

Extramedullary hematopoiesis (EMH) and other pathological conditions in canine spleens

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Summary

The aim of the study was to evaluate pathological conditions in canine spleens. The spleens of 105 dogs were examined histopathologically. The age of dogs ranged from 2 months ($n = 6$) to 21 years ($n = 99$). There were 78 male and 27 female animals. Macroscopically, 93 spleens were enlarged. During histopathological examination, different pathological conditions were found (extramedullary hemopoiesis (EMH), lymphoma, inflammation, amyloidosis, diffuse and follicular white pulp hyperplasia, red pulp hyperplasia, hematomas). Although it is a rare condition, EHM was found in the spleens of 72 dogs (68.6 percent), and further investigations are needed together with bone marrow examination. We observed that splenomegaly, especially as a focal spleen enlargement, is common in dogs, but often misdiagnosed as malignant spleen tumor.

Keywords: dog, spleen, extramedullary hematopoiesis

Diffuse canine splenomegaly or splenic masses are quite often found in small animal veterinary practice. Generalized splenomegaly may be due to various hyperplastic processes. In contrast to generalized splenomegaly, localized splenic enlargement is also common occurrence in dogs. Hematomas are some of the most common causes of an enlarged spleen in dogs, representing over 50% of splenomegaly cases. This type of splenic mass is basically an accumulation of pooled blood within the splenic tissue.

Extramedullary Hematopoiesis (EHM), especially with hyperplasia of lymphoid tissue, spleen abscess formation or chronic splenic torsion, may cause localized splenic enlargement.

The splenomegaly seen with splenic hyperplasia and extramedullary hematopoiesis reflects “work hypertrophy” resulting from the removal of abnormal blood cells from circulation, an increased activity of mononuclear phagocytic and lymphoid cells, and increased blood cell production.

Clinical signs in dogs with splenomegaly or splenic masses are often mild and nonspecific. Decreased appetite or anorexia, weight loss, vomiting, abdomen discomfort, intermittent abdomen pain, abdomen meteorism, pale mucosae, collapse, lethargy, polyuria/polydypsia, fluids in the abdominal cavity, arrhythmias

and hemoglobinuria may be seen in dogs with splenic masses or generalized splenomegaly (19).

Laboratory findings are often nonspecific and do not have significant importance for splenic pathology diagnosis. Anemia and secondary thrombocytopenia often occur. The hematocrit value may be below or within physiological ranges, accompanied by thrombocytopenia. In addition, leukocytosis may occur, accompanied by neutrophilia. Serum biochemistry is nonspecific and associated with other organ system dysfunctions (6, 9).

In some cases, computer tomography and magnetic resonance are helpful in making a definitive diagnosis because ultrasonography is limited when there are diffuse pathologies or adhesions between the damaged spleen and the surrounding organs. However, further diagnostic evaluation is needed to differentiate between benign and malignant processes in the spleen (16).

Spleen samples can be taken not only by laparotomy, but also by percutaneous fine-needle aspiration with ultrasound guidance. Serious complications of this procedure are uncommon. Fine-needle aspiration (FNA) cytology of splenic masses, nodules and enlarged spleens makes it possible to avoid an exploratory surgery and determines the following steps: surgery, other diagnostic procedures or medical therapy (5,

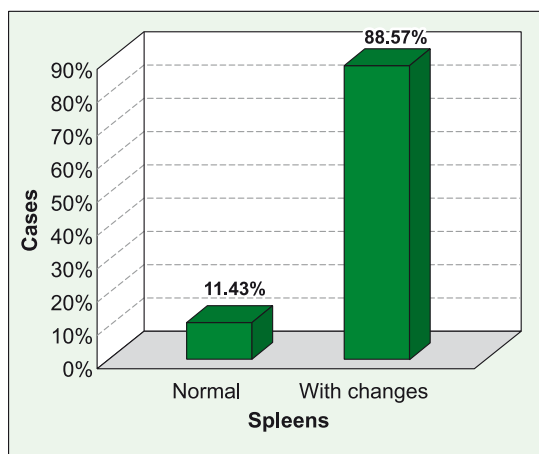


Fig. 1. Incidence of changed spleen in dogs

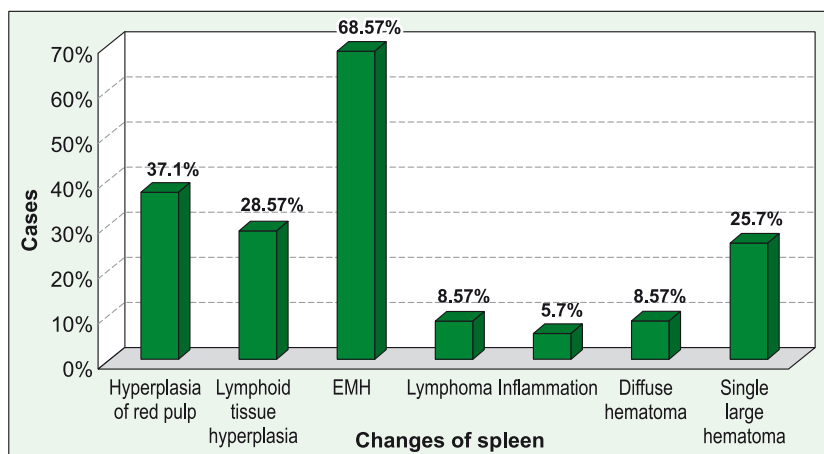


Fig. 2. Histopathological changes in canine spleens

17). Cytologic evaluation of splenic problems is not always indicated and can sometimes be contraindicated depending on certain disease processes. Because of their fragile nature, certain tumors of the spleen as well as hematomas may result in significant blood loss if stuck with a needle (25).

The aim of study was to evaluate the distribution of different types of pathology, especially of extramedullary hematopoiesis, in the canine spleen.

Material and methods

The examination of sick dogs, differentiation of disease causes, and treatment were performed at LUHS VA dr. L. Kriaučiūnas small animal clinic. For this purpose, blood serum biochemical parameters were measured with Vet Scan VS2 (Abaxis, USA) and Spotchem EZ SP-4430 (Arkray, Japan) devices. Hematological parameters (erythrocytes (RBC), hematocrit (HCT), packed cell volume (PCV), hemoglobin (Hgb), platelets (PLT), and leukocytes (WBC)) were measured with an IDEXX LaserCyte device (IDEXX laboratories, USA), peripheral blood smears and core shift in leukogram were assessed with a Nikon Eclipse E200 binocular optic microscope (Nikon Corporation, Japan) using 10 × magnification oculars and 100 × magnifying immersion oil objective lens.

Radiography and abdominal ultrasonography were performed for dogs with anemia and thrombocytopenia. Radiography was performed with a digital Medical ECONET device. Abdominal ultrasonography was performed with a stationary Mindray DP-7 device.

Splenic changes (focal masses, stasis etc.) found during ultrasound and X-ray examinations are commonly misdiagnosed as tumours. Dogs with misdiagnosed splenic changes together with concurrent diseases of other organs were more often euthanized.

After euthanasia, the spleens of 105 dogs were obtained at routine autopsy at the Pathology Centre of Veterinary Academy of Lithuanian University of Health Sciences. Each spleen was sectioned across. Tissue samples were fixed with a 10% formalin solution. The paraffin blocks were made with Shandon Pathcentre and TES 99 Medite Medizintechnik equipment. Five-micrometer sections were obtained with a Sakura Accu-Cut SRM. The sections were stained with H&E.

Results and discussion

The age of the dogs ranged from 2 months to 21 years. The group comprised 78 males and 27 females. Moderately or markedly enlarged spleens (mild or marked splenomegaly) were found at autopsy in 69 dogs (65.7%). Normal spleens without macroscopic or microscopic changes were found in 6 males (5.7%) and 6 females (5.7%) (Fig. 1).

Histopathological study revealed various pathologies in enlarged and mildly enlarged spleens. Often several pathologies were found in one spleen (Fig. 2).

Diffuse splenomegaly was revealed in 51 dogs (48.6%), and in 30 (28.6%) diffuse changes were significant. Marked focal splenomegaly due to hematoma accompanied diffuse splenomegaly in 21 dogs (20%). In 6 dogs (5.7%) hematomas were found in non-enlarged spleens.

Hematomas were found as large yellow-gray severely congested spherical masses of 6-14 cm in diameter with a tight smooth surface and red, purple or black inserts. Microscopic examination revealed decomposed red blood cells, a rich fibrin net (Fig. 3), splenic trabeculae, and hemosiderin consistently present in macrophages. There were remaining lymphoid

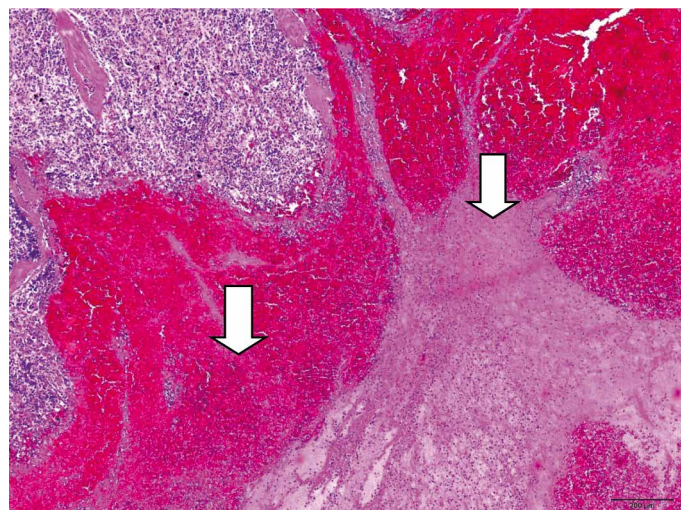


Fig. 3. Spleen hematoma. Large fibrin and erythrocyte masses (white arrows). HE. Magnification 40 ×

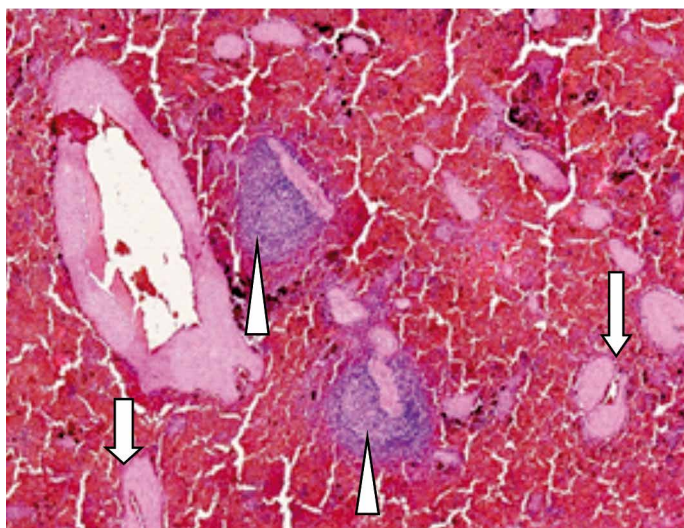


Fig. 4. Spleen hematoma. Masses of erythrocytes. Splenic cords (white arrows) and lymphoid follicles (arrow heads) are seen. HE. Magnification 40 ×

follicles (Fig. 4) and focal EMH at the edges of some hematomas. In one study, lymphoid hyperplasia was found in none of hemangiosarcoma cases and in 27% of hematoma cases. Siderotic nodules in the capsule or trabeculae were present in 25% of hemangiosarcoma cases and in 36% of hematoma cases. The authors noted that, since lymphoid hyperplasia is much more common in cases of hematoma, the presence of this feature lends support to a diagnosis of hematoma rather than hemangiosarcoma (4). Splenic hematomas not associated with an abdominal trauma are quite often found in middle-aged and senior dogs. The usual causes of spleen hematomas in dogs are nodular spleen hyperplasia and vascular tumors. Unlike in dogs, spleen hematomas are rare in cats. The usual cause of spleen hematomas in cats is an abdominal trauma (19). Nodular hyperplasia of splenic white pulp and concurrent hyperemia macroscopically are

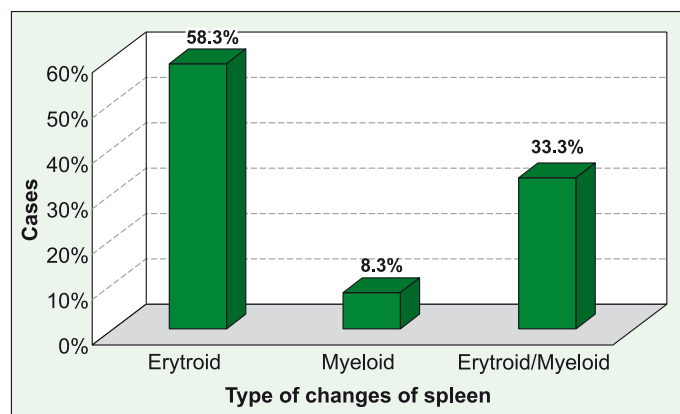


Fig. 5. Types of extramedullary hemopoiesis in canine spleens

similar to haematoma of the spleen and is often found in dogs. Hemangioma, hemangiosarcoma, lymphoma, lymphosarcoma, spleen infarction, and incomplete splenic contraction are focal changes revealed in the canine spleen. Long lasting splenic haematomas are usually massive. Due to this it is sometimes difficult to differentiate haematoma from some splenic tumors using ultrasonography or during autopsy.

Extramedullary hematopoiesis (EMH) was the most common change, revealed in 72 splenic specimens (68.6%) (Fig. 6). EMH was found in mildly enlarged spleens in 9 dogs (8.6%) without hematomas, and in 21 cases (20%) the spleen was significantly enlarged with concurrent haematoma. The spleen was moderately enlarged in 75 dogs (71.4%). According to the predominant cell type, erythroid cell proliferation was found in 42 cases (40%) (Fig. 5), myeloid cell proliferation was found in 6 cases (5.7%), and both types of cell proliferation were present in 24 cases (22.9%). Megakaryocytes were found in all cases (moderate to numerous cell count) (Fig. 7).

Other adaptive hyperplastic conditions were found in the canine spleens. Red pulp hyperplasia was present

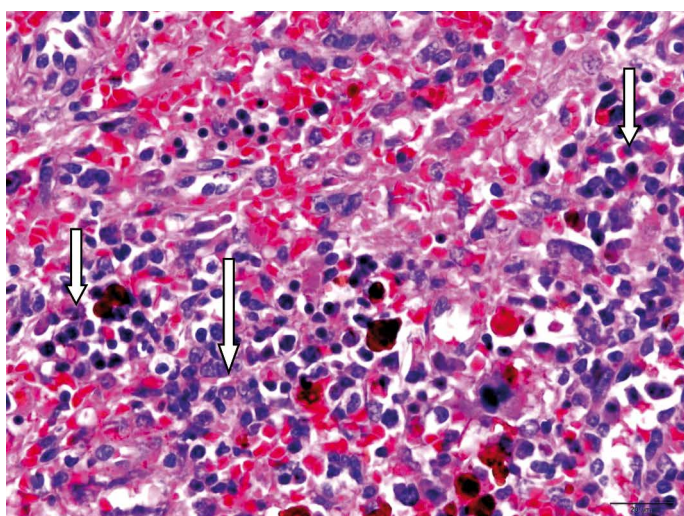


Fig. 6. EHM in a spleen, accumulation of erythroid line precursors (white arrows), megakaryocytes, hemosiderin. HE. Magnification 200 ×

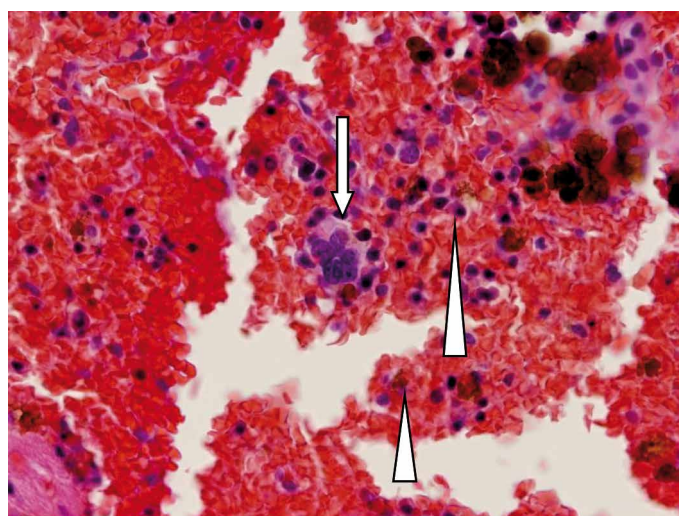


Fig. 7. EHM in spleen hematoma masses. Megakaryocytes (white arrow), sparse erythroid precursors (white arrow heads), and hemosiderophages between hemorrhagic masses. HE. Magnification 200 ×

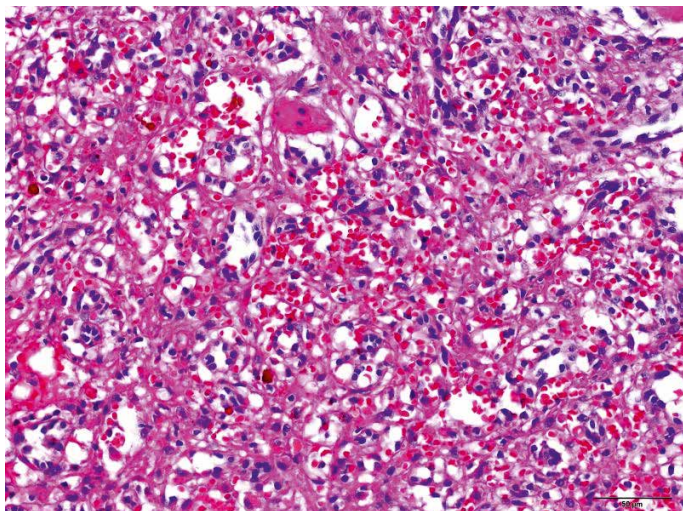


Fig. 8. Red pulp hyperplasia. Diffuse revelation of red pulp with plasmacytes and macrophages. HE. Magnification 100 ×

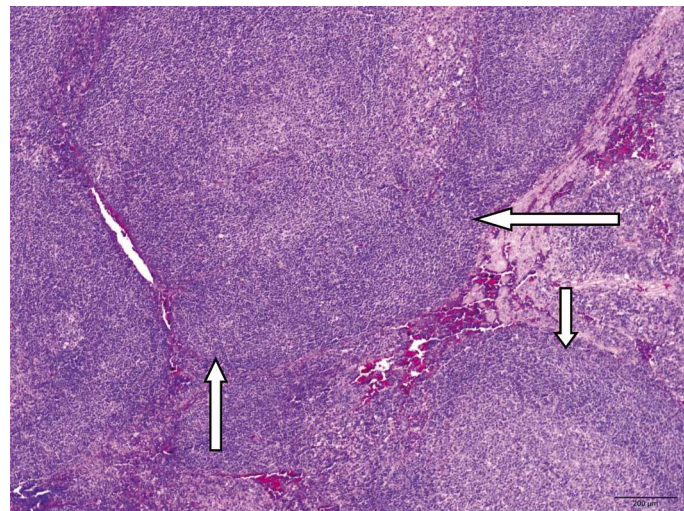


Fig. 9. Spleen lymphoma. Damanged structure of a spleen with large masses (white arrows) of proliferating tumorous lymphocytes. HE. Magnification 40 ×

in 39 dogs (37.1%), and lymphoid tissue hyperplasia was present in 30 dogs (28.6%).

Massive red pulp areas, consisting of sinusoidal blood capillaries filled with blood, and a rich reticular cell net with macrophages and plasmacytes without white pulp elements were observed in red pulp hyperplasia cases (Fig. 8).

Enlarged masses of splenic white pulp with coalescent follicles together with concurrently enlarged germinal center were found in cases of splenic white pulp hyperplasia. Therefore marked lymphocyte hyperplasia and early splenic lymphoma may be difficult to distinguish. However in cases of white pulp hyperplasia the normal splenic architecture (white pulp and red pulp compartments) should be maintained.

Significant splenomegaly was caused by lymphoma in 3 dogs (2.9%) (Fig. 9).

In many mammals, fetal hematopoiesis in the spleen ends at the end of the intrauterine period because hematopoiesis in the other organs begins. Hematopoiesis in the spleen continues long after birth in rats, mice, and minks. Because EMH is a compensatory reaction in many animals, it indicates a dysfunction of the hematopoiesis system (7, 8, 15, 20-22).

EMH is most commonly seen in the liver and spleen as a diffuse lesion. Rarely, EMH occurs as a solitary mass, posing a diagnostic dilemma (5).

Four major theories involving changes in stem cells and/or their microenvironment can explain the development of most occurrences of EMH: severe bone marrow failure; myelostimulation; tissue inflammation, injury, and repair; abnormal chemokine production (9). In dogs and cats, the most common site of EMH is the spleen. Any combination of erythroid, myeloid, and megakaryocytic cells in the spleen may be evident (3). Severe bone marrow lesions and hematological diseases in dogs and cats usually cause extramedullary hematopoiesis. Hematological diseases with EMH include chronic hemolytic anemia, myeloproliferative

processes, lymphoid tissue tumors, myelodysplasia syndrome, and canine splenic hemangiosarcoma. EMH consists of predominating erythroid cells: metarubricyte, rubricyte, prorubricyte, small amount of rubriblast and megacariocytes, which are collectively called erythroid islets. In cases of hemolytic anemia, erythroid islets are surrounded by small and large lymphocytes. The spleen contains a large number of macrophages with a large amount of hemosiderin in their cytoplasm (hemosiderophages). Decomposing erythrocytes are phagocytized by hemosiderophages.

Bone marrow diseases with EMH include myelofibrosis, myelophthisis (replacement of hematopoietic bone marrow by abnormal tissue), myelodysplasia, drug or plant toxicities, radiation, immune-mediated disease, necrosis, infection, and metastatic neoplasia (9). Some authors note that, in dogs, hematopoietic tissue is present in the spleen in pathologic conditions, but may also be present in the absence of an underlying disease (7). Ohlerth et al. found EMH foci in 6 spleens from 60 dogs.

In the myelostimulatory theory of EMH, sometimes called compensatory reactivation or reactive EMH, the upregulation of hematopoiesis in the bone marrow results in stem cell mobilization, an increased synthesis of chemokines and growth factors, and the subsequent upregulation of hematopoiesis in embryonic sites (mainly the spleen and liver) and sometimes in other tissues. Myelostimulation is a natural homeostatic response to the need for increased blood cell production that occurs in hematologic and inflammatory disorders (9). Myelostimulatory EMH usually results from disorders associated with chronic or markedly accelerated erythropoiesis, but other cell lines are occasionally affected (13, 15). Hypoxia caused by severe hemorrhagic or hemolytic anemia is the primary stimulus for myelostimulatory EMH, whereby increased erythropoietin production in the kidney stimulates the proliferation and maturation of erythroid precur-

sors. Hemolytic anemia caused by pyruvate kinase deficiency, babesiosis, immune-mediated hemolytic anemia, zinc toxicosis, and other disorders that shorten erythrocyte lifespan are reported causes of myelostimulatory EMH in animals, especially dogs (2, 14, 23). The cellular and stromal elements in granulation tissue, resolving hematomas, areas of ischemia and neovascularization, and sites of inflammation can result in the development of a niche environment capable of supporting EMH. This emerging concept posits that molecular changes associated with some types of local inflammation, injury, and tissue repair mimic those involved in hematopoiesis. Numerous cytokines and inflammatory mediators can increase the number of mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) in the peripheral circulation that subsequently can home to affected organs and tissues to participate in local regenerative or inflammatory responses (24).

Splenic EMH is often observed in animals without obvious hematologic abnormalities, suggesting that local tissue injury, inflammation, and repair are a major underlying pathogenesis of EMH in this organ (25).

A rare cause of EMH is the generation of abnormal cytokines or other hematopoietic growth factors that induce stem cell populations to differentiate into hematopoietic cells and/or simulate the marrow microenvironment.

EMH can be found within neoplasms involving both hemic and nonhemic tissues. EMH has been reported in up to 42% of hemangiosarcomas in dogs (1), in a benign mixed mammary tumor in a dog (6), and in histiocytic sarcomas in dogs (12). Hemangiosarcomas produce vascular endothelial growth factor (VEGF) to suppress bone marrow hematopoiesis and induce EMH in the spleen and liver (10, 11).

The splenomegaly seen with splenic hyperplasia and extramedullary hematopoiesis reflects "work hypertrophy" resulting from the removal of abnormal blood cells from circulation, increased activity of mononuclear phagocytic and lymphoid cells, and increased blood cell production. The chronic increased destruction of red blood cells in some non-immune-mediated hemolytic diseases also appears to cause hyperplastic splenomegaly in dogs and cats. Chronic antigen stimulation by infectious agents (e.g. bacterial endocarditis), blood parasites, or immune-mediated disease can stimulate hyperplasia of mononuclear phagocytic and lymphoid cells (3).

Splenomegaly and hematomas in dogs, especially focal, are often misdiagnosed as splenic tumour.

Conclusions:

1. Histopathological changes in the spleen include most frequently a diffuse and focal hyperplasia, especially EMH.

2. Clinical signs and laboratory findings in dogs with splenomegaly are nonspecific, and radiology and ultrasound results are controversial.

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