MANIFESTAÇÕES OFTALMOLÓGICAS E NEUROLÓGICAS EM UM CÃO COM EPENDIMOMA ANAPLASICO INTRACRANIAL. RELATO DE CASO

(OPHTHALMOLOGICAL AND NEUROLOGICAL MANIFESTATIONS OF A DOG WITH INTRACRANIAL ANAPLASTIC EPENDYMOMA. CASE REPORT)

RESUMO

Objetiva-se relatar ependimoma associado a uveíte em cão macho de quatro meses de idade, mestiço Boxer. O cão inicialmente apresentou em ambos os olhos hiperemia conjuntival, blefaroespasmo, projeção da membrana nictante e fotofobia, característicos de uveíte. Uveíte traumática foi rejeitada. Seis dias após o início dos sintomas oftálmicos ocorreram vômito, incoordenação e mudança de comportamento. Sintomas neurológicos e contração das sombrancelhas sugeriram dor de cabeça. Injúria cerebral foi investigada por tomografia computadorizada (TC) nove dias após o cão demonstrar espasmos dos membros e pescoço, seguido por parada respiratória com reversão e estabilização. A TC revelou extensa e amórfica neoformação no diencéfalo, mesencéfalo, e dentro do ventrículo lateral direito, com deslocamento à esquerda da foice cerebral. O animal foi eutanasiado, e a necropsia da cabeça revelou hidrocefalia e uma massa tumoral intracerebral consistente com a imagem da TC. A avaliação histológica por coloração hematoxilina e eosina, revelou alterações teciduais em várias áreas do Sistema Nervoso Central (SNC), mostrando vária pseudorosetas no neurópilo, mitose, e um elevado grau de atipia celular, sugerindo ependimoma. Lesões teciduais inflamatórias, hemorrágicas e necróticas foram observadas no tronco encefálico e cerebelo, devido à compressão do tumor e à hidrocefalia. A neoplasia foi fenotipicada por imunohistoquímica (IHQ), e testou positiva para os marcadores tumorais vimentina e proteína glial fibrilar ácida, confirmando ependimoma anaplásico intracranial. As mudanças comportamentais e sinais neurológicos resultaram do processo vascular, inflamatório e degenerativo no neurópilo, causados pela compressão e invasão do tecido cerebral pela neoplasia. Embora cães com ependimoma frequentemente apresentem doença neurológica, no presente caso, blefaroespasmo foi o primeiro sintoma observado pelo proprietário, e persistiu até eutanásia. Os sintomas neurológicos estão relacionados à localização do tumor e extensão das lesões secundárias devidas à sua expansão. Sintomatologia clínica e testes laboratoriais

complementares, TC, necropsia, histologia e IHQ caracterizaram ependimoma, que é raro em animais jovens.
PALAVRAS-CHAVE: Blefaroespasmo, Uveíte, Neoplasia Intracranial, Sintomas Neurológicos.

SUMMARY:

The objective is to report ependymoma associated with uveitis in a four-month-old male boxer crossbreed. The dog initially presented in both eyes conjunctival hyperemia, blepharospasm, projection of the nictitating membrane, and photophobia, characteristic of uveitis. Traumatic uveitis was rejected. Six days after onset of ophthalmic symptoms, vomiting, incoordination, and behavior changes occurred. Neurological symptoms and eyebrow contraction suggesting head pain and brain injury were investigated by computed tomography (CT) nine days after the dog showed spasms of the limbs and neck followed by respiratory arrest with reversal and stabilization. The scans revealed extensive amorphic neoformation in the diencephalon, midbrain, and within the right lateral ventricle, along with sinistral displacement of the cerebral sickle. The animal was euthanized, and necropsy of the head revealed hydrocephalus and an intracerebral tumor mass consistent with the CT imaging. Histological evaluation by hematoxylin and eosin staining revealed tissue alterations in several CNS segments, showing several pseudorosettes in the neuropil, mitosis, and a high degree of cell atypia, suggesting ependymoma. Inflammatory, hemorrhagic, and necrotic tissue lesions were observed in the brainstem and cerebellum due to compression by tumor tissue and hydrocephalus. The neoplasia was phenotyped by Immunohistochemistry (IHC), and tested positive for the tumoral markers vimentin and glial fibrillary acid protein, confirming intracranial anaplastic ependymoma. Behavior changes and neurological signs resulted from vascular, inflammatory, and degenerative processes in the neuropil caused by neoplasm compression and invasion of brain tissue. Although dogs with ependymoma often present with neurological disease, in the present case, blepharospasm was the first symptom noticed by the owner, and it persisted until euthanasia. According to the literature, and confirmed in the evolution of the current case, the symptoms are related to tumor location and extent and to secondary lesions due to tumor expansion. Clinical symptomatology and complementary laboratory testing, CT, necropsy, histology, and IHC characterized ependymoma, a rare condition in young dogs.
KEY-WORDS: Blepharospasm, Uveitis, Intracranial Neoplasia, Neurological Symptoms.

INTRODUCTION

Uveitis is a significant ocular disease in dogs and cats, with anterior uveitis being a most common symptom. Its clinical importance is due to associated vision loss and serious systemic inflammatory or infectious disease (HENDRIX, 2008; TOWSEND, 2008; NASCIMENTO, 2016), toxic, traumatic, and non-infectious systemic disease or idiopathy (RENWICK; PETERSEN-JONES, 1998; POWELL, 2002; HENDRIX, 2008; SIMON, 2008). The uvea, through its rich vascularization, can be the site of inflammatory reaction to microorganisms and toxins released in generalized infections and to local infections and subclinical metastases (WALDE et al., 1998).

Ependymoma is a primary tumor of the central nervous system (CNS) of neuro-epithelial origin, that is most often seen in middle-aged to aged animals and reported with higher incidence in brachycephalic dog breeds, and clinical signs vary depending on the location of the tumor within the CNS as well as the degree of compression of adjacent tissue (FENNER, 2004; ZACHARY, 2007; ANDRADE et al., 2015). Microscopically, ependymomas are highly cellular and well vascularized. Cells form perivascular rosettes (pseudorosettes) with nuclear polarity away from the vessel wall, or can be arranged in sheets and bands (KOESTNER; HIGGINS, 2002; ZACHARY, 2007).

The differential diagnosis of ependymomas from other CNS tumors showing pseudorosette patterns is made by the finding of true ependymal rosettes along with uniform consistent glial acidic fibrillary protein (GFAP) and vimentin immunohistochemical staining (SFACTERIA et al., 2010). A presumptive diagnosis of dog CNS neoplasia is usually made by magnetic resonance imaging (MRI) (HORTA et al., 2013; KRAFT et al., 1997; ZHAO et al., 2010) or computed tomography (CT) (HORTA et al., 2013; TURREL et al.,1986). Definitive diagnosis requires analysis of brain tissue samples (HORTA et al., 2013), and spinal cord (UENO et al., 2006).

We report a case of ophthalmological and neurological disease in a dog due to an intracranial ependymoma.

CASE REPORT

A four-month-old male boxer crossbreed was brought for ophthalmologic consultation to the veterinary clinic CEMEV, Ubatuba city, SP state, Brazil, having shown signs for three days prior of bilateral ocular discomfort. It presented with normal water and food intake, normal stools and defecation, and normal urine and urination.

General clinical condition: The dog flinched from touch and attempted to escape manipulation. It presented normal heart and respiratory rate and body temperature, normocorated gingival mucosa, hypercorated ocular mucosa, and non-reactive lymph nodes. A hemogram showed thrombocytosis.

Ophthalmological examination: Face was symmetrical and ocular position normal. Photomotor and consensual pupillary response was preserved, but reflex of exacerbated glare, photophobia, and accentuated blepharospasm were evident. Evidence of retropulsion pain, but no resistance or increase in volume of eyeballs. Intense conjunctival and bulbar hyperemia, with reactive lymphoid follicles in bilateral nictitating membranes. Anterior chamber, crystalline and fundoscopy without bilateral changes. Fluorescein negative, bilateral. Deambulation under photopic and scotopic conditions. Oral dipyrone was administered twice daily for four days and 0.1% prednisolone eye drops four times daily for four days.

At reassessment, there was no improvement of the blepharospasm, but the dog showed greater comfort after the administration of dipyrone. Again, the dog attempted escape handling, so a 24 h fast and sedation was applied before a thorough ophthalmological examination. At this second assessment, the owner reported that the dog presented apathy, had experienced

bouts of vomiting, and had fallen into a pool. Clinical examination showed normal vital signs. A hemogram to assess leukocytosis, neutrophilia, and thrombocytosis and measures of glutamic-pyruvic transaminase, alkaline phosphatase, total protein, and glycemia showed normal values. After a subsequent six day follow-up and treatment, the patient showed evidence of episodes of severe pain, and was not responsive to the administration of fentanyl, morphine, and methadone combined with dipyrone. Interaction of the animal with the environment was diminished: It began to emit cries and groans, walking around walls, and tachypnea alternated with eupnea. Contraction of eyebrows suggested head pain, and, in order to investigate a possible cerebral lesion, CT of the skull was done on day nine of investigation. Some hours before the CT examination, the patient suffered severe worsening of symptoms, including limb and neck spasms followed by respiratory arrest. The patient was stabilized and CT was conducted using a tomograph Toshiba® (Alexion 16 channel), helical method - multislice, with 16x1mm collimation, pre- and post-intravenous contrast Omnipaque® (iohexol 300 mgI/mL) at 2 mL/kg. Imaging revealed an extensive amorphous neoformation originating from the right hippocampus and progressing to the right lateral and left ventricle region, exhibiting a marked sinistral displacement of the cerebral sickle and partial loss of definition of the lateral ventricles. The mass occupied a large portion of the diencephalon and midbrain, as well as the interior of the right lateral ventricle. Administration of contrast medium revealed diffuse heterogeneous enhancement of approximately 3.8 x 3.5 x 4.3 cm (Figure 1).

Due to the severity of the clinical condition and grave prognosis, the animal was euthanized following the CFMV (2012) protocol.

Because CT indicated a tumor mass, the head of the animal was necropsied to obtain CNS tissue samples for definitive histological diagnosis (HORTA et al., 2013). Removal of the skull cap revealed hydrocephaly. The cerebral hemispheres, cerebellum, brainstem, and cervical spinal cord were removed for macroscopic analysis. A 5.5 x 3.0 x 2.5 cm gray, well-

demarcated, soft-textured mass was found, mostly located in the right ventricle, which dislocated the scythe of the brain and invaded the left ventricle and extended into the thalamus (Figure 2).

The cortical regions of the right and left cerebral hemispheres were thinner, and the right lateral ventricle was much dilated due to hydrocephalus (Figure 3).

Fragments of CNS and tumor tissue were fixed in 10% buffered formalin, and were cut into small pieces, dehydrated, cleared, and embedded in paraffin (PROPHET, 1995). Three µm sections were cut and stained with hematoxylin and eosin. Histological evaluation by light microscopy revealed tissue alterations in several CNS segments (Table 1), showing several pseudorosettes in the neuropil, mitosis, and a high degree of cell atypia (KOESTNER; HIGGINS, 2002; MAXIE; YOUSSEF, 2007; ZACHARY, 2007). Inflammatory, hemorrhagic, and necrotic tissue lesions were observed in the brainstem and cerebellum due to compression by tumor tissue and hydrocephalus.

Tumor phenotyping was performed by immunohistochemistry (IHC) (VAN DER WOERDT, 2001; VURAL et al., 2006; UENO et al., 2006).

Antigen retrieval employed citrate buffer at pH 6.0 with 18 hours incubation at 4°C. The non-biotinylated polymer amplification ENVISION/HRP (Dako®, Carpinteria, CA, USA) was used as the detection system (Avidin-Biotin Immunohistochemistry) (KEY, 2006) according to the manufacturer's protocol. The panel of primary antibodies used for tumor immunophenotyping (Table 2) were vimentin (intermediate filament of mesenchymal cells) clone V9 at 1:200 dilution, polyclonal glial fibrillar acid protein (GFAP 9) at 1:300 dilution, PAN-CK (intermediate filaments of epithelial cells) clone AE1AE3 at 1:200 dilution, E cadherin (cell adhesion molecule) clone NHC-38 at 1:50 dilution, synaptophysin (neuroendocrine cell marker) clone SY38 at 1:100 dilution, neuron-specific enolase (NSE) clone BBS/NC/HIV14 at 1:1000 dilution, and polyclonal S100 (neuroglial, ependymal,

melanocytic and Schwann cells marker) at 1:1000 dilution. For visualization of development of the reaction, the chromogen diaminobenzidine (DAB) (Dako®, Carpinteria, CA, USA) was used according to the manufacturer's protocol. The sections were counterstained with hematoxylin and eosin and examined under optical microscope to verify the positive brown staining in the cytoplasm and nuclei.

The immunolabeling of the neoplasia was positive by IHC for vimentin (Figure 4B) and GFAP 9 (Figures 4C and 4D) and negative for PAN-CK (Figure 4E), E cadherin (Figure 4F), synaptophysin (Figure 4G), and S100 (Figure 1H). The features of the neoplasm were most consistent with anaplastic ependymoma (Table 2) (KOESTNER; HIGGINS, 2002; SFACTERIA et al., 2010).

This case report was approved by the Ethics Committee on Animal Experiments on May 2008, registration number 53/08.

DISCUSSION

Uveitis is often the first manifestation of systemic disease (POWELL, 2002). The initial symptoms presented in the current case were conjunctival hyperemia, blepharospasm, projection of the nictitating membrane, and photophobia, characteristic of uveitis (RENWICK; PETERSEN-JONES, 1998).

Bilateral ophthalmic inflammatory involvement observed in the reported case could have been related to anterior uveitis resulting from the hyperviscosity syndrome present in neoplastic and paraneoplastic alterations, or even in response to chemical mediators of inflammation (Oriá et al., 2004; Gellat, 2003).

Ependymoma associated with uveitis has not been previously reported in dogs.

The condition described here in a four-month-old dog is rare and runs counter to the scientific literature, which reports ependymoma to commonly occur in middle-aged to aged

190 animals (MAXIE; YOUSSEF, 2007; TRASLAVINA et al., 2013). A higher incidence is
191 reported in the brachycephalic dog breeds (KOESTNER; HIGGINS, 2002; MARCH, 2003,
192 VURAL et al., 2006, ZACHARY, 2007). This young dog was a boxer crossbreed.

193 Although dogs with ependymoma often present with neurological disease
194 (ZACHARY, 2007), in the present case, blepharospasm was the first symptom noticed by the
195 owner, and it persisted until euthanasia. According to the literature, and confirmed in the
196 evolution of the current case, the symptoms are related to tumor location and extent and to
197 secondary lesions due to tumor expansion (FENNER, 2004; ZACHARY, 2007; ANDRADE
198 NETO et al., 2015). Computed tomography was used to investigate the structure of the dog's
199 brain (TURREL et al., 1986), and findings were confirmed by necropsy: the tumor extended
200 from the thalamus to both lateral intraventricular spaces, compressing the right and left cerebral
201 cortex and diencephalon, and causing hydrocephalus (Fig. 1, 2 and 3), as described in other
202 cases (KOESTNER; HIGGINS, 2002; MAXIE; YOUSSEF, 2007; ZACHARY, 2007; GUAN
203 et al., 2011; HORTA et al., 2013). The microscopic lesions in the CNS (Table 1) confirmed
204 compression of the cortical neurophil by tumor expansion and hydrocephalus from the frontal,
205 temporal, parietal, and occipital cortex to the diencephalon (FENNER, 2004; ZACHARY,
206 2007; ANDRADE NETO et al., 2015).

207 The tumor invasion of the thalamus caused hemorrhagia and necrosis of the neurophil.
208 The cerebellum and brainstem were compressed by hydrocephalus, causing hemorrhage and
209 necrosis of the neurophil (Table 1) (FENNER, 2004; ZACHARY, 2007; ANDRADE NETO et
210 al., 2015).

211 The gross and microscopic lesions can explain the clinical evolution of the case: The
212 blepharospasm resulted from lesions in the basal ganglia and upper midbrain, and bulbar and
213 cerebellar injury produced incoordination, vomiting, and respiratory arrest (EMOTO et al.,
214 2011).

Sudden-onset clinical signs have been previously reported in an intracranial malignant ependymoma in a boxer breed dog (BORRELLI et al., 2009).

Based on the presence of prominent perivascular pseudorosettes, ependymoma, papillary meningioma, and paraganglioma were considered in the IHC differential diagnoses. Immunohistochemical staining was conducted (KEY, 2006), showing the features of the neoplasm to be consistent with anaplastic ependymoma (Table 2). Multiple subtypes of ependymomas have been described (VURAL et al., 2006), including some that lack the characteristic papillary pattern and rosette formation expected in classic tumors. The relatively few studies that have examined biomarkers in animal ependymomas have reported positive vimentin staining and GFAP reaction, although an ependymoma in the cervical spinal cord not expressing GFAP and slightly positive for vimentin and cytokeratin was identified in an 8.5-year-old Maltese dog (MICHIMAE et al., 2004).

A case of similar oculocephalic and behavior responses and generalized ataxia in a nine-year-old German Shepherd associated with ventricular ependymoma has been reported [(VURAL et al., 2006). This case report was complemented by MRI, morphological exam, and immunohistology. The 9 x 6 x 5 mm ependymoma, localized intra-axially in the right interventricular foramen and hydrocephalus was detected by MRI. Microscopically, the tumor was composed of pseudorosettes, and immunohistochemical examination revealed vimentin and GFAP immunoreactivity in the neoplastic cells.

It is essential to differentiate primary brain tumors from secondary metastatic tumors (MOORE et al., 1996). Studies of causes of intracranial tumors in the CNS of dogs are imperative to aid in differential diagnosis, to provide a prognosis, and recommend treatment or euthanasia. Ultrasonography, computed tomography, magnetic resonance, and nuclear medicine are noninvasive and offer high-resolution cross-section images that help the clinician correlate symptoms with pathology (TUCKER; GAVIN, 1996).

In the current case, the CT was fundamental in demonstrating that the ophthalmic symptoms resulted from the irreversible expansion of intracerebral lesion, allowing a bad prognosis and the final decision of euthanasia. Neoplasms of the CNS in dogs typically cause progressive neurological signs in aged animals, with seizures and behavior changes common. Many produce localized signs detected on neurological examination and allow an anatomical diagnosis. Secondary changes such as obstructive hydrocephalus, cerebral edema, herniations, and tumor expansion may produce more wide-spread deficits. Ophthalmoscopic examination occasionally reveals papilledema. Morphological studies include IHC for phenotyping and classifying as differentiated or not.

Research is critical to understanding the molecular aspects of ependymoma, and to the development of a targeted therapeutic strategy (GUAN et al., 2011). Animals may serve as experimental models for humans, and studies of the biology of ependymoma and other CNS neoplasia should be expanded, detailing the clinical history and evolution of the disease, in light of what is known in human cases.

CONCLUSION

The comprehensive clinical investigation of an ophthalmologic case, followed through significant neurological disease evolution, culminated in diagnosis of clinical pathology via computed tomography, necropsy, CNS histology, and tumor phenotyping by immunochemistry, elucidated a rare case of ependymoma in a young dog.

REFERENCES

ANDRADE NETO, J. P.; DINIZ, S. A.; JIMENEZ, C. D.; MAIORKA, P. C. Neoplasias intracranianas. In: JERICÓ M. M., KOGIKA M. M., NETO J .P. A. (Eds). **Tratado de medicina interna de cães e gatos.** 1.ed. Rio de Janeiro: Guanabara Koogan, 2015. p. 2114-2019.

BORRELLI, A.; MATTIAZZI, L.; CAPUCCHIO, M. T.; BIOLATTI, C.; CAGNASSO, A.; GIANELLA, P.; D'ANGELO, A. Cachexia secondary to intracranial anaplastic (malignant) ependymoma in a boxer dog. **Journal of Small Animal Practice,** v. 50, n. 10, p. 554-557, 2009.

CONSELHO FEDERAL DE MEDICINA VETERINÁRIA (CFMV) [2012]. **Resolução nº 1000, de 11 de maio de 2012. Dispõe sobre procedimentos e métodos de eutanásia em animais e dá outras providências.** Avaiable in: <http://portal.cfmv.gov.br/lei/index/id/326>. Accessed on: 05/02/2019.

DIMANTAS, M. A. P.; LOWDER, C.; MUCCIOLI, C. Uveítes anteriores associadas a doenças sistêmicas. **Arquivos Brasileiros de Oftalmologia,** v. 66, n. 2, p.235-238, 2003.

EMOTO, H.; SUZUKI, Y.; KIYOSAWA, M. Blepharospasm - A review and updates. Types and symptoms. **Neuro-Ophthalmoogy Japan**, v. 28, n. 2, p. 257-266, 2011.

FENNER, W. R. Doenças do cérebro. In: ETTINGER S. J.; FELDMAN E. C. (Eds). **Tratado de medicina interna veterinária: doenças do cão e do gato.** 5.ed. Rio de Janeiro: Guanabara Koogan, 2004. p. 538-586.

GELLAT, K. N. **Fundamentos de oftalmología veterinaria.** 1.ed. Barcelona: Masson, 2003. p. 197-225.

GUAN, S.; SHEN, R.; LAFORTUNE, T.; TIAO, N.; HOUGHTON, P.; YUNG, W. K.; KOUL, D. Establishment and characterization of clinically relevant models of ependymoma: a true challenge for targeted therapy. **Neuro-Oncology ,** v.13, n. 7, p. 748-758, 2011.

HENDRIX, D. V. H. Diseases and surgery of the canine anterior uvea. In: GELLAT, K. N. (Ed.). **Essentials of veterinary ophthalmology.** 2.ed. Iowa: Blackwell Publishing, 2008. p. 189-216.

HORTA, R. S.; MARTINS, B. C.; LAVALLE, G. E.; COSTA, M. P.; ARAÚJO, R. B. Intracranial neoplasms in small animals - review. **Acta Veterinaria Brasilica**, v. 13, n. 7, n. 4, p. 272-281, 2013.

KEY, M. (Ed.) **Educational Guide: Immunohistochemical Stainning Methods.** 4.ed. Carpinteria: DAKO, 2006. p. 47-53.

KOESTNER, A.; HIGGINS, R. J. Tumors of the Ependyma and Choroid Plexus. In: Meuten, D. J. (Ed.). **Tumors in Domestic Animals.** 5.ed. Ames: Blackwell Publishing Professional, 2002. p. 707-709.

KRAFT, S. L.; GAVIN, P. R.; DEHAAN, C.; MOORE, M.; WENDLING, L. R.; LEATHERS, C. W. Retrospective review of 50 canine intracranial tumors evaluated by magnetic resonance imaging. **Journal of Veterinary Internal Medicine, v.** 11, n. 7, p. 218-225, 1997.

MARCH, P. A. Cerebropatias. In: BICHARD S. J.; SCHERDING R. G. (Ed.). **Manual Saunders: clínica de pequenos animais.** 2.ed. São Paulo: Roca, p. 1371-1394, 2003.

MASSA, K. L.; GILGER, B. C.; MILLER, T. L.; DAVIDSON, M. G. Causes of uveitis in dogs: 102 cases (1989-2000). **Veterinary Ophthalmology,** v. 5, n. 2, p. 93-98, 2002.

MAXIE, M. G., YOUSSEF, S. Ependymoma. In: MAXIE, M.G. (Ed). **Jubb, Kennedy and Palmer´s. Pathology of Domestic Animals.** 5.ed. Philadelphia: Elsevier, p.448-449, 2007.

MICHIMAE, Y.; MORITA, T.; SUNAGAWA, Y.; SAWADA, M.; OKAMOTO, Y.; SHIMADA, A. Anaplastic ependymoma in the cervical spinal cord of a Maltese dog. **Journal of Veterinary Medical Science,** v. 66, n. 9, p. 1155-1158, 2004.

MOORE, M .P.; BAGLEY, R. S.; HARRINGTON, M. L.; GAVIN, P. R. Intracranial tumors. **Veterinary Clinics of North America Small Animal Practice,** v. 26, n. 4, p. 759-777, 1996.

NASCIMENTO, H. M. [2016]. Uveítes: revisitando o tema. **e-Oftalmo. Conselho Brasileiro de Oftalmologia: Revista Digital de Oftalmologia, v.** 1, n. 5, p. 1-26, 2016. Avaiable in:<http://eoftalmo1.hospedagemdesites.ws/Content/imagebank/pdf/v2n1a02.pdf>. Accessed on: 10/11/2016.

ORIÁ, A. P.; PEREIRA, P. M.; LAUS, J. L. Uveitis in dogs infected with *Ehrlichia canis.* **Ciência Rural,** v. 34, n. 4, p. 1289-1295, 2004.

Powell, C. C. Uveal Disease: Uveitis in general. In: RIIS, R. C. (Ed.). **Small animal ophthalmology secrets.** Philadelphia: Hanley & Belfus, 2002, p. 177-183.

PROPHET, E. B. Métodos histotecnológicos. In: PROPHET, E.B.; MILLS, B.; ARRINGTON, J. B.; SOBIN, L. H. (Eds.). **Laboratory methods in histotechnology.** 1.ed. Washington: American Registry of Pathology, Armed Forces Institute of Pathology, 1995. p. 31-34.

RENWICK, P. W.; PETERSEN-JONES, S. M. Dolor orbital y ocular. In: PFEIFER, R. L., PETERNSEN-JONES, S. M. (Eds). **Oftalmologia de animales pequeños: una aproximacion orientada hacia el problema.** 2.ed. Buenos Aires: Editorial Intermédica, 1998. p. 167-194.

SFACTERIA, A.; MACRÍ, F.; PERILLO, L.; RAPISARDA, G.; LANTERI, G.; MAZZULLO, G. Cytologic and histologic features of spinal cord ependymoma in a young dog: a case report. **Veterinarni Medicina,** v. 55, n. 1, p. 35-38, 2010.

SIMON, M. Afecções do trato uveal. In: HERRERA, D. (Ed.). **Oftalmologia clínica em animais de companhia.** 1.ed. São Paulo: Medvet, 2008. p. 173-193.

SUMMERS, B. A.; CUMMINGS, J. F.; DE LAHUNTA, A. **Veterinary Neuropathology.** 1.ed. St. Louis: Mosby, 1995. p. 351-401.

TOWSEND, W. M. Canine and feline uveitis. **Veterinary Clinics of North America Small Animal Practice,** v. 38, n. 2. p. 323-346, 2008.

TRASLAVINA, R. P.; KENT, M. S.; MOHR F. C.; DICKINSON P. J.; VERNAU, K. M.; BOLLEN, A. W.; HIGGINS, R. J. Clear cell ependymoma in a dog. **Journal of Comparative Pathology,** v. 149, n. 1, p. 53-56, 2013.

TUCKER, R. L.; GAVIN, P. R. Brain imaging. **Veterinary Clinics of North America Small Animal Practice,** v. 26, n. 4, p. 735-758, 1996.

TURREL, J. M. ; FIKE, J. R.; LE COUTEUR, R. A.; HIGGINS, R. J**.** Computed tomographic characteristics of primary brain tumors in 50 dogs. **Journal of the American Veterinary Medical Association,** v. 188, n. 8, p. 851-856, 1986.

UENO, H.; MORIMOTO, M.; KOBAYASHI, Y.; HIZUME, T.; MURAYAMA, N.; UZUKA, Y. Surgical and radiotherapy treatment of a spinal cord ependymoma in a dog. **Australian Veterinary Journal,** v. 84, n. 1-2, p. 36-39, 2006.

VAN DER WOERDT, A. Management of intraocular inflammatory disease. **Clinical Techniques in Small Animal Practice**, v. 16, n. 1, p. 58-61, 2001.

VURAL, S. A.; BESALTI, O.; ILHAN, F.; OZAK, A.; HALIGUR, M. Ventricular ependimoma in a German Shepherd dog. **Veterinary Journal**, v. 172 n. 1, p. 185- 187, 2006.

WALDE, I., SCHÄFFER, E.H.; KÖSTLIN, R.G. (Eds.) 1998. Afecções da câmara anterior e da úvea anterior. **Atlas de clínica oftalmológica do cão e do gato.** 2.ed. São Paulo: Manole, 1998. p. 133-145.

ZACHARY, J. F. Ependimomas. In: MCGAVIN, M. D.; ZACHARY, J. F.(Eds.). **Pathologic Basis of Veterinary Disease.** 4. ed. St Louis: Mosby Elsevier, 2007. p. 950.

ZHAO, Q.; LEE, S.; KENT, M.; SCHATZBERG, S.; PLATT, S. Dynamic contrast-enhanced magnetic resonance imaging of canine brain tumors. **Veterinary Radiology and Ultrasound,** v. 51, n. 2, p. 122-129, 2010.

412 **Table 1** CNS anatomical segments and microscopic description of observed lesions

CNS Anatomical segment	Microscopic observations
Cervical spinal cord	dilatation of the ependymal canal, neuropil vessels and meningeal congestion
Obex and cerebellar Peduncle	multiple hemorrhagic foci on the neuropil of the 4th ventricle floor.
Cerebellum	multiple hemorrhagic foci in the cerebellar convolutions. Purkinje cell degeneration and necrosis. Meningeal congestion.
Caudal and rostral colliculus	Discrete perivascular hemorrhagic foci. Meningeal and neuropil congestion.
Thalamus	Dark and retracted neurons, edema and neuropil congestion. Meningeal congestion. Tumoral tissue arranged in pseudorosettes, neovascularization, mitotic figures, evident nucleoli, laterally located nucleus, and high degree of cellular pleomorphism (anisocytosis and anisocariosis).
Right frontal, parietal and occipital cortex	dark and retracted neurons, cortical spongiosis, neuropil and meningeal congestion, discrete mononuclear inflammatory infiltrate, meningeal hemorrhage.
Right tumor adhered in cortex temporal	tumor formations arranged in pseudorosettes with neovascularization at the edge of the neuropil (cortical region), mitotic figures, evident nucleoli, laterally located nucleus, high degree of cellular pleomorphism (anisocytosis and anisocariasis)
Right diencephalon	Meningeal and neuropil congestion, cortical spongiosis, discrete mononuclear inflammatory infiltrate.
Left frontal, temporal, parietal and occipital cortex	Meningeal and neuropil congestion, dark and retracted neurons, cortical spongiosis and discrete mononuclear inflammatory infiltrate.
Left diencephalon	Meningeal and neuropil congestion, hemorrhagic foci with dark and retracted neurons, cortical spongiosis and discrete mononuclear inflammatory infiltrate.

Tumoral mass inside both ventricles	tumor formations arranged in pseudorosettes with small central lumen and in leaflets or cell groups without a discernable distribution pattern. Tumor cells with high degree of cellular pleomorphism (anisocytosis and anisocariasis), circular or oval nucleus and moderately hyperchromatic eosinophilic cytoplasm with poorly defined borders, neovascularization, mitotic figures, evident nucleoli.

413

414

416 **Table 2** Antibodies, clone, dilution, and results of tumor cell immunostaining

Antibodies	Clone	Dilution	Immunostaining in neoplastic cells
Vimentin	V9	1:200	Positive
GFAP 9	Polyclonal	1:300	Discrete and sparse positivity
PAN-CK	AE1AE3	1:200	Negative
E cadherin	NHC-38	1:50	Negative
Synaptophisyn	SY38	1:100	Negative
NSE	BBS/NC/VIH14	1:1000	Negative
S100	Polyclonal	1:1000	Negative

417

418

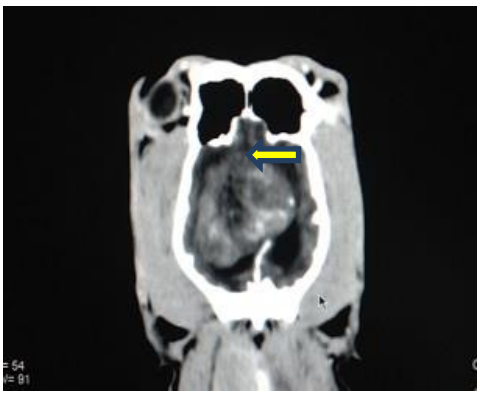


FIGURE 1 Computed tomography. Amorphous neoformation originating in the right hippocampus and progressing to the right lateral ventricle and to the left side, producing displacement of the cerebral sickle to the left and partial loss of definition of the lateral ventricles (yellow arrow). Helical-multislice method. Collimation 16 x 1 mm

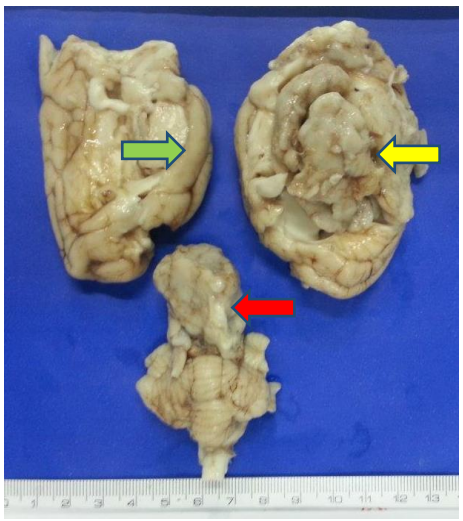


FIGURE 2 The neoplasia in the right ventricle (yellow arrow) dislocated the scythe of the brain, invaded the left ventricle (green arrow), and extended to the thalamus (red arrow)

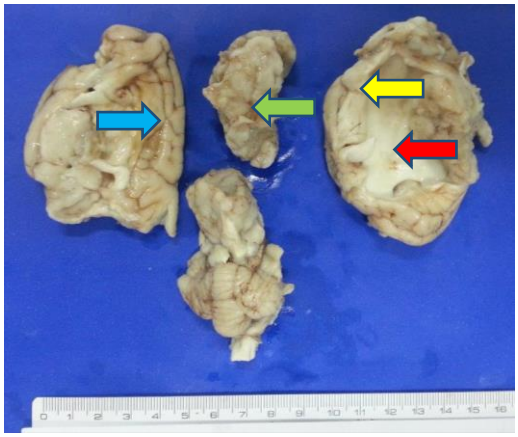
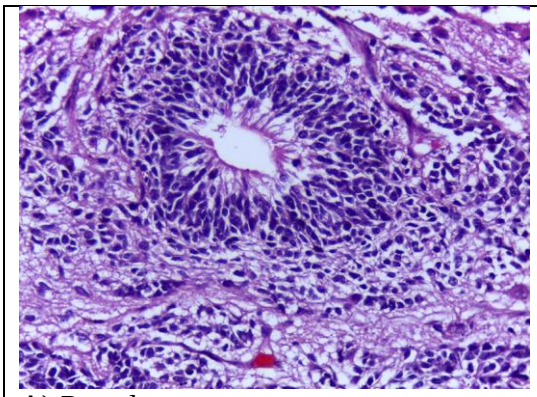
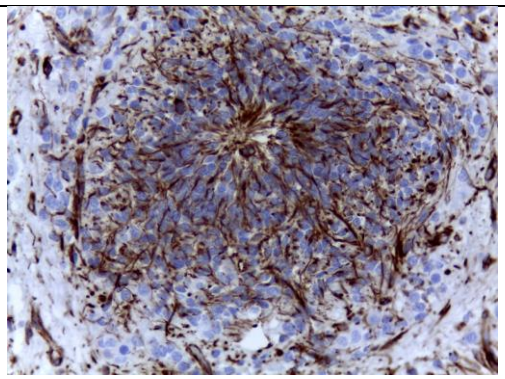


FIGURE 3 The cortical regions of the right (yellow arrow) and left cerebral (blue arrow) hemispheres were less thickened. The right lateral ventricle was much dilated due to hydrocephalus (red arrow). The tumoral mass was withdrawn from the right ventricle (green arrow)

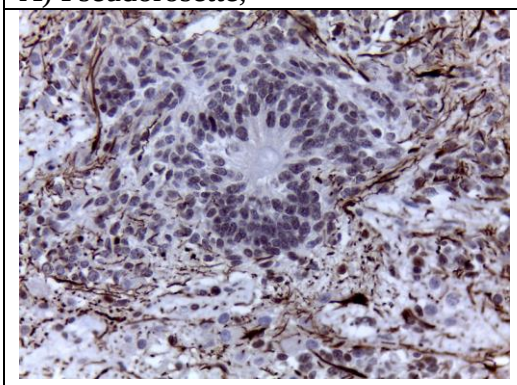
455



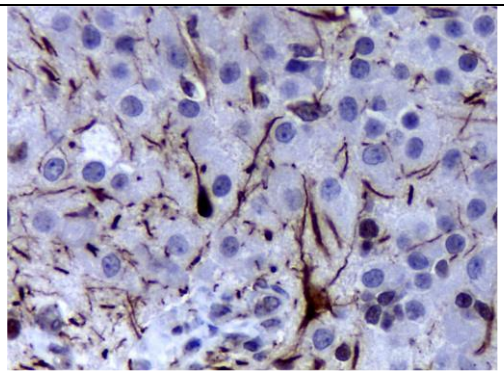
A) Pseudorosette,



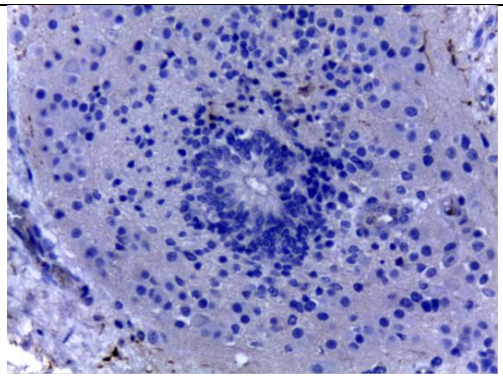
B) Pseudorosette, positive vimentin. 400x.



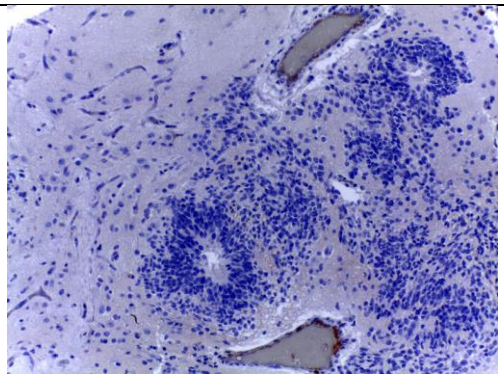
C) Pseudorosette, positive and discrete GFAP 9. 400x.



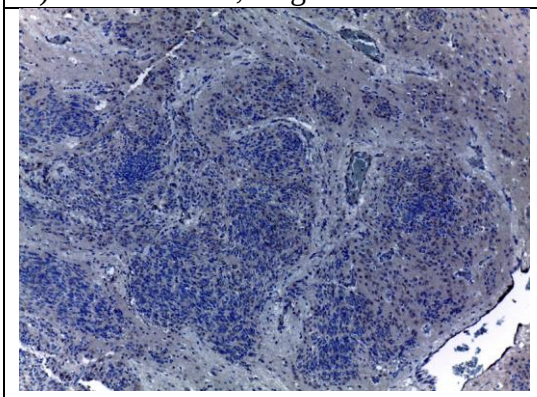
D) Neurophil, positive and discrete GFAP 9. 600x.



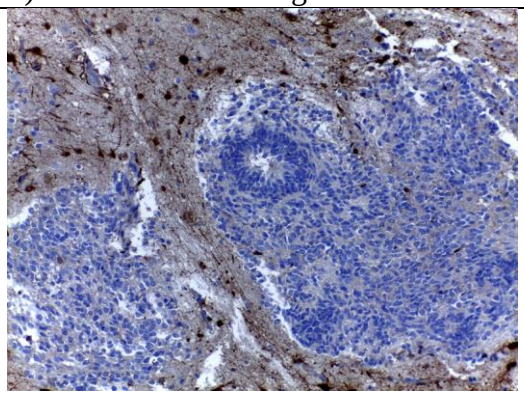
E) Pseudorosette, negative CKPan. 400 x



F) Pseudorosettes - negative E cadherin. 200x.



G) Pseudorosette - negative synaptophysin. 100x



H) Pseudorosette - negative S100. 100x.

456 **FIGURE 4** (A) tumor H&E staining, positive immunostaining by DAB chromogen
457 (B-D) (CHI) for target antibodies, negative immunostaining by DAB chromogen (E-H).