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Comparative pathological characterization of canine splenic haemangioma and haemangiosarcoma

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Abstract
The common vascular endothelial neoplastic conditions are haemangioma and haemangiosarcoma, specifically in the spleen, with variable clinical presentations and outcomes. This study presents a comparative analysis of three cases of canine splenic haemangioma and two cases of haemangiosarcoma, examining them in gross, cytological, and histopathological aspects. The gross morphology between the two has a wide range of differences like solitary nodule to irregular multiple red raised areas, and the histopathology of haemangioma showed blood-filled vascular channels lined by well-differentiated endothelial cells, with minimal atypia and focal haemorrhage and for haemangiosarcoma revealed irregular vascular spaces lined by plump to flattened hyperchromatic endothelial cells, coagulative necrosis, fibrin deposition, haemosiderin pigmentation, and anaplastic changes. The CD31 marker was applied to haemangiosarcoma splenic tissues and it showed strong immunoreactivity in the neoplastic endothelial cells.

Keywords: Canine spleen, CD31 Marker, gross, haemangioma, haemangiosarcoma

Introduction
The splenic masses are frequently encountered in canine patients and may arise from various tissue types, including lymphoid tissue, vascular endothelium, fibrous connective tissue, and smooth muscle. The benign lesions comprise haematomas, haemangiomas, and nodular hyperplasia, whereas malignant neoplasms are most often of vascular origin, with haemangiosarcoma and lymphoma being the most common [1-3]. The haemangiomas are grossly well-defined nodular, the nodules ranged from 7 to 20 cm (11 cm) in diameter and microscopic features are variable-sized blood-filled spaces lined by single layer of slightly plumpy vascular endothelium, together with large areas of haemorrhages [4], whereas haemangiosarcomas appeared soft to firm, size ranges more than 10cm and ranged in color from reddish-black to black-brown, with areas of haemorrhage and necrosis present on the cut surfaces. The free fluid stained with blood of about 300 mL was detected in the abdominal cavity during necropsy. Histopathological features are irregular vascular spaces, lined by elongated, multiple layers of plump or anaplastic endothelial cells. The nuclei were large and hyperchromatic and mitotic figures were often seen [5].
Cytologically, the findings of splenic haemangiosarcoma contained a large amount of blood and the neoplastic cells were variable sized and fusiform to squamous-shaped. The cytoplasm was basophilic, with indistinct cytoplasmic borders; in some cells, small vacuoles were observed. The nuclei were oval to elongated, with coarsely clumped chromatin and contained prominent nucleoli [6]. CD31 antigen is regarded as a useful marker for endothelial cells because (1) it is constantly expressed on all types of endothelial cell, (2) it is expressed only by endothelial cells, platelets and macrophages, and (3) it is conserved in neoplasia arising from endothelial cells. The immunohistochemistry of HSA using CD 31, or platelet endothelial cell adhesion molecule-1 (PECAM), is an adhesion receptor expressed by endothelial cells involved in regulating apoptosis and maintaining vascular permeability and integrity, indicating the variations in the neoplastic endothelium [7-8]. CD 31 IHC was performed on the same tissue section.

Materials and Methods

The complete demographic information on canine carcasses submitted to the Department of Veterinary Pathology is given the Table 1. The clinical presentation mentioned in case sheets are anorexia, fever, pale mucus membrane, weight loss, vomition and abdominal distension. The final needle aspiration cytology (FNAC) and impression cytology of spleen was taken as prescribed by authors Cowell and co-authors [9]. The spleen tissue samples were collected and fixed in 10 per cent neutral buffered formalin for 48 hours, embedded in paraffin and 4-5µm thick sections were cut and stained with haematoxylin and eosin [10]. The CD 31(Endothelial cell marker) monoclonal l(JC/70A), Mouse, IgG1 k from BioGenex (Cat. No. AM232-5M) with secondary kit Supersensitive^{TM}polymer-HRP IHC Detection system/DAB (Ready-to-use system) were used in the same tissue section of the splenic masses.

Results and Discussion

The demographic information of these cases was in agreement with the sex and age predeliction of about 6-18 years [2, 11, 12]. Three necropsy cases were diagnosed as cavernous haemangioma in the spleen. It was seen in a 12-year-old female Cocker Spaniel, 11 year 11-year-old female Doberman and 8 year 8-year-old female GSD. Grossly, the first dog had a 1.2 x 1.0 cm size raised red brown nodule in the head of the spleen (Fig. 1), while the second and third dogs had enlarged spleen. In the third dog, diffuse and interspersed colours of dark black brown to red areas (Fig. 2) were noticed all over the spleen. On the cut section, dark red and grey areas with multifocal pale white foci were observed 13. Cytologically, neoplastic endothelial cells were oval, spindle shaped cell (Fig. 3) have light to medium basophilic cytoplasm and contained round to slightly oval nucleus and lacy chromatin [9, 13]. Histopathologically, first case showed large, intercommunicating blood-filled spaces showing RBC's and second case showed lymphoplasmacytoid cell appearance with margination of chromatin. In addition, vascular spaces lined by single layer of plumpy endothelial cells (Fig. 4) were observed. The sinus was dilated largely, filled with RBC's and thickened trabeculae [13, 14].

Two cases of haemangiosarcoma were recorded in a 12-year-old male Golden Retriever and an eight-year-old GSD. Grossly, the spleen was enlarged (weighed 1.190 kg) three to four times larger than normal spleen in the first case and only slightly enlarged in the second. In the first case, a large grey white mass measuing 17 x 11 cm (Fig. 5) was found ventrally on the body and tail region of the caudal border. The incised large and smaller masses were grey white (Fig. 6) with blood filled cavities. Whereas in the second case, an irregular red fleshy haemorrhagic mass (28 x 10 cm) (Fig. 7) was found attached to the caudal border by mesenteric folds. Except for the large mass in the first case, all the nodules on the mesentery were soft on incision and contained blood and blood clots, necrosis and haemorrhagic borders (Fig. 8). About 2 litres of blood (hemoperitoneum) was recorded in the GSD, which was in agreement with Gulbahar *et al.* (1998). Cytologically, on aspiration neoplastic cells showed indistinct cell borders, punctate vacuolated cytoplasm (Fig. 9), round to oval nucleus and multiple prominent nucleoli (Fig. 10), anisocytosis, anisokaryosis and mitotic figures. Numerous inflammatory cells, predominantly neutrophils were also seen [15]. Histopathologically, thickened capsule, areas of coagulative necrosis, infarct and fibrin were noticed. Plumpy neoplastic endothelial cells lined the blood vessels. The neoplastic cells, formed irregular vascular spaces filled with RBC's (Fig. 11). The endothelial cells were flattened to plumpy (Fig. 12) nuclei were large and hyperchromatic and mitotic figures (Plate 23d) were seen. In one case, haemosiderin pigmentation was noticed. Anaplastic cells with anisokaryosis were also seen [15, 16].

The monoclonal antibody CD31 had immunoreaction with the cell membrane and/or cytoplasm of neoplastic endothelial cells lining the blood vessels below the capsular area. The cytoplasm of the atypical endothelial cells showed patchy staining with CD31 near the blood vessels and the trabeculae. Strongly positive staining with CD31 was observed in spindle shaped to flattened or plumpy endothelial cells cytoplasm (Fig. 13-14) lining the blood-filled spaces. The Strong immunoreactivity of CD31 were in concordance the previous authors for the neoplastic endothelial cells [7, 16, 17]. The overall comparative characterization of the cases of benign haemangioma and malignant haemangioma were given in the Table 2.

Table 1: Demographic information on canine carcasses with splenic haemangioma and haemangiosarcoma

	Haemangioma			Haemangiosarcoma	
	Case 1	**Case 2**	**Case 3**	**case 1**	**Case 2**
Breed	Cocker spaniel	Dobermann	GSD	Golden Retriever	GSD
Age	12	11	8	12	8
Sex	Female	Female	Female	Male	Female

Table 2: Comparative pathological characters of canine splenic haemangioma and haemangiosarcoma

Feature	Haemangioma	Haemangiosarcoma (HSA)
Number of cases	3 (all females, mixed breeds)	2 (1 male Golden Retriever, 1 male GSD)
Age range	8-12 years	8-12 years
Breed predisposition	No specific breed predisposition (only 3 different breeds recorded)	Recorded in Golden Retriever and GSD; known predisposition in large breeds
Gross appearance	Solitary small red-brown nodules (~1.2 × 1.0 cm) in the spleen and the literature reports larger nodules (7-20 cm)	Spleen markedly enlarged in one case (1.190 kg; 3-4× normal) and mildly enlarged in another; nodules 1-4 cm; large grey-white mass (17 × 11 cm) with blood-filled cavities or irregular red, haemorrhagic mass (28 × 10 cm)
Consistency	Soft, spongy	Soft, blood-filled, large irregular mass, firm in some areas
Cytology	Endothelial cells with round-oval nuclei, light blue cytoplasm and minimal pleomorphism	Spindle-shaped cells, Indistinct cell borders, punctate vacuolated cytoplasm; round-oval nuclei, multiple prominent nucleoli, anisocytosis, anisokaryosis, mitotic figures, neutrophilic infiltration
Histopathology	Blood-filled vascular channels lined by well-differentiated endothelial cells, minimal atypia, focal haemorrhage	Thickened capsule, coagulative necrosis, infarcts, fibrin, irregular vascular spaces filled with RBCs, plump-flattened endothelial cells with large hyperchromatic nuclei, mitoses, anaplastic cells, haemosiderin pigmentation
Immunohistochemistry	Not applied	Strong positive for CD31 endothelial marker
Biological behaviour	Benign, non-metastatic	Malignant, aggressive, high metastatic potential

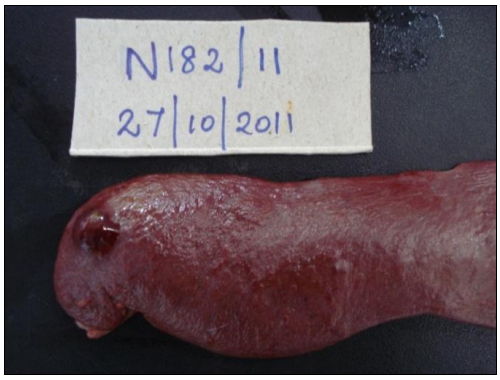

Fig 1: Spleen - Haemangioma - Solitary red raised nodule in the head region

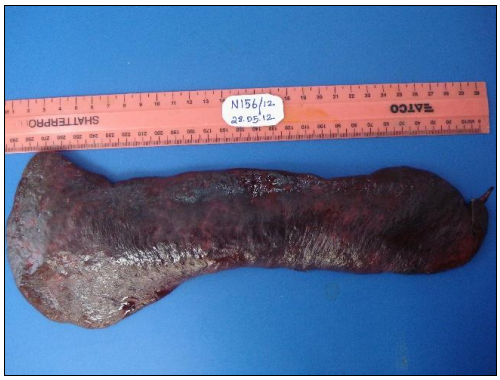

Fig 2: Spleen - Haemangioma - Diffusely enlarged - Dark red to black brown areas

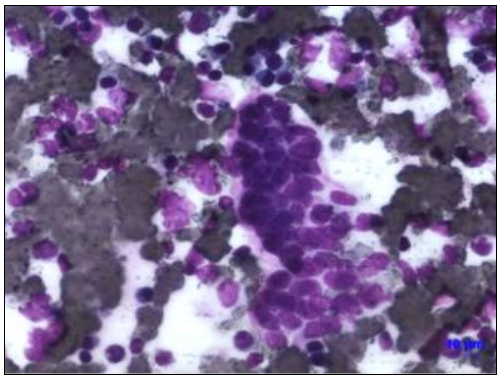

Fig 3: Cytology - Haemangioma - Clusters of endothelial cells - Nucleus - Round to oval - Slightly basophilic cytoplasm with heavy RBC infiltration Leishman bar - 10µm

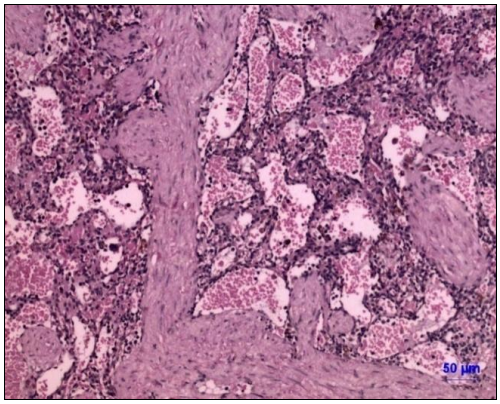

Fig 4: Cavernous Haemangioma - Large dilated space with minimal atypia in the endothelial cells and the thickened trabeculae H&E bar - 20µm

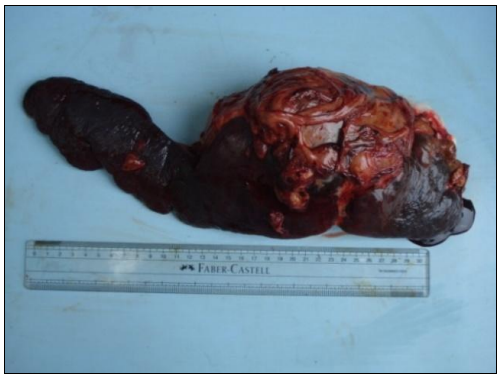

Fig 5: Spleen - Haemangiosarcoma - Irregularly enlarged - greyish yellow mass with mesenteric attachment in the body and tail region

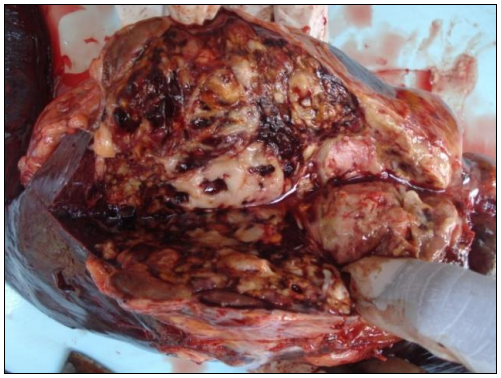

Fig 6: Spleen - Haemangiosarcoma - Cut section - Greyish white nodules with necrosis and blood-filled cavities

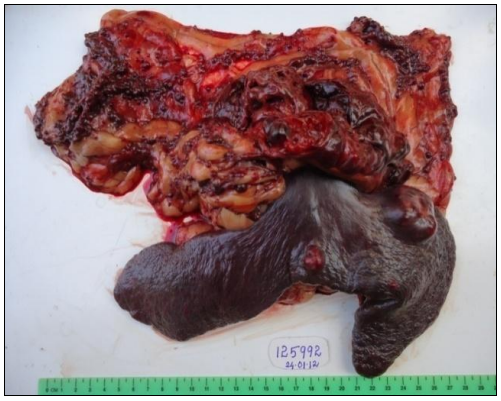

Fig 7: Spleen - Haemangiosarcoma - Two reddish brown nodules - large mass with mesenteric attachment with blood clots

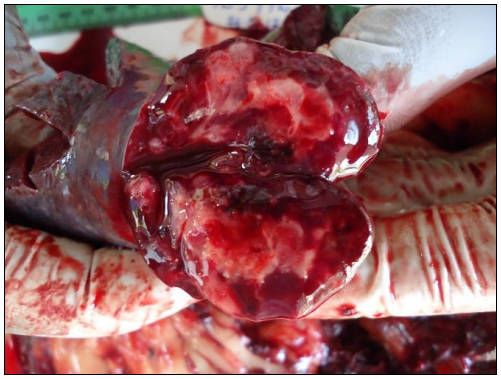

Fig 8: Spleen - Haemangiosarcoma - cut section - necrotic areas with haemorrhagic borders

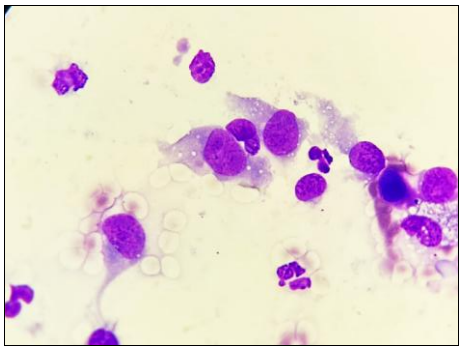

Fig 9: Cytology - Haemangiosarcoma - Neoplastic spindle or plumpy cells - Nucleus - oval to elongated - vacuolated basophilic cytoplasm Leishman bar - 10µm

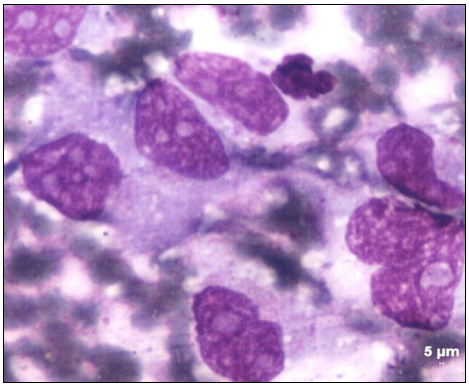

Fig 10: Cytology - Haemangiosarcoma - Neoplastic spindle or plumpy cells - Nucleus - oval to elongated - coarse chromatin - multiple prominent nucleoli Leishman bar - 5µm

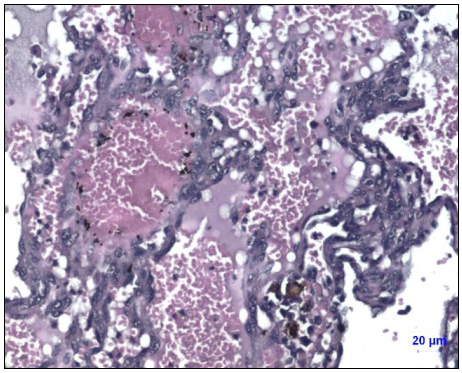

Fig 11: Spleen - Haemangiosarcoma - Irregular vascular spaces lined with neoplastic endothelium and filled with RBCS H&E bar - 20µm

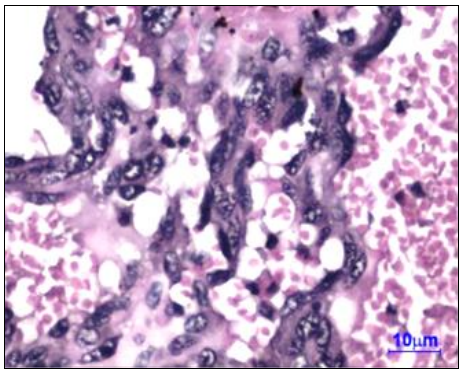

Fig 12: Spleen - Haemangiosarcoma -Neoplastic endothelium flattened to plumpy with prominent nucleoli and filled in RBCs H&E bar - 10µm

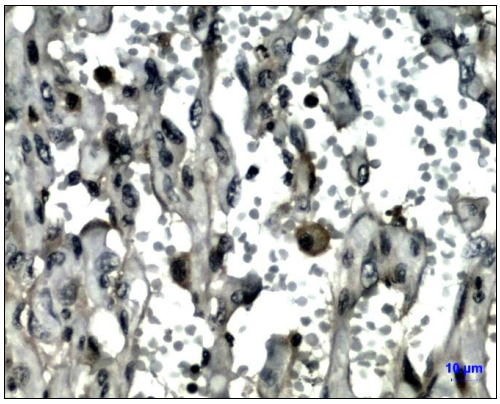

Fig 13: Spleen - Haemangiosarcoma - IHC - CD31 (DAB Brown) - Cytoplasmic immunoreactivity in neoplastic endothelial cells Scale bar= 10µm

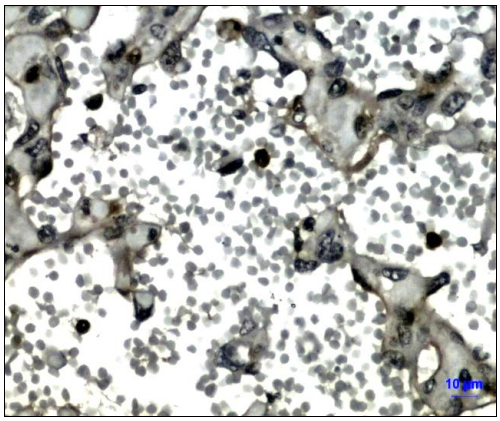

Fig 14: Spleen - Haemangiosarcoma - IHC - CD31 (DAB Brown) - Cytoplasmic immunoreactivity in neoplastic endothelial cells Scale bar= 10µm

Conclusion

The splenic vascular tumors are much common in dogs with very vague clinical presentations. Hemangiomas are presented in a milder form with a solitary nodule, well circumscribed, well-differentiated endothelial lining and minimal atypia. In contrast, Haemangiosarcoma is characterized by marked splenomegaly, large or multiple haemorrhagic masses, significant cytological atypia, high mitotic activity, and histological evidence of aggressive vascular proliferation, necrosis, and anaplasia, consistent with their malignant nature. The Endothelial marker CD 31 aids in the confirmation of the canine splenic vascular tumors.

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Conflict of interest

No conflict of interest.

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