Case (with diagnosis)

A DOG WITH A GASTRIC FOREIGN BODY AND LEISHMANIASIS

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Signalment
An 8-year-old male, English setter, dog.

History and clinical findings
The dog was referred to the San Francesco Veterinary Hospital in Milan, to perform an ultrasonography for a suspicious gastric foreign body, detected by rx.
He was adopted from Calabria (Southern Italy) and, from then, he was vaccinated and treated for both endo and ectoparasites with imidacloprid+permetrine-based product (Advantix® spot on). Before the presentation, he was already diagnosed with canine leishmaniasis (antibody title 1:160) and he was treated with miltefosine and allopurinol (the latter one was ongoing at the moment of the referral).
He had an history of lethargy and dysorexia, with intermittent hyperthermia from three days before the admittance. At the admission, the general health status was good but confirmed the hyperthermia (39.7°C) and the lethargy.

Diagnostic procedures
Due to the aspecific signs and clinical findings, several analyses were performed in order to better investigate the cause for this illness. Since the dog was referred for a foreign body suspicion, an abdomen ultrasonography was performed. Biochemical analyses with BT1500vet (Futurlab, Limena, Padua, Italy) and complete blood count (CBC) using ProCyte DX Hematology Analyzer (IDEXX Laboratories Italia s.r.l., Milan, Italy) were performed at the admission. Moreover, since the dog was already diagnosed with leishmaniasis, in the following days, urinalysis, *Leishmania* antibody titer with IFAT and serum protein electrophoresis were performed. Due to CBC results and the geographical area in which the dog lives (Lombardy, a region where different tick species are present), a rapid immunochromatographic test for vector-borne diseases (SNAP® 4DX® Test, IDEXX Laboratories Italia, Milan, Italy) and a coagulation profile (ACL7000, Instrumentation Laboratory, Munich, Germany) were performed.

Ultrasonography confirmed the presence of a gastric foreign body associated with signs of gastritis (probably due to a mechanic damage). Moreover, there was splenomegaly, abdominal lymph nodes enlargement and signs of chronic nephropathy.

Biochemistry was unremarkable except for a mild hypoalbuminemia and hyperglobulinemia, increased C-reactive protein (CRP) and slightly increased amylase (**TABLE 1**).

TABLE 1. Biochemical results (BT1500vet, Futurlab, Limena, Padua, Italy)
***** = Abnormal results

ALT (U/L)	25	15-78
AST (U/L)	42	10-44
ALP (U/L)	77	16-119
γGT (U/L)	3	0.0-11
Amylase (U/L)	**2035***	338-1800
Lipase (U/L)	124	20-160
LDH (U/L)	78	45-233
CK (U/L)	121	40-150
Total Bilirubin (mg/dL)	0.17	0.0-0.45
Cholesterol (mg/dL)	234	156-369
Triglycerides (mg/dL)	85	30-112
Glucose (mg/dL)	91	74-120
Total Protein (g/dL)	6.5	5.7-8.0
Albumin (g/dL)	**1.7 ***	2.8-4.0
Globulin (g/dL)	**4.8 ***	2.4-4.5
A/G	**0.35 ***	0.5-1.3
Creatinine (mg/dL)	0.96	0.5-1.8
Urea (mg/dL)	27	15-50
Calcium (mg/dL)	10.3	7.3-11.3
Phosphorus (mg/dL)	4.1	2.6-6.2
Na^+ (mmol/L)	146	140-154
K^+ (mmol/L)	4.3	3.8-5.6
Cl^- (mmol/L)	117	102-117
CRP (mg/dL)	**3.51 ***	0.00-1.07

Complete blood count revealed a severe thrombocytopenia, mild normocytic normochromic anemia, leukopenia with neutropenia and mild monocytosis and eosinopenia (**TABLE 2**).

TABLE 2. Hematological results (Procyte DX, IDEXX Laboratories Italia, Milan, Italy)
***** = Abnormal results

RBC (10^{12}/L)	**5.08 ***	5.65 - 8.87
HCT (%)	**31.9 ***	37.3 - 61.7
HGB (g/dL)	**11.4 ***	13.1 - 20.5
MCV (fL)	62.8	61.6 - 73.5
MCH (pg)	22.4	21.2 - 25.9
MCHC (g/dL)	35.7	32.0 - 37.9
RDW (%)	15.5	13.6 - 21.7
%RETIC	0.2	
RETIC (10^9/L)	12.2	10.0 - 110.0
WBC (10^9/L)	**4.43 ***	5.05 - 16.76
%NEUTROPHILS	44.9	
%LYMPHOCYTES	27.1	

%MONOCYTES	26.4	
%EOSINOPHILS	0.9	
%BASOPHILS	0.7	
NEUTROPHILS (10^9/L)	**1.99 ***	2.95 - 11.64
LYMPHOCYTES (10^9/L)	1.20	1.05 - 5.10
MONOCYTES (10^9/L)	**1.17 ***	0.16 - 1.12
EOSINOPHILS (10^9/L)	**0.04 ***	0.06 - 1.23
BASOPHILS (10^9/L)	0.03	0.00 - 0.10
PLT (10^9/L)	**21 ***	148 - 484

Urinalysis performed by cystocentesis underlined a frank proteinuria (**TABLE 3**) and the presence of granular casts.

TABLE 3. Urinalysis results. Urine collected by cystocentesis
In bold the abnormal results

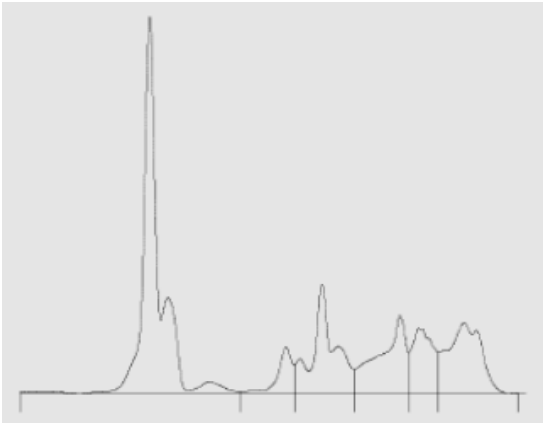
PHYSICAL – CHEMICAL EXAMINATION (dipstick)		
Visual examination:		golden yellow, turbid
USG:	1032	1015 - 1045
pH:	7	5,5 - 7,5
Protein:	**+++**	negative/+
Glucose:	negative	negative
Ketones:	negative	negative
Blood:	**++**	negative
Bilirubin:	negative	negative/+
UP/UC:	**2.6**	< 0,5
MICROSCOPIC SEDIMENT EXAMINATION		
Leukocytes:	absent	<5/HPF
Erythrocytes:	absent	<5/HPF
Casts:	**granular ++**	absent - rare
Crystals:	absent	absent
Epithelial cells:	**+**	rare
Bacteria:	absent	absent

Leishmania antibody titer evaluated with IFAT was 1:1280 (negative until 1:80) and it was increased with respect of the previous one.

The capillary serum protein electrophoresis, performed with Minicap Sebia (Sebia Italia s.r.l., Bagno a Ripoli, Florence, Italy) showed a mild decrease of albumin, a slight increase of alpha 1 and beta 1 fraction and a decrease of the albumin/globulin ratio (**FIGURE 1**).

FIGURE 1. Capillary zone electrophoresis of the serum proteins (Minicap Sebia, Sebia Italia s.r.l., Bagno a Ripoli, Florence, Italy)
***** = Abnormal results

Total Protein (g/dl) **5.60*** (5.70 - 7.70)
A/G **0.69*** (0.80 - 1.90)



Albumin %	40.80	44.40 - 65.70	Albumin (g/dL)	**2.28***	2.40 - 4.90
Alpha total %	20.50	9.30 - 24.00	Alpha total (g/dL)	0.26	
Alpha 1 %	4.60	2.00 - 7.30	Alpha 1 (g/dL)	**0.89***	0.17 - 0.40
Alpha 2 %	15.90	6.20 - 16.70	Alpha 2 (g/dL)	0.76	0.40 - 1.00
Beta total %	22.90	9.20 - 31.80	Beta total (g/dL)	0.53	
Beta 1 %	13.50	2.90 - 11.10	Beta 1 (g/dL)	**1.15***	0.10 - 0.80
Beta 2 %	9.40	6.30 - 20.70	Beta 2 (g/dL)	1.28	0.40 - 1.60
Gamma %	15.80	4.50 - 20.10	Gamma (g/dL)	0.88	0.20 - 1.20

A rapid immunochromatographic assay (SNAP® 4DX® Test, IDEXX Laboratories Italia, Milan, Italy) for the detection of vector-borne diseases was also performed and resulted negative for all the tested pathogens (*Ehrlichia spp.*, *Anaplasma spp.*, *Borrelia burgdorferi* and *Dirofilaria immitis*).

The coagulation profile (ACL7000, Instrumentation Laboratory, Munich, Germany) showed a slight increase of aPTT (**TABLE 4**)

TABLE 4. Coagulation results.
***** = Abnormal results

aPTT (sec,):	**13,1 ***	8,6 - 12,8
PT (sec.)	8,1	5,8 9,5
FDP's (µg/mL)	<2,5	0 - 2,5
D-Dimer	0,4	0,01 - 0,50
Fibrinogen (mg/dL)	305	100 - 400
Antithrombin III (%)	110	100 - 150

What is your opinion about this case?

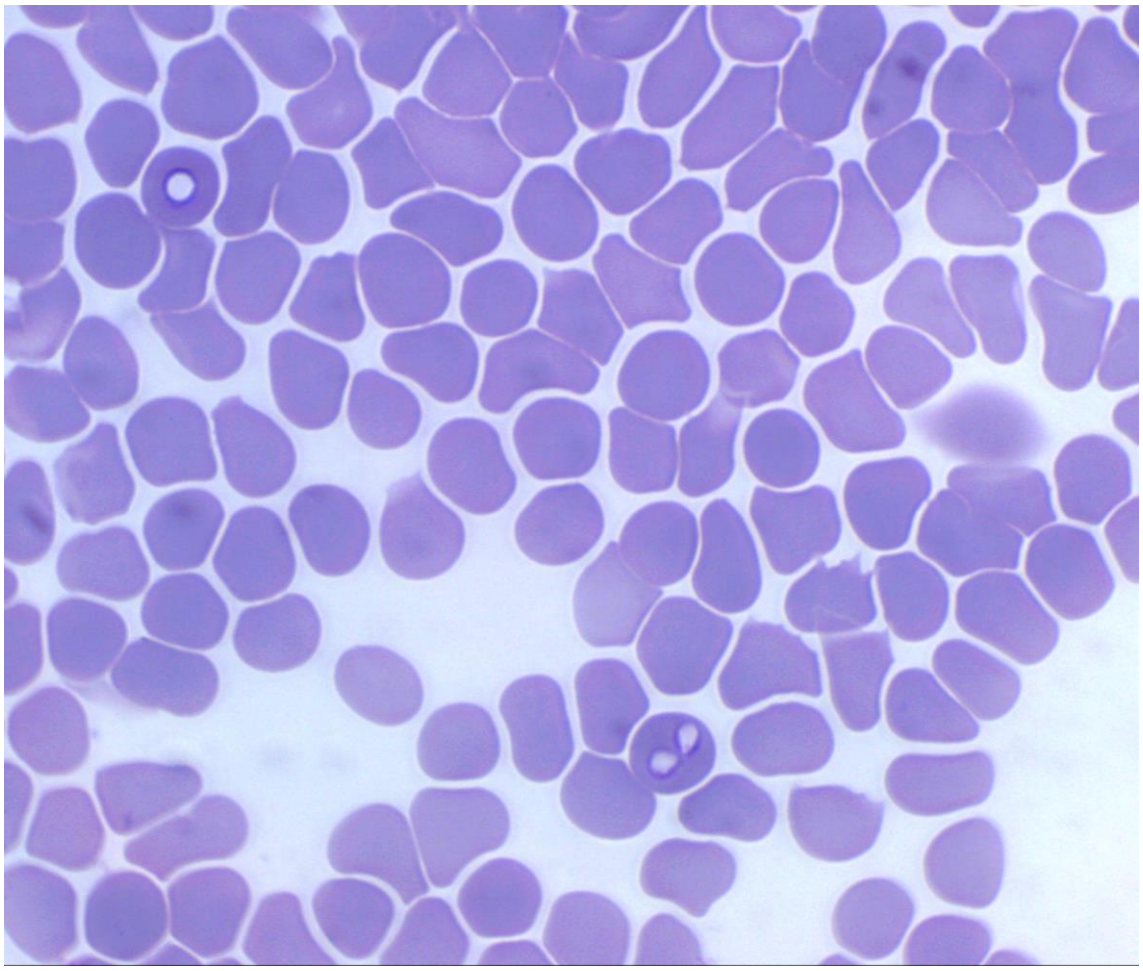
Is the foreign body causing all the clinical signs?

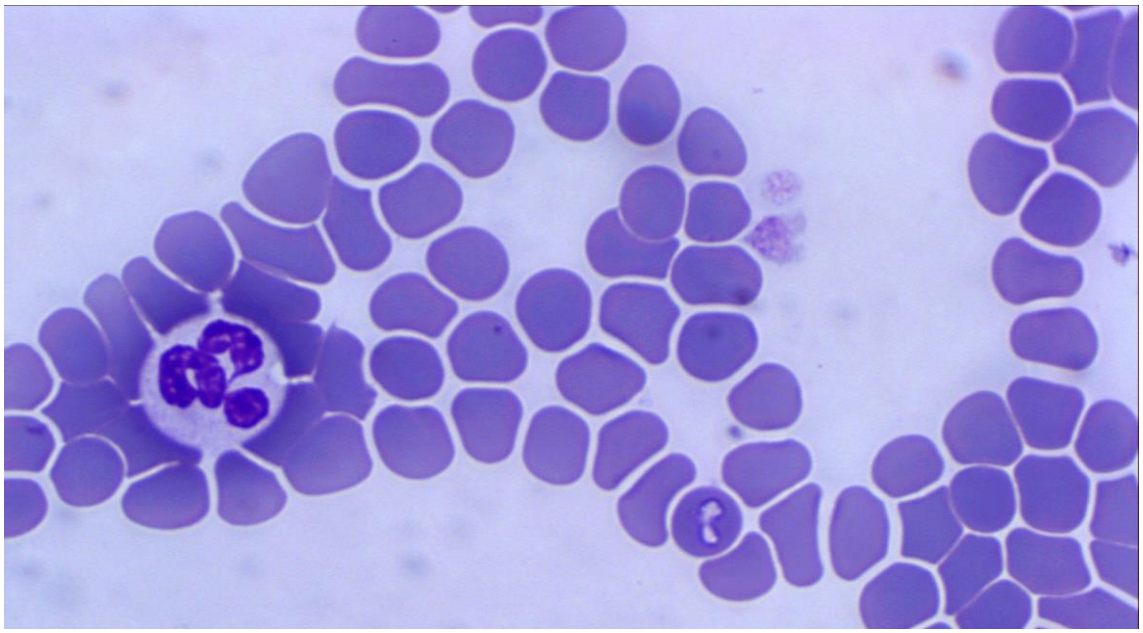
Is it *Leishmania* responsible for them?

Do we need some more information about the diagnostic procedures?

Actually, something from the previous analyses was missing, and that is to say the microscopic blood smear evaluation. This confirmed the thrombocytopenia and revealed a mild anisocytosis. Moreover, as you can see from the **FIGURE 2**, it revealed the presence of intraerythrocytic microorganisms, consistent with *Babesia* spp.

FIGURE 2. *Babesia* spp. in blood smear





The dog was treated with imidocarb dipropionate 6.6 mg/Kg IM and the treatment was repeated after two weeks, as recommended by literature[6,8]. After two weeks from the first treatment the dog was in good condition, thrombocytopenia and leukopenia were no longer present, the anemia improved but it was still persistent. *Babesia* was no longer detectable in the blood smear evaluation. After one month from the first treatment, the dog was no longer anemic.

Discussion

Babesiosis is a vector-borne disease transmitted by ticks. In northern Italy, *Babesia canis* is the most frequently described species. It is transmitted by *Dermacentor reticulatus* and it is considered a large *Babesia* (2.5x4.5 µm)[1]. *B. canis* is infective only 2-3 days after the tick's bite[2], probably due to the change of temperature correlated with the blood meal, which activates the maturation of the infective sporozoite[3]. Clinical signs of babesiosis can be very different, depending on the species and the strain involved. However, some of them are common across all the *Babesia* spp. infection, such as depression, weakness, anorexia, fever, spleen and lymph nodes enlargement, anemia, thrombocytopenia, jaundice and pigmenturia[4,5]. Hypoalbuminemia, hyperbilirubinemia and increased CRP are frequently found [1,6]. In addition, increased urinary protein/creatinine ratio is reported in the majority of severe *B. canis* infections, with associated hemoglobinuria and occasional granular casts[6]. It is though that systemic hypotension, leading to vasoconstriction, might be the most important cause of renal hypoxia in *B. canis* infections, but anemia may also contribute to inadequate oxygenation[7].

In light of all this, the signs and clinical findings observed in this case are consistent with babesiosis.

Depending on the species, anemia could be regenerative or nonregenerative (pre-regenerative)[4], but the latter one is more typical of the infection caused by *B. canis*, and it is caused by a combination of intravascular and extravascular hemolysis resulted from both rupture of red blood cells, increased osmotic fragility and the activity of secondary immune-mediated processes[3,6]. Thrombocytopenia is due to immunity, spleen sequestration or consumption due to hemolytic or vascular injury. However, abnormal coagulation parameters have not been reported frequently[4].

As already described in literature, microscopic blood smear examination is a useful tool for diagnosis of babesiosis in dogs and it is very easy to perform, even if the identification of the protozoa requires some experience. *Babesia*'s merozoite are evident inside the erythrocyte usually as a pair of pear-shaped parasites [3]. The sensitivity of this method is lower than the molecular diagnosis, in particular for *Babesia canis*, in which parasitemia is often low[5]. However, it is 100% specific, especially for what concern the large *Babesia*, which is easier to be identified[6]. When history and clinical signs are very suggestive for babesiosis, but the microscopic blood smear evaluation is negative for the presence of the parasite, it is possible to repeat the exam on the buffy coat of the centrifuged sample, or on a new sample collected from the peripheral capillary (ear tip or nail bed)[5].

For what concern treatment, as already said, imidocarb dipropionate is the treatment of choice for canine babesiosis. The most frequently described side effects are pain at the injection site and cholinergic signs (anorexia, hypersalivation, epiphora, abdominal pain, vomiting and diarrhea) which disappear quickly. Usually, the majority of dogs infected with large *Babesia* improve clinically in 1-7 days after the treatment[6]. However, owners should be aware that dogs may remain subclinically infected and may suffer a relapse[8].

In the present case, without an accurate screening of the blood smear, all the clinical findings could be related with leishmaniasis. In fact, normocytic normochromic anemia, thrombocytopenia, hypoalbuminemia, hyperglobulinemia, proteinuria and increased CRP are consistent with leishmaniasis, especially with regards of the IFAT result[9]. It must be underlined that no cross-reaction had been observed between *Babesia* and *Leishmania* when using IFAT as a diagnostic test, so the increased title is reliable[10]. However, severe thrombocytopenia, in dogs affected by leishmaniasis, is usually associated with a co-infection with other vector-borne pathogens[9].

So, in light of the excellent improvement after imidocarb therapy, it is possible to say that *Babesia* was the causative agent for the dog illness and for almost all its alterations in laboratory parameters. The presence of *Babesia* could have caused a relapse of *Leishmania* and this may explain the increased IFAT and the severe proteinuria. In fact, even if proteinuria could be present even in dogs affected by babesiosis, it is usually associated with more severe clinical signs and macroscopic hemoglobinuria[7]. In this case, clinical signs are moderate and it is possible to suppose that there is just a microscopic hemoglobinuria. In fact, urine dipstick revealed the presence of “blood” (which could mean both red blood cell or haemoglobin), but in the microscopic sediment examination there was no erythrocyte, so we could suppose that “blood” positivity in the dipstick actually was related to the presence of haemoglobin in urine.

The take-home message of this case is that, even when the diagnosis seems obvious, based on history, clinical examination and laboratory results, we should always be careful and do not underestimate the importance of each laboratory data. In particular, as already state, CBC should always go along with an accurate microscopic blood smear evaluation, at least for clinically ill patients[11].

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