psalla³, Nektarios Soubasis², Timoleon Rallis², Maria Kritsepi-Konstantinou¹

¹Diagnostic Laboratory, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
²Clinic of Companion Animal Medicine, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
³Laboratory of Pathology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

*Corresponding author: Email address: economidis.john@gmail.com
CASE PRESENTATION

Specimen

Giemsa-stained peripheral blood smear (Figure 1).

Signalment

Dog, Poodle cross, 5-year-old, intact male, 6.7 kg.

History

The dog was presented with intermittent vomiting, anorexia and weight loss for the previous 3 weeks. It was housed indoors and was not fully vaccinated and dewormed.

Clinical signs

Physical examination revealed mild hypothermia, emaciation, lethargy, moderate dehydration, and respiratory distress. Inspection of the oral cavity disclosed the presence of an ulcerated lesion on the tongue, while abdominal palpation suggested the presence of splenomegaly. Arterial blood pressure was normal.

Clinicopathologic investigation

Abnormal findings identified in the complete blood count and serum biochemistry panel analysis are presented in Table 1. A Giemsa-stained blood smear was microscopically examined for the assessment of white blood cells differential count and morphology.
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Units</th>
<th>Result</th>
<th>Reference intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>$10^6/\mu L$</td>
<td>5.14</td>
<td>5.50-8.50</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>g/dL</td>
<td>11.8</td>
<td>12.0-18.0</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>%</td>
<td>35.5</td>
<td>37.0-55.0</td>
</tr>
<tr>
<td>White blood cells</td>
<td>$10^3/\mu L$</td>
<td>35.7</td>
<td>6.0-17.0</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>$10^3/\mu L$</td>
<td>29.7</td>
<td>3.9-8.0</td>
</tr>
<tr>
<td>Monocytes</td>
<td>$10^3/\mu L$</td>
<td>1.6</td>
<td>0.2-1.1</td>
</tr>
<tr>
<td>Total proteins</td>
<td>g/dL</td>
<td>4.7</td>
<td>5.5-8.0</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/dL</td>
<td>1.3</td>
<td>2.9-4.0</td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td>mg/dL</td>
<td>81</td>
<td>10-38</td>
</tr>
<tr>
<td>Creatinine</td>
<td>mg/dL</td>
<td>1.2</td>
<td>0.7-1.3</td>
</tr>
<tr>
<td>ALT</td>
<td>IU/L</td>
<td>21</td>
<td>18-62</td>
</tr>
<tr>
<td>ALP</td>
<td>IU/L</td>
<td>679</td>
<td>32-149</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>mg/dL</td>
<td>2.2</td>
<td>0.2-0.6</td>
</tr>
<tr>
<td>Total calcium</td>
<td>mg/dL</td>
<td>7.0</td>
<td>8.6-10.9</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>mg/dL</td>
<td>6.5</td>
<td>2.2-6.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>mEq/L</td>
<td>3.9</td>
<td>3.7-5.9</td>
</tr>
<tr>
<td>Sodium</td>
<td>mEq/L</td>
<td>150</td>
<td>144-158</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>mg/dL</td>
<td>363</td>
<td>125-296</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>mg/dL</td>
<td>207</td>
<td>24-102</td>
</tr>
</tbody>
</table>

Table 1: Clinicopathologic investigation at presentation.

Questions/tasks:

Evaluate the following images and provide your diagnostic interpretation (Figure 1A and 1B).
Figure 1A (63x objective)

Figure 1B (100x objective)
Cytologic description

The microscopic examination of the peripheral blood smears revealed a mild left shift (band neutrophils: 1,785/μL). Several mainly intracytoplasmic (neutrophils, monocytes) and extracellularly located round to oval organisms, 2-5 μm in length, with discrete cell membrane, eccentrically-placed nucleus and a distinct kinetoplast, consistent with *Leishmania* spp. amastigotes, were observed during the microscopic examination of plain and buffy coat blood smears. A few microfilariae were also found. After a thorough evaluation of plain blood smears (review of 1,000 neutrophils, 200 monocytes and 50 eosinophils), 0.2% of neutrophils were found to be parasitized by *Leishmania* spp. amastigotes. The evaluation of buffy coat blood smears (review of 1,000 neutrophils, 1,000 monocytes and 200 eosinophils) indicated a 0.5% and 0.1% percentage of infected neutrophils and monocytes, respectively.

The cytologic examination of Giemsa-stained bone marrow aspiration smears revealed hypercellularity with moderate myeloid and mild plasmacytic hyperplasia, while histiocytes percentage was at the upper normal range. Numerous *Leishmania* spp. amastigotes were observed extracellularly or phagocytized by macrophages, segmented neutrophils, neutrophil precursors and even eosinophils (Figure 2 and 3).
Figure 2 (100x objective)

Figure 3 (100x objective)
Diagnostic imaging
Thoracic radiographs disclosed the presence of diffuse interstitial lung infiltrates, while abdominal ultrasonography indicated a peritoneal effusion and a diffusely hypoechogenic splenic parenchyma.

Serology
IFA titer for anti-leishmanial antibodies: 1/1,600 (cut-off titer: ≥1/100).
Point-of-care ELISA for Dirofilaria immitis antigen and antibodies to Ehrlichia spp., Borrelia burgdorferi and Anaplasma spp.: negative.

Knott's test for microfilariae
A few microfilariae, consistent with Dirofilaria immitis, were found.

Additional serum biochemical analysis
Lipase: 195 IU/L (reference intervals: 5-32 IU/L).
Bile acids (fasting sample): 9 mg/dL (reference intervals: 0-12 mg/dL).
Canine pancreatic lipase immunoreactivity: 1471 μg/L (reference intervals: 0-200 μg/L, pancreatic inflammation: >400 μg/L).

Urinalysis
Specific gravity: 1.020.
Protein-to-creatinine ratio: 11.5 (reference intervals: <0.5).
Microscopic examination of the sediment: unremarkable.

Peritoneal fluid analysis
Total nucleated cell count: 100/μL.
Red blood cells count: 0/μL.
Total proteins: 0 g/dL.
Creatinine: 1.0 mg/dL.
Potassium: 2.7 mEq/L.
Lipase: 1,579 IU/L.
Cytologic examination of the sediment: Rare non-degenerated neutrophils and small lymphocytes were observed. No microorganisms were found.

**Microbiology**

Cultures of urine and peritoneal fluid were negative for bacteria.

**Short-term clinical and laboratory follow-up**

A tentative diagnosis of nephrotic syndrome, chronic kidney disease of IRIS stage I (with proteinouria, without blood hypertension) and pancreatitis, as well as a definitive diagnosis of leishmaniosis and dirofilariasis was established. The dog was hospitalized for 4 days, during which crystalloids, antibiotics, allopurinol, tramadol and acetylcysteine were administered.

**Outcome**

Due to the deteriorating clinical condition and poor long-term prognosis, the dog was euthanized with its owner’s consent. Necropsy was subsequently performed.

**Gross pathology**

During heart evaluation, a few adult heartworms were found in the right ventricle.

**Histopathology**

Histologic examination of the pancreas revealed few focally extensive areas of necrosis, characterized by cellular debris admixed with few degenerated neutrophils, surrounded by
macrophages, lymphocytes, plasma cells and a peripheral rim of reactive fibroblasts. The inflammatory infiltration was focally extending to the adjacent adipose tissue. The morphologic diagnosis was chronic moderate granulomatous pancreatitis (Figure 4).

![Figure 4 (10x objective)](image)

**Discussion**

The present case is noteworthy because of the visualization of *Leishmania* spp. amastigotes during routine peripheral blood smear examination in a dog. Although the molecular detection of *Leishmania* spp. in peripheral blood samples is commonly reported,¹ documentation of amastigotes during the routine cytologic examination of peripheral blood smears is rare² and to the authors’ knowledge, it has been previously reported in nine dogs²-⁷. The diagnostic sensitivity of blood buffy coat smears for the detection of *Leishmania* spp. amastigotes is reportedly 50%.¹

The presence of *Leishmania* spp. amastigotes in peripheral blood suggests a hematogenous systemic spread of the infection. No correlation has been found between the incidence of
Leishmania spp. amastigotes in the peripheral blood and the clinical or laboratory status of the affected dog.\(^1\) In this case, Leishmania spp. amastigotes were seen in plain and buffy coat blood smears both extracellularly and phagocytized by neutrophils and monocytes, as has been reported in the relevant literature.\(^2\)\(^-\)\(^7\) When cytologically found, Leishmania spp. amastigotes are mostly phagocytized by macrophages, since the latter cell type constitutes the typical cellular host of the parasites. In contrast, in peripheral blood, Leishmania spp. amastigotes may frequently be seen phagocytized by neutrophils.\(^1\) Although the role of neutrophils in the pathogenesis of leishmaniosis has not been completely clarified, there is solid evidence supporting the so-called "Trojan horse" model, which suggests a "silent entry" of Leishmania spp. amastigotes into the macrophages via phagocytosis of infected apoptotic neutrophils, that were initially used as a temporary shelter for the protozoa.\(^7\) Interestingly, in the present case, Leishmania spp. amastigotes were also found inside eosinophils and neutrophil precursors during bone marrow cytologic examination. To the best of our knowledge, the phagocytosis of Leishmania spp. amastigotes by the aforementioned cells has not been previously described.

Importantly, in the present case, the dog exhibited clinical and clinicopathologic findings consistent with pancreatitis, which was eventually confirmed via histopathologic examination of the pancreas. Although a direct association between pancreatitis and leishmaniosis is not adequately substantiated by the current literature, in our case, chronic granulomatous pancreatitis could be attributed to leishmaniosis, since granulomatous inflammation is a primary pathogenic mechanism in canine leishmaniosis.\(^9\)

In conclusion, the detection of Leishmania spp. amastigotes in peripheral blood is rare, while its associated clinical relevance has yet to be fully investigated. Routine cytologic screening of plain peripheral blood smears in dogs suspected of leishmaniosis is not currently advised for establishing a diagnosis, due to the lower diagnostic sensitivity compared with bone marrow or lymph node cytology. However, evaluation of peripheral blood buffy coat smears may be of higher diagnostic sensitivity.
References


