

BABESIOSIS AND DIROFILARIASIS - TRIGGERS OR RISK FACTORS FOR CANINE LYMPHOMAS?

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Abstract

Lymphodysplasia is the reversible process by which antigen-stimulated adult B lymphocytes dedifferentiate into peripheral lymphoblasts, then into immunoblasts, plasmablasts and then plasma cells secreting antibodies against the antigen that stimulated them. In lymphoma, malignancy occurs in the intermediate stages of dedifferentiation with monoclonal immunoblastic or plasmablastic proliferation in Plasmacytoma with the secretion of enormous amounts of chimeric antibodies.

The pathogenic mechanism consider in this paper consists of prolonged antigen hyperstimulation with parasitic and cell-destructive proteins that cause an accentuated proliferation of undifferentiated adult lymphoblasts at the level of organized lymphoid structures: the spleen for centroblastic lymphoma and the mediastinal, mesenteric, peripheral submandibular, prescapular, superficial inguinal lymphocenters and polyploidy in multicentric lymphoma.

In oncology, it is a well-known fact that chronic inflammation is an important risk factor on the scale of carcinogenesis and the Babesia spp. and Dirofilaria spp. parasites determine exacerbated inflammatory reactions, their components and toxic excretions being the antigen in the puzzle.

Thus, we ask: canine lymphoma has not been demonstrated to have a clear trigger among the known etiological factors, but should we start looking for proof?

Key words: canine lymphoma, babesiosis, dirofilariasis, antigen hyperstimulation, inflammatory reaction.

INTRODUCTION

Canine malignant lymphomas are classified into a group of hemopathies originating in the reticuloendothelial system, which are divided into 2 forms: Hodgkin lymphomas and non-Hodgkin lymphomas.

Malignant non-Hodgkin lymphomas (MNL) represent malignant monoclonal proliferations of B lymphocytes in most cases and in rare cases of T lymphocytes, in various degrees of lymphocyte differentiation and maturation, the most common form being multicentric or generalized lymphoma.

The bacterial etiology with *Helicobacter pylori* in humans has been incriminated in the development of gastric diffuse large B-cell lymphoma (Keikha et al., 2022).

Bridgford et al., theorized in 2008, that there is a strong connection between “a positive *Helicobacter heilmannii* Hhe status with the

presence of feline gastric lymphoma, especially lymphoblastic lymphoma”.

Dirofilaria immitis (Heartworm disease) is transmitted by culicids (mosquitoes) and it is a helminthiasis that affects canids, manifested by the presence of the nematode in the heart and in the pulmonary arteries

This parasite's toxins take a toll on the liver, kidneys and secondarily on haematopoiesis and an intensely antigenic action, evidenced by the increase in serum immunoglobulins and the number of lymphocytes, monocytes and eosinophils in the peripheral blood.

“In addition, the simultaneous death of groups of adult worms can trigger an acute disease characterized by the exacerbation of inflammatory reactions and the emergence of serious thromboembolic events” (González et al., 2012).

Babesiosis is caused by unicellular endoglobular parasites that can be found in the erythrocytes.

The vectors are ticks (*Ixodes ricinus*) that transmit the larger *Babesia canis* and the smaller *Babesia gibsoni*. Babesias cause hemolytic anaemia through metabolic toxins and mechanical effects, causing agglutination of dead erythrocytes and adhesion to the capillary endothelium with thrombus formation and disseminated intravascular coagulation (DIC). In addition to the Babesia-induced haemolytic anaemia and hypoxia, some dogs develop a complicated to the babesiosis characterized by immune-mediated haemolytic anaemia (IMHA) and/or signs of inflammatory reactions (Ionita et al., 2023). This is explained by the fact that the structural antigenic components of the babesia are destroyed following specific therapy and this determines an antigenic hyperstimulation effect, which causes hyperplasia of the splenic white pulp and an intense lymphoproliferative and macrophagic reaction in all lymphoid tissues, especially in the lymph nodes - similar to the Macrophage activation syndrome (MAS) - a severe inflammatory systemic abnormality (Mechtounne et al., 2021).

MATERIALS AND METHODS

The evaluation of the 53 patients spanned over 38 months.

The canine patients were both male and female. The inclusion criteria for the study were:

- current diagnosis of malignant lymphoma (3 cases of centrocytic lymphoma, 23 cases of centroblastic lymphoma and 27 cases of lymphoblastic lymphoma);
- Dirofilariasis - 54% of dogs (chronic Dirofilariasis), 37% of dogs Babesiosis (at least 3 infestations/dog), 9% patients with Dirofilariasis and Babesiosis (Figure 1);
- breed - 48 dogs, breeds with thicker skin (Salmon et al., 1994) and a control group of 5 dogs, breeds with thinner skin (all male, all aged $\approx$ 6 years, all black);
- coat colour - dogs with black or brindle coats - brown with black and a control group of 5 dogs, breeds with thick skin (Affolter & Moore, 1994), male, all aged $\approx$ 8 years, all light fur).

Complete anamnesis, history and clinical evaluation were recorded in the study sheet. Most of the time the lymphoma was asymptomatic and was either discovered during

the evaluation of the parasitic disease (76% of the cases included in the study) or through the lymph node reaction identified during clinical control (24%) (Figure 2).

Microscopic detection of parasites on blood smears.

Imaging evaluation by both radiography and ultrasound (Esaote Veterinary MyLab Six Crystal Line).

Laboratory determinations were performed with the IDEXX VetTest biochemistry device, the IDEXX BioAnalytics was used for measuring the cytokines and inflammatory markers, the IDEXX Vet Autoread/IDEXX LaserCyte haematology device, IDEXX VetLab Station for proteinogram, SNAP 4DX Plus Tests.

Oncological diagnostic confirmed by cytopathological examination - fine needle aspiration.

RESULTS AND DISCUSSIONS

Autoimmune and chronic inflammatory syndromes are associated with increased risks of Lymphoma.

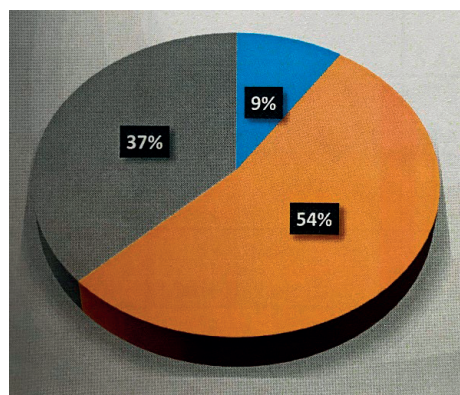


Figure 1. Percentage of lymphoma cases correlated with haemoparasites:

9% patients with Dirofilariasis and Babesiosis
54% patients with Dirofilariasis
37% patients with Babesiosis

As we proceed, our study demonstrates the constant or repetitive effect of the parasites on the immune system of the host and the results that a chronic Dirofilariasis or a repetitive Babesias can have, keeping in mind that chronic inflammation is one of the risk factors of the carcinogenesis.

Because different lymphoma subtypes develop at different stages of lymphocyte differentiation, associations of autoimmune and inflammatory disorders could help oncologists have better understanding of the mechanisms that lead to a lymphoma diagnosis.

This study is an important step to the initiation of a prevention protocol for one of the most frequent types of cancer.

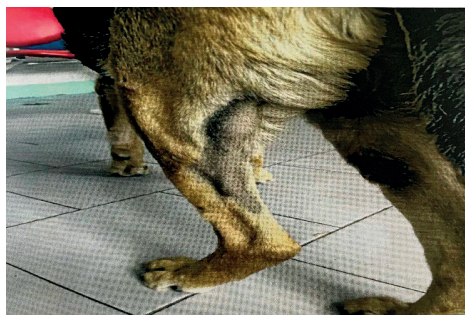


Figure 2. Clinical evaluation - in this case, the popliteal enlargement was the only symptom of centrocytic lymphoma. The owners had brought the dog in for testing for Babesiosis

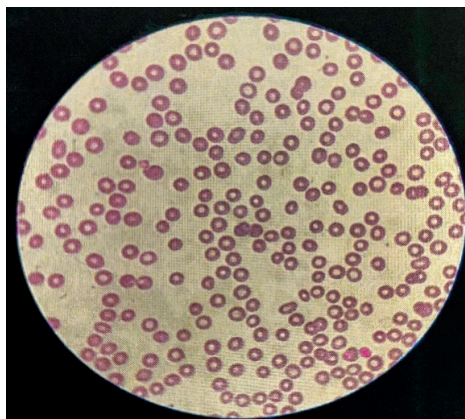


Figure 3. *Babesia* spp.

Microscopic detection of parasites in the blood smears is essential for the inclusion in the study. Pathological values on peripheral blood smears:

- normocytic erythrocytes, polychromasia (++);
- leukocytes: segmented and hypersegmented neutrophils (+++), eosinophils (+++), monocytes and reactive lymphocytes (++);
- platelets: 0-5HPF* (low number), large platelets (+), small platelet aggregates (+).

*High Power Field; (+) low numbers; (++) medium numbers; (+++) high numbers.

The bone marrow releases a larger number of immature red blood cells (reticulocytes) than it physiologically should, without them having reached the last step of the erythropoiesis (Kim et al., 2020).

The need that determines the presence of a larger than normal number of reticulocytes is linked to one cause: a haemolytic anaemia. (Ray et al., 2023). In our study, the aetiology of the haemolytic anaemia is either due to the large number of *Babesia* spp. (Figure 3) parasites that have taken over a large number of red blood cells or have died after specific treatment and destroyed a large number of red blood cells and/or immune-mediated haemolytic anaemia (IMHA) that can be triggered by severe inflammatory reactions (Garden et al., 2019).

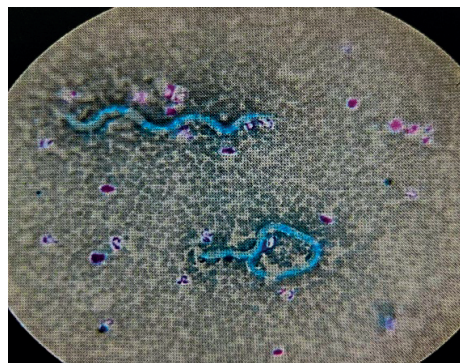


Figure 4. Microfilariae (*D. immitis*)

For patients diagnosed with regenerative anemia due to *Dirofilaria* spp. (Figure 4) the ethiology is based on complicated mechanisms triggered by the toxic proteins that the parasite secretes: intravascular haemolysis and erythrophagocytosis - the macrophage activation by inflammatory cytokines due to chronic inflammatory disease (Nemeth & Ganz, 2014; Mendez et al., 2014).

Regarding the leukocyte values, we correlated the segmented and hypersegmented neutrophils with the presence of macrophages in cytopathological interpretations. Inflammatory cytokines trigger the release of older neutrophils (hypersegmented) from the bone marrow storage pool (Raskin et al., 2004). In this case as well, the demand for a large number of neutrophils is initiated by the severe

inflammatory syndrome that concurrent with the parasitic infestation (Leisewitz et al., 2023). The immune stimulated lymphocytes that we identified in large numbers on the haematology reports, the ultra-specialized eosinophils and the monocytes are in response to the aggressive antigen stimulation effect in both *Dirofilaria* and *Babesia* cases (Wezyk et al., 2023; Milanovic et al., 2017). There have been many studies regarding the implication of platelets in parasitic infestation and the reason for the associated thrombocytopenia (Alonso & Cox, 2015; Cox & McConkey, 2010; Jinna & Khandhar, 2023). The low platelet count is frequent in parasitic infestations and in our study, we associated the thrombocytopenia, the haemolytic anaemia and the high quantity of blood clots to the platelets binding to the ruptured red blood cells or infested erythrocytes, forming micro-aggregates.

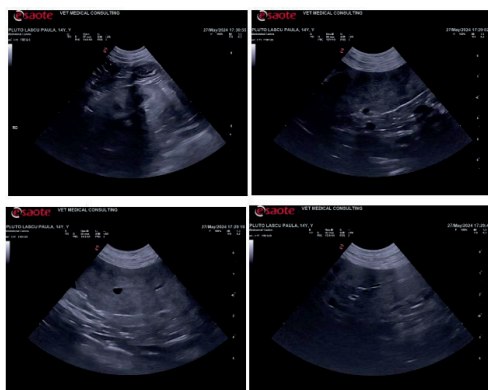


Figure 5. Spline: evident splenomegaly, with regular borders but present of more poorly delimited hypoechogenic infiltrations, disseminated in the mass of the splenic parenchymal; Obvious hepatomegaly, with moderate vein distention and multiple nodular lesions up to 1.5 cm in diameter, disseminated in the hepatic mass

Pathological values on biochemistry analysis:

- liver markers: elevated (+++) ALT, ALKP, Bilirubin, TP, LD;
- renal function markers: elevated (+++) CREA, BUN;
- Calcium levels: elevated (+++);



Figure 6. Soft tissue radiopacity in the projection area of the caudal oesophagus pushing the ventral main bronchus. Presence of air in the pleural space. Increased radiopacity in the caudal right lung lobe. Soft tissue radiopacity superimposed on the cardiac silhouette in the caudal mediastinum. Presence of a radiolucent region in the caudal portion of the right cranial lung lobe

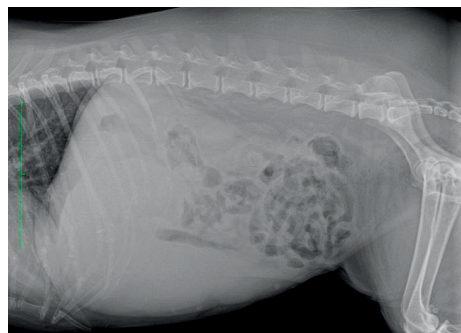


Figure 7. Significant hepatomegaly and splenomegaly

We also registered 2 cases of allergy due to sever infection with *Babesia*, dogs presenting with pulmonary infiltration with eosinophilia and serum globulins are increased.

Ultrasonography and radiography are vital tools both in parasitic evaluation (Corda et al., 2022) and oncology, giving valuable information in diagnosis, staging and monitoring (splenomegaly, hepatomegaly, glomerular nephropathies) (Sitprija & Boonpucknavig, 1994) cardiac modifications, fluid accumulations in the pericardium, the pleura, the peritoneum etc.). Figure 5 presents changes caused by *Babesia* spp., and Figures 6 and 7 show changes caused by a centroblastic lymphoma.

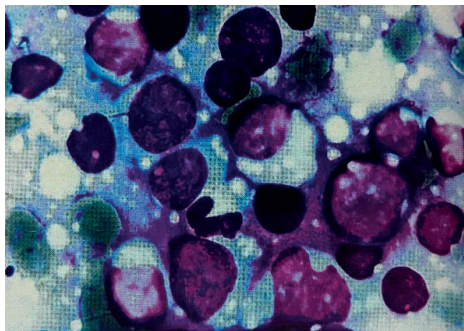


Figure 8. Malignant lymphoblasts

Cytopathological examination through fine needle aspiration offers the oncological pathology confirmation (Figure 8), although it can give false negative or false positive in cases of low-grade lymphoma.

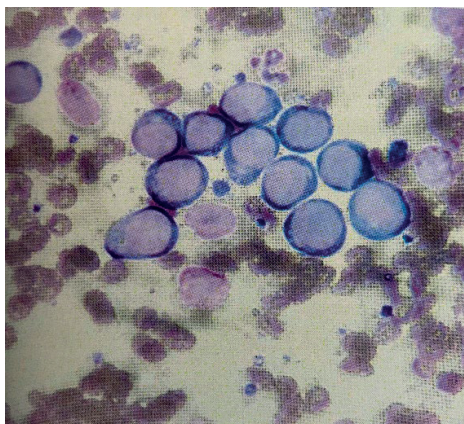


Figure 9. Lymphoblasts

CONCLUSIONS

The increase of immunoglobulins in lymphomas associated with *Dirofilariasis* and *Babesiosis* confirms antigenic hyperstimulation along with the associated eosinophilia syndrome.

The parasitic lymphoproliferative and macrophagic reaction in all lymphoid organs that determines a severe systemic inflammatory reaction can be due either to a chronic (in *dirofilariasis*) or a repetitive (in *babesiosis*) exposure. In both cases, over time, the inflammation and intense immune reaction can cause DNA damage and lead to carcinogenesis. The pathogenesis mechanism consists of the hyperstimulation of the antigen with parasitic proteins and cell destruction that causes a

pronounced proliferation of undifferentiated adult lymphoblasts at the level of organized lymphoid structures: the spleen for centroblastic lymphoma and the mediastinal, mesenteric, peripheral submandibular, prescapular, superficial inguinal lymph nodes and polyploidy in multicentric lymphoma.

Lymphodysplasia is the reversible process by which antigen-stimulated adult B lymphocytes differentiate into peripheral lymphoblasts (Figure 9), then into immunoblasts, plasmablasts and then plasma cells secreting antibodies against the antigen that stimulated them.

All 53 dogs included in the study has a history of several diagnosis of *Babesia* spp. and/or chronic infestation with *Dirofilaria* spp. In the 38 months of observation, all dogs experienced at some point a severe inflammatory episode with exaggerated symptoms of immune reaction which leads us to concur that pets with chronic or repeat diagnosis of parasitosis will fall under the category of risk factors (chronic inflammation) and of trigger (autoimmune like reaction) for malignant lymphoma.

Lymphoma seems to have a seasonal character, namely early spring starting in March with the appearance of hematophagous insects, that determine either an immune response, a severe systemic inflammatory response or a disease relapse due to organ toxicity; late autumn in October correlated with prolonged exposure to the sun during the summer and the seasonal pattern of decline of the immune system (Wyse et al., 2021).

Skin thickness has proven to be a predictability marker for prognosis and response to treatment. Further study regarding predictability markers is necessary.

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