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MORPHOLOGY AND EPIDEMIOLOGICAL ASPECTS OF SPLENOMEGALY IN DOGS – RETROSPECTIVE STUDY

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Abstract

Splenomegaly in dogs is frequently diagnosed in veterinary practice. Establishing its morphological substrate is of major importance in choosing the therapeutic course and establishing the prognosis. The current study analyzed 194 dog spleens (190 surgically removed), submitted to the Pathology department, between August 2005 and October 2020. Macroscopic, cytological and histopathological examinations were performed. According to our results, localized/asymmetric splenomegaly represents 78.35% of the total cases examined. Splenomegaly was diagnosed mainly in mixed-breed dogs (22.68%), among the purebreed dogs the most affected being those of medium and large size, such as German Shepherd (11.86%) and Rottweiler (10.3%). Old age is a risk factor, 51% of the subjects being over 10 years old; 53% were males and 47% females. In 55% of all cases the splenomegaly had a non-neoplastic substrate, in 45% being represented by neoplastic processes. The most frequently diagnosed tumor was hemangiosarcoma (50.57%); the most common non-neoplastic diseases were hematoma (40.19%) and splenic congestion (26.17%).

Key words: *dog; epidemiology; splenomegaly; tumoral and non-tumoral lesions.*

INTRODUCTION

The spleen is a secondary lymphoid organ that performs multiple functions in the body, the most important being hematopoiesis, blood filtration, immune response, blood storage and iron metabolism.

The diagnosis of splenic lesions in canine patients is increasingly required in current veterinary practice, mainly in old dogs. Imaging exams represent the main toll to identify the changes in the splenic parenchyma, while the histopathological examination is used to establish the definite diagnosis (Vulpe et al., 2015).

Splenic lesions most often cause splenomegaly, which can be localized/asymmetrical or diffuse/uniform. Localized splenomegaly is most often diagnosed in dogs, while in cats the diffuse form is more common (Zachary, 2017).

Localized splenomegaly is characterized by the presence of one or more nodules, grouped into two types - bloody nodules and firm nodules. Bloody nodules appear in case of splenic hematomas, acute infarcts or hemangiosarcoma, while firm nodules occur most frequently in nodular lymphoid hyperplasia and in some primary and secondary neoplastic processes (lymphoma, fibrosarcoma, histiocytic sarcoma, lipoma, liposarcoma, leiomyosarcoma etc.) (Spangler & Kass, 1998; Șahînduran et al., 2016). Congested splenomegaly may be mainly the consequence of acute hyperemia and stasis. Noncongested splenomegaly occurs in case of metaplasia, generalized lymphoid hyperplasia, inflammatory and neoplastic processes (lymphoma, visceral mast cell tumors, histiocytic sarcoma) and splenic amyloidosis (Jubb et al., 2017; Zachary, 2017).

Studies have described multiple aspects which could possibly indicate an underlying malignant tumor in case of splenomegaly, in an attempt to develop a long-term prognosis. The presence of hemoperitoneum and the size of the splenic masses may indicate a malignant process and decrease the post-splenectomy survival rate. According to Mallinckrodt & Gottfried (2011), compared to the dogs with splenic hemangiosarcoma, those with benign splenic masses had a higher mean mass-to-splenic volume ratio and also a higher mean splenic weight as a percentage of body weight. Cleveland & Casale (2016) showed that the

median life expectancy of dogs with benign splenic lesions is 436 days and 110 days in the case of malignant splenic lesions. In Spangler and Kass's study (1997) on the survival rate of post-splenectomy in dogs, only 7% of patients diagnosed with hemangiosarcoma were still alive one year after the surgery.

The aim of this retrospective study is to review the main types of lesions that cause splenomegaly in dogs, with the identification of a possible susceptibility regarding breed, age and sex. Secondly, we emphasized the value of cytological examination in establishing and confirming a diagnosis of the underlying cause of splenomegaly.

MATERIALS AND METHODS

For this study we reviewed records of 194 spleens surgically removed or after necropsic examinations between August 2005 and October 2020, from canine patients diagnosed with splenomegaly. The spleens were examined and processed within the Pathological Department of the Faculty of Veterinary Medicine Bucharest. Splenomegaly was mainly diagnosed by imaging examination. After excision, the spleens were grossly examined. In some cases, cytologic smears were made preoperatively using the fine-needle aspiration technique and by impression and scraping in case of surgically-removed spleens. The smears were stained using the May-Grünwald Giemsa (M-G.G.) method. Subsequently, the samples collected from representative areas were fixed 10% neutral buffered formalin, dehydrated in alcohol and paraffin-embedded. Histopathology slides were stained with hematoxylin and eosin (HE) and also with Perls staining, which was used to highlight the siderophages.

RESULTS AND DISCUSSIONS

Between August 2005 and October 2020, at the Pathological Department of the Faculty of Veterinary Medicine Bucharest, 194 dog spleens were examined, of which 98% (n = 190) were surgically removed and 2% (n = 4) were collected during necropsies.

From the 194 spleens analyzed, 78.35% (n = 152) had localized splenomegaly and 21.65% (n = 42) had diffuse splenomegaly (Table 1). Malignant tumors represent 41% of the total cases, the benign tumoral and non-neoplastic lesions being the most common findings in diffuse (73.8%) and localized splenomegaly (55.26%). Similar results were obtained in a retrospective study conducted at the University of Londrina, Brazil (Olegário da Silva et al., 2016), where out of a total of 71 spleens examined, 67.8% (n = 59) had localized splenomegaly and the remaining 16.9% (n = 12) had diffuse splenomegaly. In this study, non-neoplastic lesions were also more common in the mentioned subcategories. Cleveland & Casale (2016) also noted that localized splenomegaly is most frequently associated with benign and non-tumoral masses.

Table 1. Type of splenomegaly according to gross examination and distribution of malignant, non-tumoral and benign tumoral lesions

Type of splenomegaly	**Number of cases (%/total cases)**	**Number of cases with malignant lesions (%/cases)**	**Number of cases with non-tumoral and benign tumoral lesions (%/cases)**
Localized	152 (78.35%)	68 (44.74%)	84 (55.26%)
Diffuse	42 (21.65%)	11 (26.2%)	31 (73.8%)
Total	**194 (100%)**	**79 (41%)**	**115 (59%)**

Regarding to the breed predisposition of splenomegaly, our data shows that most cases corresponded to mixed-breed dogs (22.68%), followed by German Shepherd (11.86%), Rottweiler (10.31%), Bichon (5.67%), Cocker Spaniel (5.67%), Poodle (5.15%), German Shorthaired Pointer (4.64%), Labrador Retriever (3.09%), Pekingese (3.09%), Golden Retriever (2.58%), Husky (2.58%), Boxer (2.06%), Romanian Mioritic Shepherd Dog (1.55%), Fox Terrier (1.55%) and Shih Tzu (1.55%); medium-sized and large-sized dogs are thus the majority. Similar results have been described in other articles (Bandinelli et al., 2011; Olegário da Silva et al., 2016). Given the fact that splenomegaly occurs mainly in large-breed dogs, Corbin et al. (2017) decided to study the splenomegaly in small-breed dogs. In their study, the most affected breeds were

Wheaton Terrier, Bichon Frize, Cocker Spaniel and Pembroke Welsh Corgi.

In our study, 53% of the spleens were from males and 47% from females. Therefore splenomegaly has no sex predisposition. The distribution of cases according to age reveals a preponderance of splenomegaly in the category of dogs aged between 6 and 10 (43%) and especially those over 10 (51%). Dogs under 6 years of age represented 6% of the cases.

The identified splenic lesions in the current study were classified into non-neoplastic lesions and neoplastic lesions (Tables 2 and 3). Out of the total cases, 55% (n = 107) represented non-neoplastic lesions, while 45% (n = 87) were neoplastic, of which 9.2% (n = 8) were benign and 90.8% (n = 79) were malignant. The diagnosis was established by histopathological examination in 87% of cases (n = 168) and by cytological examination in 13% of cases (n = 26). In 51% (n = 99) of the cases the cytological examination was followed by the histopathological one. Of these 99 cases, in 82.83% (n = 82) the cytological diagnosis corresponded with the histopathological one. Also, the cytologic examination could easily identify the malignant processes. In case of 9 patients (9/82), the cytological examination indicated a non-tumoral lesion, without being able to establish its origin, and a histopathological examination was needed to establish the definitive diagnosis. We can conclude that in case of splenomegaly, the cytological examination is useful in differentiating the malignant processes from the benign ones. All 99 cytopathological examinations were performed on surgically excised spleens, and none of the fine-needle aspirates (n = 26) of the spleen were followed by a histological examination. Thus, we could not determine the accuracy of the cytological examination performed by ultrasound-guided fine-needle aspiration. Yankin et al. (2019) analyzed 125 smears from samples collected through ultrasound-guided aspiration from splenic nodules and identified a clinically relevant diagnosis in only 20% of cases. However, in O'Keefe and Couto's study (1987) about the utility of cytological examination by fine-needle aspiration in the diagnosis of splenomegaly, in all 14 cases in which both cytological and histopathological examinations were performed, the diagnoses corresponded completely. Also, Ballegeer et al. (2007) obtained a correlation between the cytological and histopathological examinations in 61.3% of the 31 cases.

Of the total splenic lesions diagnosed in the present study, 55% were non-neoplastic. The most common lesions were hematoma (40.19%), splenic congestion (26.17%) and reactive hyperplasia (24.3%). These types of lesions were also the most common in Lee et al.'s study (2018), in which non-neoplastic lesions represented 68.8% (n = 32) of total cases, with 18 reactive hyperplasias, 4 hematomas and 4 splenic congestions. In our study, 5 cases consisted of non-specific changes, including hemorrhage, extramedullary hematopoiesis and hemosiderosis, along with 2 cases of splenitis, 2 cases of splenic infarction and one case of accessory spleen (Table 2). The diagnoses were classified according to the predominant lesion, as there were cases in which several types of lesions coexisted in the same histopathological sample. Being a long-term retrospective study, no information could be collected on the post-splenectomy survival rate of the canine patients.

Table 2. Type and distribution of non-neoplastic lesions

Diagnosis	Number of cases (%)
Hematoma	43 (40.19%)
Congestion	28 (26.17%)
Reactive hyperplasia	26 (24.30%)
Non-specific changes	5 (4.67%)
Splenitis	2 (1.87%)
Splenic infarction	2 (1.87%)
Accessory spleen	1 (0.93%)
Total non-neoplastic lesions	**107 (100%)**

We diagnosed only 2 types of benign tumors: hemangioma (n = 7) and myelolipoma (n = 1).

The most common malignant tumor was hemangiosarcoma (50.57%), followed by histiocytic sarcoma (13.78%), splenic lymphoma (9.2%), splenic fibrosarcoma (6.9%) and malignant fibrous histiocytoma (4.6%). Although it is a rare malignant tumor (Soare et al., 2012), our cases included 12 dogs diagnosed with splenic histiocytic sarcoma. Hemangiosarcoma is identified in many studies as the most common malignant splenic tumor (Bettini et al., 2001; Cleveland & Casale, 2016; Day et al., 1995; Leyva et al., 2018), the data of

our study being in accordance with these studies. Other malignant tumors from our cases were splenic mmast cell tumor (2.3%) and metastases (3.45%) (Table 3).

Table 3. Type and distribution of neoplastic lesions

Diagnosis	Number of cases (%)
Hemangioma	7 (8.05%)
Myelolipoma	1 (1.15%)
Total benign lesions	**8 (9.20%)**
Hemangiosarcoma	44 (50.57%)
Histiocytic sarcoma	12 (13.78%)
Splenic lymphoma	8 (9.20%)
Splenic fibrosarcoma	6 (6.90%)
Malignant fibrous histiocytoma	4 (4.60%)
Splenic mast cell tumor	2 (2.30%)
Splenic metastasis of adenocarcinoma	2 (2.30%)
Splenic metastasis of mesothelioma	1 (1.15%)
Total malignant lesions	**79 (90.80%)**
Total neoplastic lesions	**87 (100%)**

Hemangiosarcoma was the most common splenic tumor in the examined cases (n = 44). The highest incidence was registered among mixed-breed dogs (27.27%), and among the pure breeds, the most affected were German Shepherd (20.45%), Bichon (9.09%) and Siberian Husky (6.82%).

Out of a total of 44 cases of hemangiosarcoma, 54.55% of the patients studied were females, and the remaining 45.45% were males. The dogs' age ranged from 6 to 14 years, the average age being 10.68 years. In another retrospective study conducted at the Faculty of Veterinary Medicine Cluj-Napoca (Biriş et al., 2019), hemangiosarcoma was mainly diagnosed in German Shepherds (23%), mixed-breed dogs (22%) and Rottweillers (11%); the average age of the animals was 10.91 years, and in terms of sex distribution, 67% were males and 33% females. Another study, made between 2007 and 2010 (Tăbăran et al., 2010), found that the main cause of neoplastic splenomegaly in dogs was the hemangiosarcoma. It mainly affected the older animals, over 9 years, while the most affected breeds were German Shepherd and Rottweiller; there was no sex predilection. The results of our study are similar to those of the studies mentioned above, the differences between the results regarding gender predilection emphasizing the need for further research on this subjects.

Regarding the spleens in our study, on gross examination, the hemangiosarcoma appeared predominantly as a single nodular mass, of variable size, with the characteristic „bloody nodule" appearance (Figures 1 and 2).

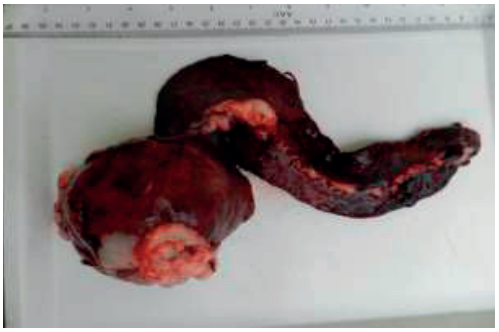

Figure 1. Localized splenomegaly - hemangiosarcoma, German Shepherd male

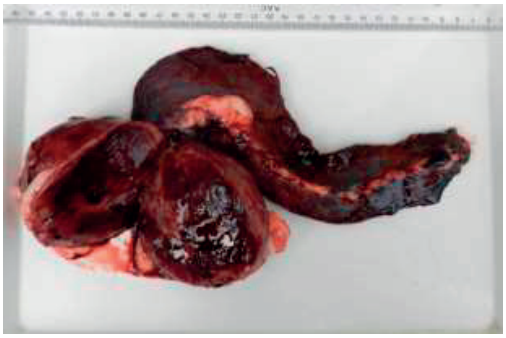

Figure 2. Splenic hemangiosarcoma, German Shepherd male - cut section surface

Cytological examination of splenic hemangiosarcomas (Figures 3 and 4) reveals a cellularity of mesenchymal type, with cell enlargement, pleomorphism and features of malignancy. The nuclei are round or oval, with coarse chromatin, and the nucleoli are proeminent and numerous. The cytoplasm of neoplastic cells is basophilic, occasionally with punctate vacuolation (Christopher, 2003). Siderophages may be present in large numbers, this feature also being observed in the smears examined by us.

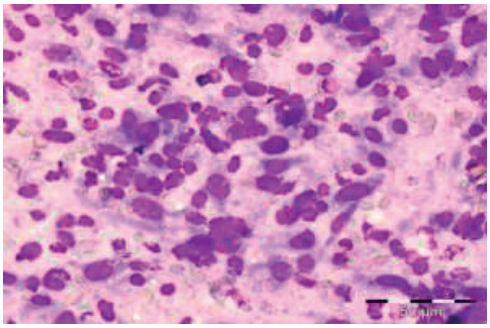

Figure 3. Cytological examination of splenic hemangiosarcoma - spindle cells, with elongated nucleus, basophilic cytoplasm, evident nucleoli, M-G.G., x 400

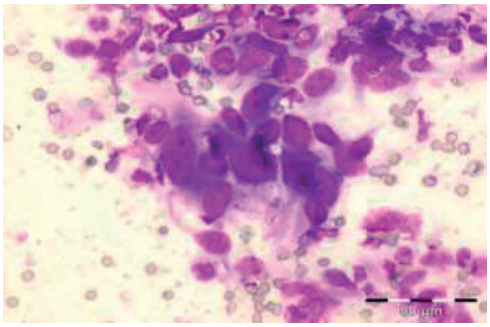

Figure 4. Cytological examination of splenic hemangiosarcoma - mesenchymal spindle-shaped cells with anisocytosis and anisocaryosis, M-G.G., x 400

Histopathological examination revealed the splenic parenchyma replaced by a tumoral proliferation, not encapsulated, represented by tissue of mesenchymal origin, respectively angioblasts, conjunctival stroma and sclerosis.
The vascular spaces are lined by tumoral cells, are anastomosed and filled with numerous erythrocytes.
Tumoral angioblasts are medium sized, polygonal or spindle-shaped, have a moderately abundant basophilic cytoplasm and a round or oval nucleus, centrally positioned.
Cellular atypia is generally moderates and represented by anisocytosis, anisokaryosis, cariomegaly and evident nucleoli (Figures 5 and 6).

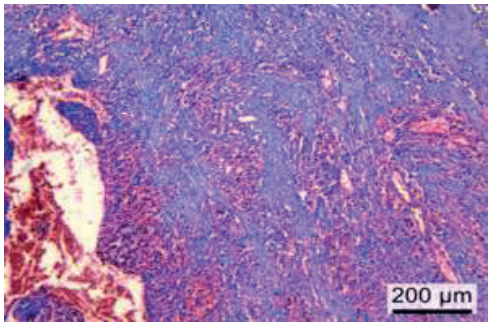

Figure 5. Splenic hemangiosarcoma - Vascular spaces of variable size and shape, anastomosed and filled with numerous erythrocytes, HE, x 100

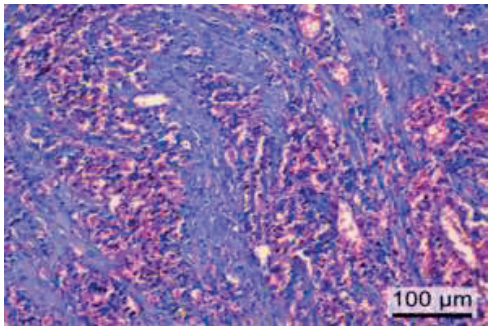

Figure 6. Well-differentiated splenic hemangiosarcoma - Cellular atypia, anisocytosis, anisokaryosis, caryomegaly, evident nucleoli, HE, x 200

Splenic hematoma is the most common non-neoplastic lesion underlying the splenomegaly (n = 43) in our study (Figures 7 and 8). On gross examination, the hematoma appears as a nodular mass, well delimited, of different sizes.

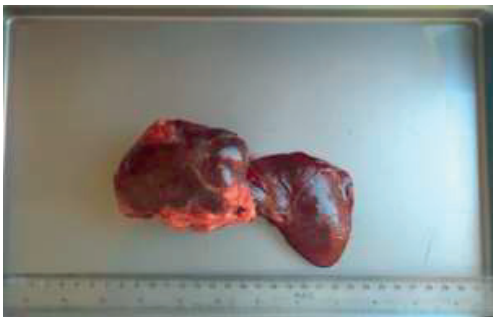

Figure 7. Splenic hematoma, male Jack Russel Terrier- Prominent large nodular mass (10/9 cm), with well-defined borders

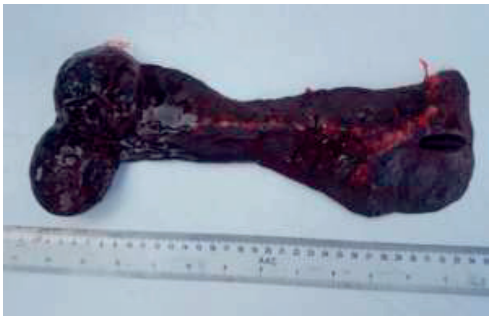

Figure 8. Splenic hematoma, male German Shorthaired Pointer - cut section surface of a compact, homogeneous blackish red mass

Cytologically, the splenic hematoma is characterised by the presence of numerous erythrocytes within the background, most of them being altered and lysed, numerous siderophages and magakaryocytes (Figure 9 and 10). In old hematomas inflammatory cells are present (neutrophils, lymphocytes, plasma cells), as well as fibroblasts who contribute to the formation of the capsule.

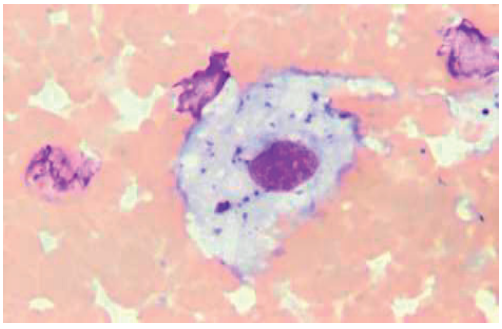

Figure 9. Cytological examination of splenic hematoma - many lysed erythrocytes in the background and a siderophage, M-G.G., x 1000

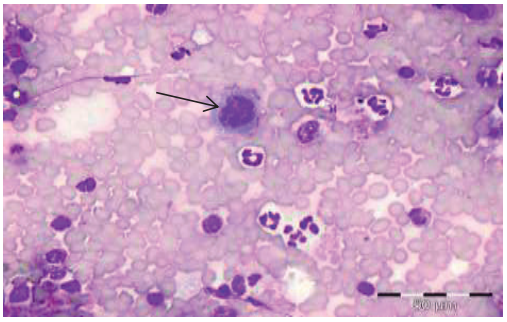

Figure 10. Cytological examination of splenic hematoma - Numerous erythrocytes, lymphocytes, neutrophils; megakaryocyte (arrow), M-G.G., x 400

On histopathological examination, vascular changes are predominant, with the accumulation of excess erythrocytes in the splenic pulp and the presence of many siderophages, the latter being an important indicator of a chronic pathological process, focused on erythrophagocytosis. The hematoma contains multiple fibrin networks. We also observed many megakaryocytes in the histological sections of splenic hematomas. Their presence in large numbers in splenic hematomas is also described in the study of Zamokas et al. (2016).

In order to highlight the siderophages, Perls staining was used, in which the hemosiderin granules from the splenic macrophages acquire a bluish-black color, corresponding to the iron deposits (Figures 11 and 12).

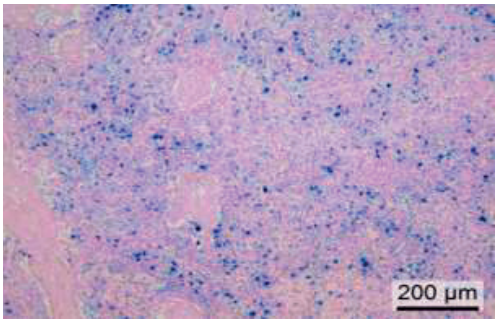

Figure 11. Splenic hematoma - Congestion and hemorrhage, numerous siderophages and fibrin network, PERLS, ob. x100

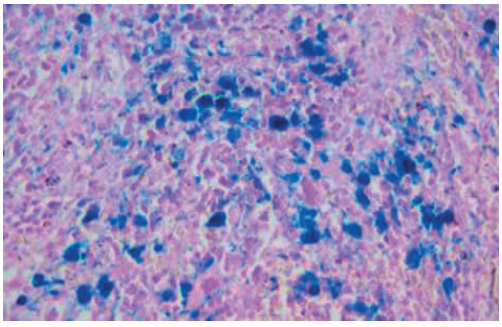

Figure 12. Splenic hematoma - Siderophages - Blackish blue hemosiderin granules, PERLS, ob. x400

Analyzing our datas, we observed that mixed-breed dogs (23.26%), followed by Rottweilers (9.3%), Boxers (6.98%), Cockers (6.98%) and Labrador Retrievers (6.98%) were most commonly diagnosed with splenomegaly due to

hematoma. Females diagnosed with splenic hematoma represented 51.16% of the cases, while males constituted 48.84%. The mean age of dogs with splenic hematoma was 11.27 years, higher than those with hemangiosarcoma (10.68 years). These results do not match to those in another study (Bettini et al., 2001), where the mean age of dogs with splenic hemangiosarcoma (10.3 years) is higher than that of those with splenic hematoma (8.2 years), proving the need for more detailed studies on this subject.

CONCLUSIONS

Splenomegaly in dogs appeared in 78.35% of the 194 cases examined as asymmetric/ localized splenomegaly.

Splenomegaly occurred in 51% of the examined cases in dogs over 10 years of age, older dogs being more predisposed to this condition. Most cases were registered in mixed-breed dogs (22.68%), German Shepherd (11.86%), Rottweiller (10.3%) and Bichon (5.67%), with no sex predilection.

In 55% of cases the splenomegaly had a non-neoplastic substrate and in 45% it was caused by a neoplastic process. Regarding neoplastic processes, 90.8% were malignant and 9.2% were benign. The most common neoplastic diseases were hemangiosarcoma (50.57%), histiocytic sarcoma (13.78%) and splenic lymphoma (9.2%); the most common non-neoplastic diseases were hematoma (40.19%), splenic congestion (26.17%) and reactive hyperplasia (24.3%).

In case of splenomegaly in dogs, cytological examination is useful in differentiating malignant from benign tumors, with confirmation of diagnosis by histopathological examination.

Hemangiosarcoma is the most common splenic tumor and it affects mixed-breed dogs (27.27%), German Shepherds (20.45%) and Bichons (9.09%); the average age of the subjects is 10.68 years.

Hematoma is the most common non-neoplastic splenic lesion. It is frequently found in mixed-breed dogs (23.26%), Rottweilers (9.3%) and Boxers (6.98%). The average age of the dogs with splenic hematoma is 11.27 years.

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