BJVP
Brazilian Journal of Veterinary Pathology

Case Report

Intravascular Lymphomatosis in the Central Nervous System of Dogs: Immunohistochemical Investigation in Two Cases

Gisele F. Machado[1]*, Márcio B. Castro[2], Guilherme D. Melo[1], Wagner L. Ferreira[1], Eliana G. Aylon[3], Antonio C. Alessi[4]

[1]Departamento de Clínica, Cirurgia e Reprodução Animal, FMV-UNESP – Univ Estadual Paulista, Araçatuba, SP, Brazil
[2]Departamento de Anatomofisiologia e Reprodução, FAV-UNB – Univ de Brasília, Brasília, DF, Brazil
[3]UNIFRAN – Univ de Franca, Franca, SP, Brazil
[4]Departamento de Patologia Veterinária, FCAV-UNESP - Univ Estadual Paulista, Jaboticabal, SP, Brazil
* **Corresponding author:** Departamento de Clínica, Cirurgia e Reprodução Animal. UNESP- Faculdade de Medicina Veterinária, Rua Clóvis Pestana, 793, Araçatuba, São Paulo, Brasil – CEP: 16050-680. Tel: +55 18 3636-1420.
E-mail: giselem@fmva.unesp.br

Submitted December 21st 2010, Accepted January 28th 2011

Abstract

We report here two *postmortem* cases of dogs with intravascular lymphomatosis affecting the central nervous system. Intravascular lymphomatosis is represented by an exclusively intravascular proliferation of neoplastic lymphoid cells. To characterize the origin of the neoplastic cells, we have proceeded with immunohistochemical analysis to identify B and T lymphocytes and endothelial cells. The results showed predominance of cells from the T cell lineage, and no evidence of B cell origin was found. Few cells from one dog also exhibited cytoplasmatic staining for vimentin and Von Willebrand factor. Although in one case some immunophenotype diversity was observed, the massive presence of CD3 positive cells confirmed these neoplasms as intravascular lymphomatosis of T cell origin.

KeyWords*:* cell marker, immunohistochemistry, lymphoproliferative disorder, neoplasia, neuropathology, T cell

Introduction

Intravascular lymphomatosis (IVL) is a rare form of non-Hodgkin lymphoma characterized by neoplastic proliferation of lymphoid cells within the lumen of capillaries, small veins and arteries, with no or minimal involvement of the parenchyma (20). IVL was first described as angioendotheliomatosis proliferans systemisata (13) in a human patient with the cutaneous form of the disease. Initially this term and others, such as malignant angioendotheliomatosis and neoplasic angioendotheliosis, referred to the proliferation of endothelial cells which obliterated the lumen of small vessels with predilection for skin and brain, but possibly present in all organs, such as the adrenal glands, lymph nodes and lungs (3, 4, 19).

Immunocytohistochemical and cytogenetic studies revealed the lymphoid origin of the malignant cells (10). In humans, IVL is recognized as a distinct subtype of extranodal diffuse large-B cell lymphoma (DLBCL) in the World Health Organization (WHO) classification (6). Retrospective reviews of IVL in cats and dogs identified the neoplasic cells as T and B lymphocytes, non-T non-B lymphocytes, and also endothelial cells (9, 14, 16). The diverse origin of the neoplastic cells suggested that some features of this disease remain to be elucidated (15).

IVL exclusively confined to the central nervous system (CNS) is extremely rare, and symptoms are heterogeneous and related to vascular occlusion of the affected tissues, due to thromboses and infarctions (12, 17). The disease is not fully recognized by clinicians because the unspecific symptoms and although the presence of neoplastic cells inside blood vessels, they are not observed in routine blood samples (5, 8). With one exception (2), in all reported feline and canine cases, diagnosis of IVL was made at the necroscopic examination (8, 9, 14, 15, 16, 18, 19).

In the report presented herein, we describe two cases of IVL affecting the CNS of dogs, focusing on the

 All rights reserved 2007.

pattern of histopathological lesions as well as on the immunophenotype of the neoplastic cells.

Case report

Case No. 1: A nine-year-old female mixed breed dog, nulliparous, was referred to Franca University Veterinary Hospital with a day history of motor incoordination on the hind limbs and progression to spastic tetraplegia. The dog presented episodes of generalized tonic-clonic seizures, hyporexia, alterated behavior, opistotonus and bilateral mydriasis not responsive to the light. A complete blood count revealed normocytic-normochromic anemia, neutrophilic leukocytosis and elevation of the total plasmatic protein. The clinic case rapidly evolved to conscience level depression and death. A necropsy was performed and tissues were collected for histological examination.

Case No. 2: A brain of a dog was sent to the Department of Veterinary Pathology – UNESP-FCAV-Jaboticabal for histopathological examination and rabies' differential diagnosis. No day history was provided.

The whole brain of both cases and samples of multiple organs of case No. 1 were formalin-fixed and embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin-eosin (HE). For immunohistochemistry (IHC), paraffin-embedded sections of brains were immunostained using the antibodies mouse anti-canine CD3 (Serotec, MCA1774S), mouse anti-human CD79α (Dako, M7051), rabbit anti-dog IgG (Sigma, A6792), mouse anti-vimentin (Dako, M0725), and rabbit anti-human Von Willebrand factor (formerly factor VIII-related antigen; Dako, A0082). For all antibodies, negative and positive controls (lymph node) were used in conjunction with brain sections. Antigen retrieval was made with microwave and citrate buffer (pH 6.0). Immunolabelling was performed with streptavidin-biotin-horseradish peroxidase (Dako, K0690). The reaction was developed with 3,3´diaminobenzidine (Dako, K3468) and counterstained with Harry's hematoxylin.

In case No. 1, gross inspection of the brain revealed prominent and congested leptomeningeal vessels and hemorrhages mainly at left cerebral hemisphere and both piriform lobes (Figure 1A-B). In case No. 2 there was no evident gross alteration.

Histopathological examination of both cases demonstrated atypical mononuclear cells filling variably sized cerebromeningeal arteries, veins and capillaries. In both cases, the neoplastic cells are large, with pleomorphic and large nuclei, with one to several nucleoli, variable chromatin pattern, and scant amphophilic cytoplasm, and moderate quantity of mitotic figures. The neoplastic cells are exclusively confined to the lumen of vessels (Figures 2A-B). In some areas adjacent to affected blood vessels there are moderate amounts of macrophages with abundant foamy cytoplasm (Figure 2C), probably consequence of ischemic injury. Vessels, sometimes dilated, show thin walls and thrombosis, mostly within the meninges. Some blood vessels present hypertrophied endothelial cells (Figure 2D). In the affected blood vessels there are scarce erythrocytes along with the neoplastic lymphocytes. Intravascular lymphomatous cells were also found in heart, kidneys and lungs in case No. 1. Bone-marrow involvement was not detected.

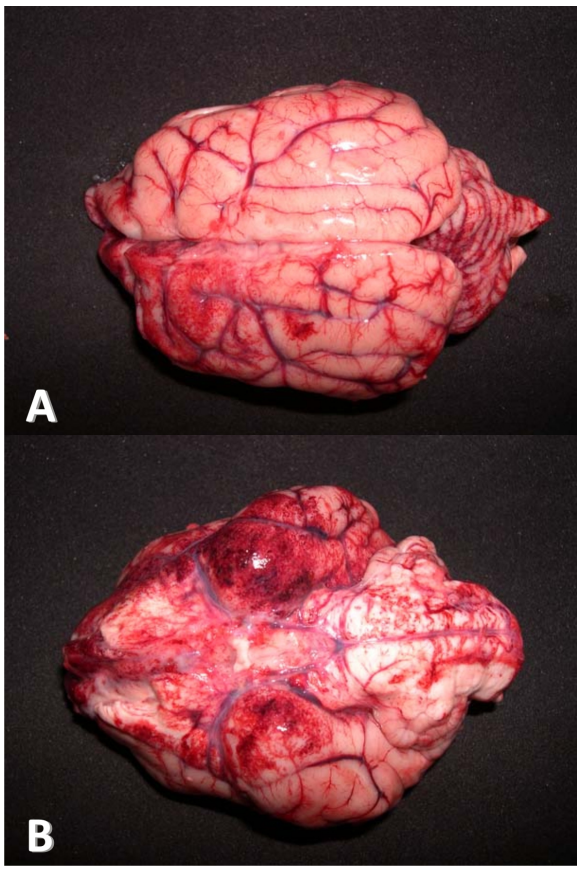

Figure 1. Brain, dog, intravascular lymphomatosis, case No. 1. A: Dorsal view of the brain showing diffuse meningeal vascular congestion and a focal area of hemorrhage in the left frontal lobe. B: Ventral view of the brain exhibiting a focally extensive area of hemorrhage in the left piriform lobe and multifocal areas of hemorrhages in the right piriform lobe, in the olfactory tract and in the brain stem.

Immunohistochemical analysis showed that the neoplastic cells presented a strong cytoplasmatic CD3 immunostaining (Figure 3A). The immunostaining for CD3 was present exclusively in the cells confined to the vessels lumina. Staining was negative for CD79 (Figure 3B) and IgG. Vimentin was also positive in both cases and presented a strong cytoplasmatic staining of the normal endothelial cells and a pale staining in few intravascular cells (Figure 3C). Immunodetection of the Von Willebrand factor was evident only in case No. 1, in the cytoplasm of rare intravascular cells and in blood vessel walls (Figure 3D).

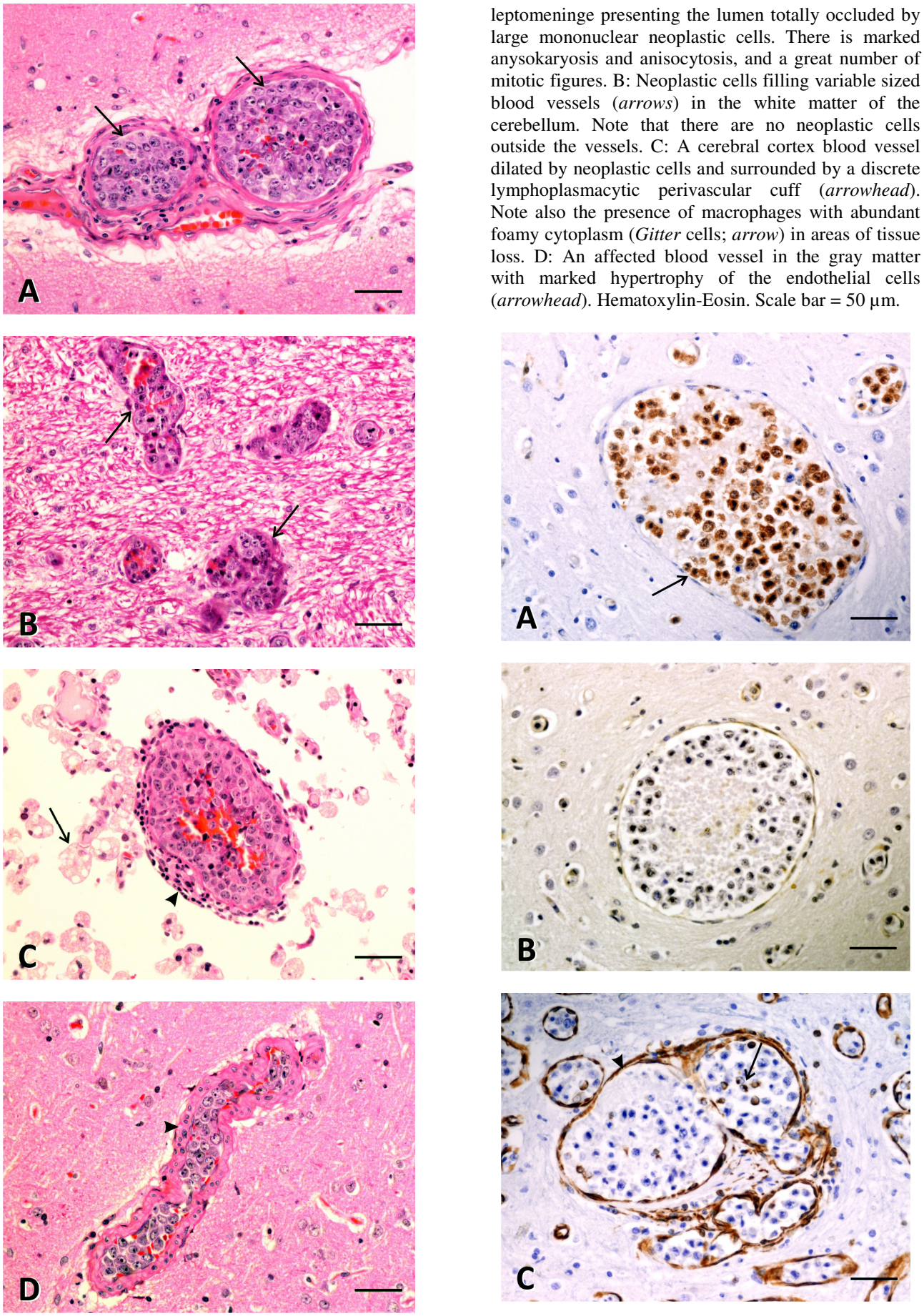

Figure 2. Brain, dog, intravascular lymphomatosis. A: Two blood vessels (*arrows*) in the cortical leptomeninge presenting the lumen totally occluded by large mononuclear neoplastic cells. There is marked anysokaryosis and anisocytosis, and a great number of mitotic figures. B: Neoplastic cells filling variable sized blood vessels (*arrows*) in the white matter of the cerebellum. Note that there are no neoplastic cells outside the vessels. C: A cerebral cortex blood vessel dilated by neoplastic cells and surrounded by a discrete lymphoplasmacytic perivascular cuff (*arrowhead*). Note also the presence of macrophages with abundant foamy cytoplasm (*Gitter* cells; *arrow*) in areas of tissue loss. D: An affected blood vessel in the gray matter with marked hypertrophy of the endothelial cells (*arrowhead*). Hematoxylin-Eosin. Scale bar = 50 µm.

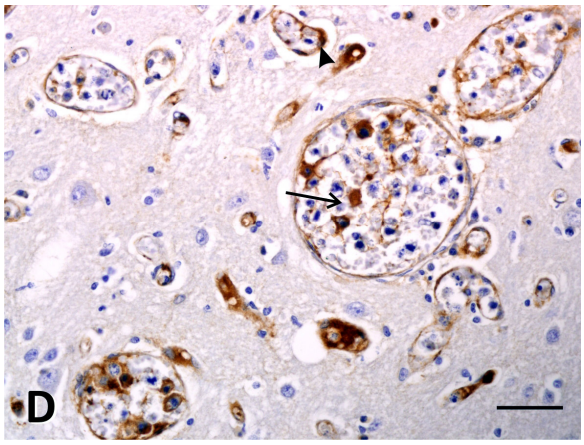

Figure 3. Brain, dog, intravascular lymphomatosis. A: Immunolabelled intravascular cells (cytoplasmatic staining) showing positive phenotype for CD3 marker (*arrow*) in the gray matter. B: Section of the gray matter stained with anti-CD79. Observe lack of stain. C: Endothelial cells (*arrowheads*) and some few intravascular cells (*arrows*) presenting positive cytoplasmatic staining for vimentin marker in the white matter. D: Case No. 1. Cytoplasmatic staining for Von Willebrand factor (formerly factor VIII-related antigen) exhibiting rare positive intravascular (*arrows*) and endothelial cells (*arrowheads*) in the gray matter. Streptavidin–biotin peroxidase complex method. Scale bar = 50 µm.

Discussion

The histopathological lesions observed in these dogs are consistent with a disease characterized by the proliferation of cells within blood vessels. IVL involving CNS is a rare but important differential diagnosis of encephalopathies and encephalitis. A retrospective review of veterinary medical records identified 17 cases of canine IVL and the clinical presentation was variable including spinal cord ataxia, posterior paralysis, seizures and vestibular disease (9).

McDonough *et al.* (9), found T cells positive for $\alpha_d\beta_2$ leukointegrin suggesting a splenic origin and they hypothesized that the cells arise in red pulp, or other immune compartment. The reason why the neoplastic cells exhibit an exclusively intravascular pattern of proliferation is still unclear, but there are evidences that these cells express molecules to adhere to the vascular endothelium, such as CD44 and hyaluronan, and at the same time they lack the expression of important receptors involved in diapedesis, such as ICAM-1, β_1-integrin, CD11a and CD18 (11).

The clinical and pathological features of canine IVL closely resembled those of the human disease. However, in prominent contrast to human cases, which are most often B cell lymphomas, the immunophenotype of the canine IVLs is rather heterogeneous. Canine IVLs are described as derived primarily from T cells and from non-T non-B lymphocytes, and B cells being found in only a single instance (9).

Usually in routine of veterinary pathology diagnosis, the classic CD3 marker identifies cells with T lymphocyte phenotype, while CD20, CD79α and IgG can be used to recognize cells derived from the B cell lineage (9, 11). Moreover, as endothelial cells might, sometimes, be involved in this disorder, identification of these cells can be achieved by immunodetecting the Von Willebrand factor (formerly factor VIII-related antigen), which also identifies megakaryocytes and platelets (7). There are a great number of other cellular markers that can be used to further classify the lymphomatous neoplastic cells from humans and mice (1), but those that are applicable to canine tissue are infrequent or unavailable.

In the cases presented herein, the immunohistochemical staining demonstrated that neoplasic cells had intense cytoplasmatic staining for CD3 and no evidence of B cell origin was found (negative staining with CD79 and IgG markers). This result characterized both cases as IVL of T cell origin, in agreement with previous descriptions (9). Further, in case No. 1, contrasting with other reports (18, 19), we found a small number of neoplastic cells positive for the Von Willebrand factor marker, which is used to study angiogenesis in neoplasms. This finding suggests an endothelial precursor of some neoplastic cells and is indicative of a heterogeneous origin of the neoplasm in case No. 1; however, in a report of IVL in humans, Kano *et al.* (7), described that 28% of IVLs presented factor VIII positive cells and they suggested that this finding occurs due to a non-specific absorption of factor VIII from the thrombi.

The results of IHC validated the prevalence of IVLs from the T cell type in dogs, even though the positivity of few cells for the Von Willebrand factor gives evidences of the heterogeneity of immunophenotypes among intravascular cells of the same animal.

In conclusion, we report two cases of IVL affecting the CNS of dogs, and with IHC, we were able to classify these neoplasms into two subtypes: while case No. 1 was an IVL with predominating T cells; case No. 2 was clearly an IVL of the T cell lineage type. Further, these findings highlight the importance of IHC to aid the correct classification of neoplasms and also emphasize the necessity of IVL to be included in the differential diagnoses of encephalitis and encephalopathies.

Acknowledgements

We wish to thank M.Y.I. Campos and F.A. Ardisson from UNESP–FCAV–Jaboticabal for excellent histotechnical support.

References

1. ARDIGHIERI L., LONARDI S., VERMI W., MEDICINA D., CERRONI L., FACCHETTI F. Intralymphatic atypical T-cell proliferation in a

cutaneous hemangioma. J. Cutan. Pathol., 2010, 37, 497-503.

2. BUSH WW., THROOP JL., MCMANUS PM., KAPATKIN AM., VITE CH., VAN WINKLE TJ. Intravascular lymphoma involving the central nervous system in a dog. J. Am. An. Hosp. Associat., 2003, 39, 90-6.
3. CHAPIN JE., DAVIS LE., KORNFELD M., MANDLER RN. Neurological manifestations of intravascular lymphomatosis. Acta Neurol. Scand., 1995, 91, 494–9.
4. DROBACHEFF C., BLANC D., ZULTAK M., HUMBERT P., CARBILLET JP., DUPOND J.L., LAURENT R. Malignant angioendotheliomatosis reclassification as an angiotropic lymphoma. Int. J. Dermatol., 1989, 28, 454–6.
5. FERRERI AJM., DOGNINI GP., CAMPO E., WILLEMZE R., SEYMOUR JF., BAIREY O., MARTELLI M., DE RENZO A., DOGLIONI C., MONTALBAN C., TEDESCHI A., PAVLOVSKY A., MORGAN S., UZIEL L., FERRACCI M., ASCANI S., GIANELLI U., PATRIARCA C., FACCHETTI F., DALLA LIBERA A., PERTOLDI B., HORVATH B., SZOMOR A., ZUCCA E., CAVALLI F., PONZONI M., ON BEHALF OF THE INTERNATIONAL EXTRANODAL LYMPHOMA STUDY GROUP (IELSG). Variations in clinical presentation, frequency of hemophagocytosis and clinical behavior of intravascular lymphoma diagnosed in different geographical regions. Haematologica., 2007, 92, 486-92.
6. GATTER KC., WARNKE RA. Intravascular large B-cell lymphoma. JAFFE ES., HARRIS NL., STEIN H., VARDIMAN J. Eds. Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press, 2001: 177-8.
7. KANO M., YOSHIDA J., HASHIZUME Y., SUGITA K. Factor VIII can be positive in a special type of malignant lymphoma, intravascular malignant lymphomatosis: an immunohistochemical investigation. Noshuyo Byori., 1994, 11, 135-141.
8. LAPOINTE JM., HIGGINS RJ., KORTZ GD., BAILEY C.S., MOORE PF. Intravascular malignant T-cell lymphoma (malignant angioendotheliomatosis) in a cat. Vet. Pathol., 1997, 34, 247-50.
9. McDONOUGH SP., VAN WINKLE TJ., VALENTINE BA., VAN GESSEL YA., SUMMERS BA. Clinicopathological and immunophenotypical features of canine intravascular lymphoma (malignant angioendotheliomatosis). J. Comp. Pathol., 2002, 126, 277-88.
10. MOLINA A., LOMBARD C., DONLON T., BANGS C., DORFMAN RF. Immunohistochemical and cytogenetic studies indicate that malignant angioendotheliomatosis is a primary intravascular (angiotropic) lymphoma. Cancer., 1990, 66, 474–9.
11. NAKAMICHI N., FUKUHARA S., AOZASA K., MORII E. NK-cell intravascular lymphomatosis – a mini-review. Eur. J. Haematol., 2008, 81, 1-7.
12. PASSARIN M., WEN P., VATTEMI E., BUFFONE E., GHIMENTON C., BONTEMPINI L., OTTAVIANI S., MUSSO A., PEDERSINI R. Intravascular lymphomatosis and intracerebral haemorrhage. Neurol. Sci., 2010, 31, 793-7.
13. PFLEGER L., TAPPEINER J. On the recognition of systematized endotheliomatosis of the cutaneous blood vessels (reticuloendotheliosis?). Hautarzt., 1959, 10, 359-63.
14. 3RIDGE L., SWINNEY G. Angiotrophic intravascular lymphosarcoma presenting as bi-cavity effusion in a dog. Aust. Vet. J., 2004, 82, 616-8.
15. ROTHWELL TL. Angiotrophic intravascular lymphosarcoma. Aust. Vet. J., 2005, 83, 207.
16. ROTHWELL TL., XU FN., WILLIS FJ. Unusual multisystemic vascular lesions in a cat. Vet. Pathol., 1985, 22, 510-2.
17. SIPS GJ., AMORY CF., DELMAN BN., KLEINMAN GM., LIPSEY LR., TUHRIM S. Intravascular lymphomatosis of the brain in a patient with myelodysplastic syndrome. Nat. Rev. Neurol., 2009, 5, 288-92.
18. SUMMERS BA., DeLAHUNTA A. Cerebral angioendotheliomatosis in a dog. Acta Neuropathol., 1985, 68, 10–4.
19. VAN GESSEL YA., McDOUNOGH SP., McCORMICK HJ., SUMMERS BA. Cutaneous presentation of canine intravascular lymphoma (malignant angioendotheliomatosis). Vet. Dermatol., 2000, 11, 291-7.
20. VIEREN M., SCIOT R., ROBBERECH W. Intravascular lymphomatosis of the brain: a diagnostic problem. Clin. Neurol. Neurosurg., 1999, 101, 33-6.

 All rights reserved 2007.