Brief Communication
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Clinicopathologic Features of Intracranial Central Neurocytomas in 2 Dogs

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Background: In humans, central neurocytomas are rare and typically benign intracranial tumors found within the lateral ventricles, although extraventricular variants have been reported. Intracranial central neurocytomas have not been previously recognized in domestic animals.

Objectives: To describe the clinicopathologic features of canine intracranial central neurocytomas.

Animals: Two dogs with spontaneous intracranial and intraventricular neoplasms.

Results: Both dogs experienced seizures, rapid neurological deterioration, and death from tumor-associated complications within 5 days of the onset of clinical signs, and had neoplastic masses within the lateral ventricles. A brain MRI was performed in 1 dog, which revealed a T1-isointense, heterogeneously T2 and FLAIR hyperintense, and markedly and heterogeneously contrast-enhancing mass lesions within both lateral ventricles. Histologically, the neoplasms resembled oligodendrogliomas. The diagnosis of central neurocytoma was supported by documenting expression of multiple neuronal markers, including neuron-specific enolase, synaptophysin, neural-cell adhesion molecule, and neuronal nuclear antigen within the tumors, and ultrastructural evidence of neuronal differentiation of neoplastic cells.

Conclusions and Clinical Importance: Central neurocytoma should be a differential diagnosis for dogs with intraventricular brain masses. Morphologic differentiation of central neurocytoma from other intraventricular neoplasms, such as ependymoma or oligdendroglioma, can be difficult, and definitive diagnosis often requires immunohistochemical or ultrastructural confirmation of the neural origin of the neoplasm.

Key words: Brain tumor; Canine; CNS disorders; Neuroanatomy; Neurology; Neuropathology; Oncology; Pathology.

Central neurocytomas are rare neoplasms in humans, accounting for 0.25–0.5% of all brain tumors.[1] Central neurocytomas are typically well-differentiated intraventricular neoplasms that have a favorable prognosis after surgical resection or radiotherapeutic treatment, although atypical, extraventricular variants have been reported.[1–3] Central neurocytomas were first described as a distinct neuropathological entity in 1982,[4] and often present a diagnostic challenge as their histopathologic phenotype can resemble oligodendroglioma, ependymoma, or pineocytoma.[1–4] Although their histiogenesis is still controversial, they are currently characterized by the World Health Organization as neuronal or mixed neuroglial origin tumors based on their expression of multiple neuronal markers such as synaptophysin (SYN), neuronal nuclear antigen (NeuN), and neuron-specific enolase (NSE), along with ultrastructural features of neuronal differentiation.[5–7]

Central neurocytomas are poorly described in domestic animals.[7] This report details the clinicopathologic features of 2 canine supratentorial intraventricular central neurocytomas.

Abbreviations:

ABC	avidin-biotin complex detection method
ALP	alkaline phosphatase detection method
CNP-ase	2′, 3′-cyclic nucleotide 3′-phosphodiesterase
CPT	choroid plexus tumor
FLAIR	fluid attenuated inversion recovery
GFAP	glial fibrillary acidic protein
IHC	immunohistochemistry
MBP	myelin basic protein
MRI	magnetic resonance imaging
N-CAM	neural-cell adhesion molecule
NeuN	neuronal nuclear antigen
NF	neurofilament
NSE	neuron specific enolase
Olig2	oligodendrocyte transcription factor 2
PNET	primitive neuroectodermal tumor
SYN	synaptophysin
WB	western blot

Case Histories

Dog 1 was a 5-year-old, male, 44-kg Bull Terrier that experienced 3 generalized seizures the week before referral. Abnormalities detected on neurological examination included right nasofacial hypalgesia, visual field deficits consistent with a left thalamocortical lesion, and delayed conscious proprioception in the right thoracic and pelvic limbs with normal spinal reflexes. The examination was consistent with a left prosencephalic neuroanatomic diagnosis.

An MRI examination of the brain was obtained under general anesthesia (Fig 1). The dog was treated

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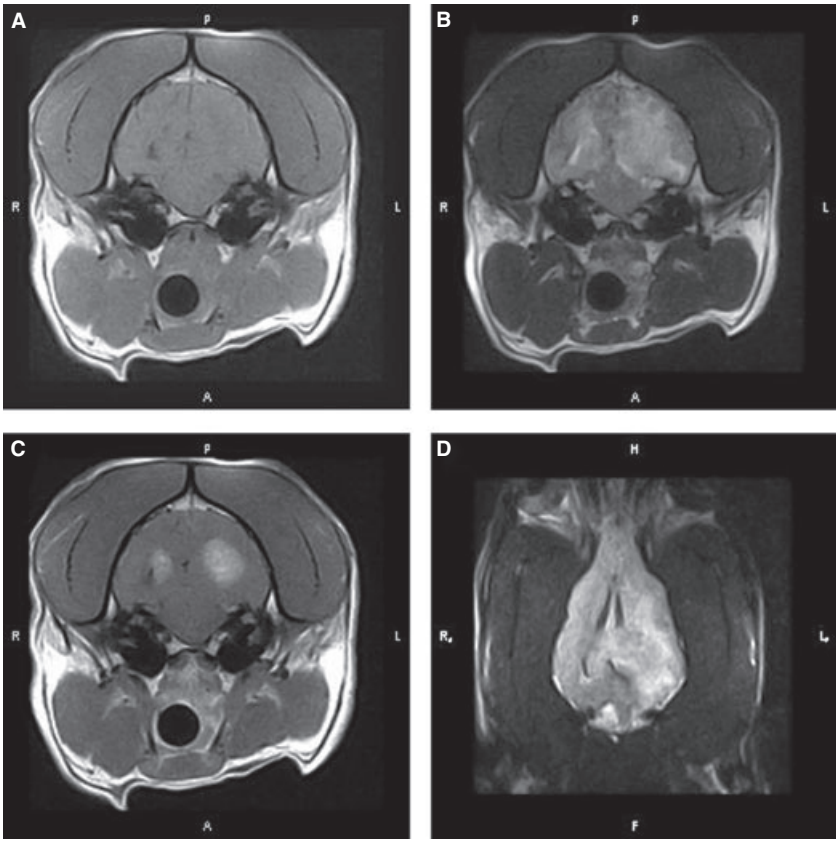

Fig 1. Brain MRI of Dog 1. Transverse images **(A–C)** at the level of the tympanic bullae and a dorsal planar FLAIR **(D)** image demonstrate predominantly T1-isointense (A), heterogeneously T2 (B) and FLAIR (D) hypo- to hyperintense, and heterogeneously contrast enhancing (C), mass lesions occupying both lateral ventricles. Also visible are mass effects manifested as a falx shift (A–C) and periventricular white matter edema (B and D).

with 20% mannitol (1 g/kg, IV over 30 minutes), furosemide (0.75 mg/kg, IV), and dexamethasone (0.27 mg/kg, IV) during the MRI examination. Albuminocytologic dissociation was the only abnormality observed (total protein 85 mg/dL; reference interval <25 mg/dL) during analysis of cerebrospinal fluid.

Upon anesthetic recovery the dog had a generalized seizure, which abated after administration of diazepam (1.6 mg/kg, IV). Surgical and radiotherapeutic treatments were declined by the owner. Zonisamide (6.8 mg/kg, PO, q12h) and prednisone (0.5 mg/kg, PO, q12h) therapies were initiated and the dog discharged. Two days later, the dog developed status epilepticus and died. A necropsy was performed.

Dog 2 was a 6-year-old, neutered male, 11-kg Boston Terrier that was observed having a partial facial seizure the morning of presentation. Four hours after the seizure, the dog developed ataxia of all limbs and a left head turn, which rapidly progressed to lateral recumbency. Upon examination, the dog was semi-comatose, responding only to noxious nociceptive stimuli, bradycardic (HR = 44 bpm), and hypertensive (indirect Doppler systolic pressure = 220 mmHg). The oculovestibular reflex was absent. The history and clinical examination were suggestive of intracranial hypertension, possible brain herniation, and an associated Cushing's reflex. The dog experienced respiratory arrest during administration of 20% mannitol (1.5 g/kg, IV, over 30 minutes) and the owner declined further resuscitative interventions. The dog was euthanized and a necropsy examination performed.

Immunohistochemical (IHC), Western Blot (WB), and Ultrastructural Methods

Formalin-fixed, paraffin embedded, 5 μm brain tissue sections were used for all IHC procedures. Brain sections were stained according to manufacturer's instructions with primary antisera against glial fibrillary acidic protein (GFAP; Dako[a] polyclonal, 1 : 150), myelin basic protein (MBP; Dako polyclonal, 1 : 200), NSE (Dako clone BBS/NC/VI-H14, 1 : 300) SYN (Dako clone SY38, 1 : 100), vimentin (Dako clone V9, 1 : 300), neurofilament (NF, Dako clone 2F11, 1 : 100) chromogranin A (Dako polyclonal, 1 : 200), S-100 (Dako polyclonal, 1 : 200), NeuN (Millipore[b] clone A60, 1 : 50), and 2′, 3′-cyclic nucleotide 3′-phosphodiesterase (CNP-ase, Millipore, clone 11-5B, 1 : 150), oligodendrocyte transcription factor 2 (Olig2, Millipore polyclonal, 1 : 100) with an alkaline phosphatase detection (ALP) method and fast red counterstain (GFAP, MBP, NSE, vimentin, NF,

chromogranin A, S-100 and NeuN) on an automated staining system[c] or avidin-biotin complex (ABC) method and Mayer's hematoxylin counterstain (SYN, CNP-ase, Olig2). Western blot detection of SYN and N-CAM was performed with frozen tumor tissue from both dogs, as previously described.[6] Briefly, 40 μg of protein obtained from lysed and homogenized tissue extracts was subjected to 12% SDS-PAGE, transferred to an Immobilon-P[b] membrane, and reacted with anti-SYN (Abcam[d] clone SY38, 1 : 500) and anti-N-CAM (Abcam polyclonal 1 : 200) antibodies at room temperature for 2 hours. Positive control tissues used for IHC and WB consisted of the components of normal murine and canine brains that constitutively express the antigen of interest.

Brain samples fixed in buffered 2.5% glutaraldehyde were postfixed in buffered 1% osmium tetraoxide, dehydrated and embedded in epoxyplastic. Tissue sections were then sectioned at 50 nm, stained treated with lead citrate and uranyl acetate, and examined with a transmission electron microscope.[e]

Neuropathological Examinations

In both dogs, abnormalities were limited to the central nervous system. In dog 1, gross examination of the brain revealed a multilobular, gray, and friable mass originating within the left lateral ventricle and extending into the right lateral ventricle via the interventricular foramen. The left corona radiata and internal capsule were markedly swollen, and a left transtentorial brain herniation was present. In dog 2, a gray and hemorrhagic intraventricular mass appeared to be arising from the septum pellucidum. The mass was admixed with free blood within the ventricle, which resulted in marked expansion and near total obliteration of the left lateral ventricular lumen (Fig 2). The dorsocaudal aspect of the cerebellar vermis and overlying meninges were hemorrhagic and herniated through the foramen magnum.

Intraventricular masses from both dogs were composed of a monomorphic population of small cells with a round central nucleus, finely stippled chromatin, and indistinct cytoplasm. Tumor cells were clustered into variably dense sheets and islands separated by a perivascular, cell-free fibrillary stroma (Fig 3A and B). Densely cellular regions from both dogs contained areas where tumor morphology closely resembled that of an oligodendroglioma, with perinuclear halos, distinct cytoplasmic borders, and delicate networks of branching vasculature being evident (Fig 3C). Mitotic figures were rare in both dogs. Occasional perivascular or stromal calcifications were observed in Dog 2. Multifocal areas of glomeruloid vascular tufts were also seen in Dog 2 (Fig 3C).

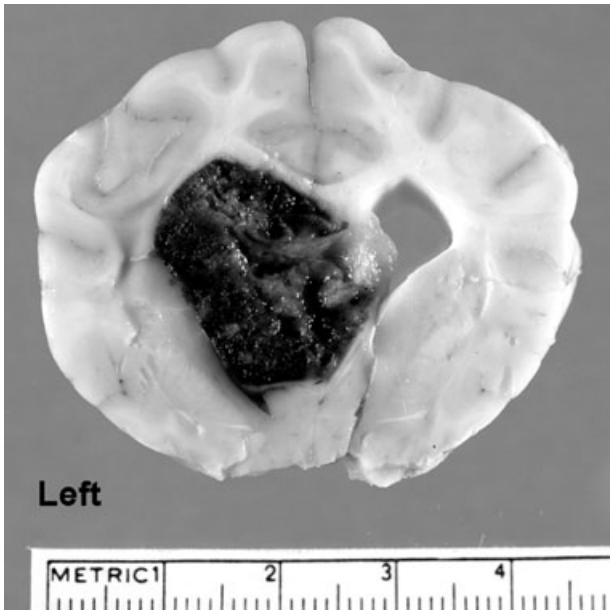

Fig 2. Gross brain specimen at the level of the cruciate sulcus of Dog 2 demonstrating hemorrhagic mass lesion obliterating the left lateral ventricle.

Tumor cells from both dogs demonstrated marked immunoreactivity to NSE (Fig 4A) and SYN (Fig 4B), especially in fibrillary, neuropil-like regions. Positive immunoreactivity to NeuN was present in <10% of neoplastic cells from both dogs (Fig 4C), and was observed in tumor nuclei and cytoplasm. Patchy, multifocal areas of perivascular immunoreactivity to

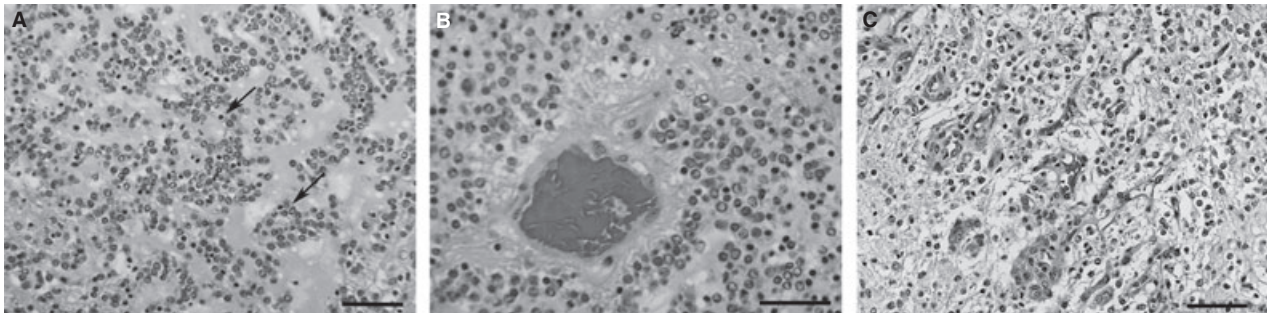

Fig 3. Histopathologic features of canine central neurocytoma, H&E, bar = 100 μm in all panels. **(A)** Dog 1, small, uniform neoplastic cells interspersed throughout a fibrillary stroma, with irregular rosette formation surrounding fibrillary areas (arrows). **(B)** Dog 2, perivascular, neuropil-like fibrillary island, largely devoid of cells. **(C)** Dog 2, oligodendroglial morphology and glomeruloid vascular profiles within the tumor.

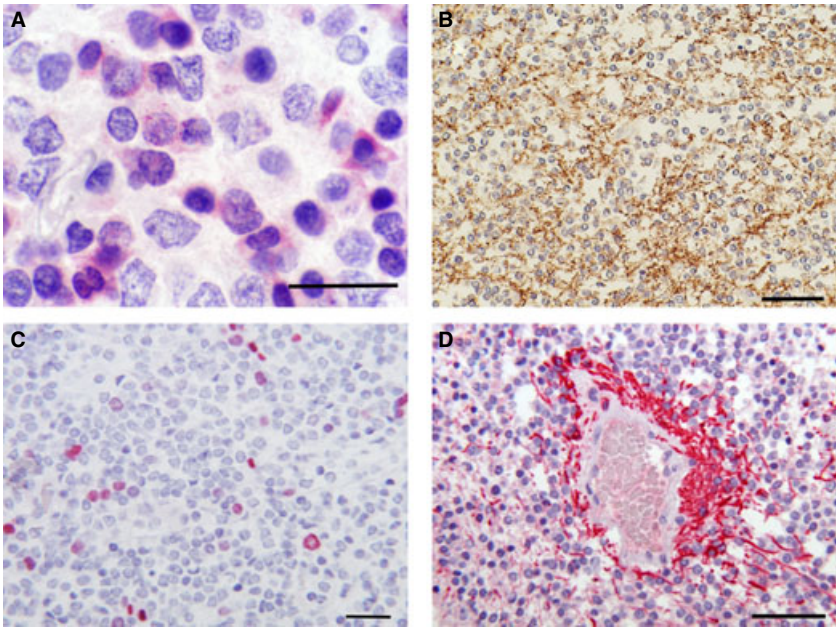

Fig 4. Immunohistochemistry of central neurocytoma, bar = 50 μm in all panels. **(A)** Dog 1—Positive NSE immunohistochemical labeling of tumor cells. **(B)** Dog 2—Positive SYN immunohistochemical labeling of tumor cells displaying oligodendroglial morphology. **(C)** Dog 1—Sparse positive immunoreactivty of neoplastic cells to NeuN. **(D)** Dog 2—Perivascular cytoplasmic processes demonstrate GFAP immunoreactivity. Panels A, C, and D: ALP detection method with fast red counterstain. Panel B: ABC detection method with Mayers' hematoxylin counterstain.

GFAP were noted in Dog 2 (Fig 4D). No immunoreactivity to MBP, Olig2, vimentin, S-100, NF, CNPase, or chromogranin A was observed in either case. Western blots confirmed the intratumoral presence of bands corresponding to the predicted molecular weights of SYN (38 kDa), as well as multiple major isoforms (120-, 140-, and 180-kDa) of N-CAM (Fig 5) in each dog.

Neuronal differentiation of neoplastic cells, characterized by a fine and dispersed nuclear chromatin, Golgi bodies, osmiophilic lysosome-like inclusions, elongated cytoplasmic processes containing microtubules, and normal (Fig 6) and aberrant synapses were observed in electron photomicrographs of tumor samples of both dogs. Aberrant synapses contained clear and dense core vesicles, and presynaptic boutons with no apparent synaptic adhesions.

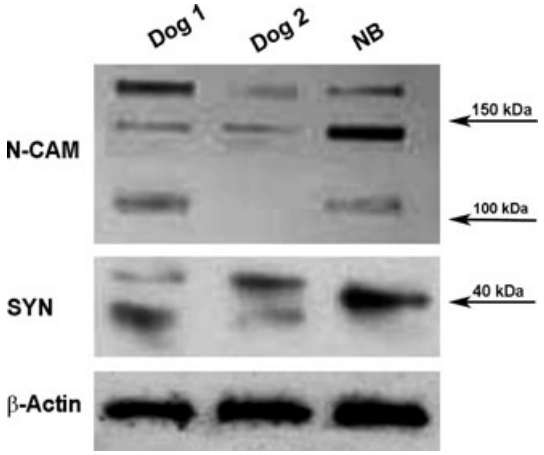

Fig 5. Western blot detection of SYN and multiple N-CAM isoforms in canine central neurocytomas. NB, normal canine brain.

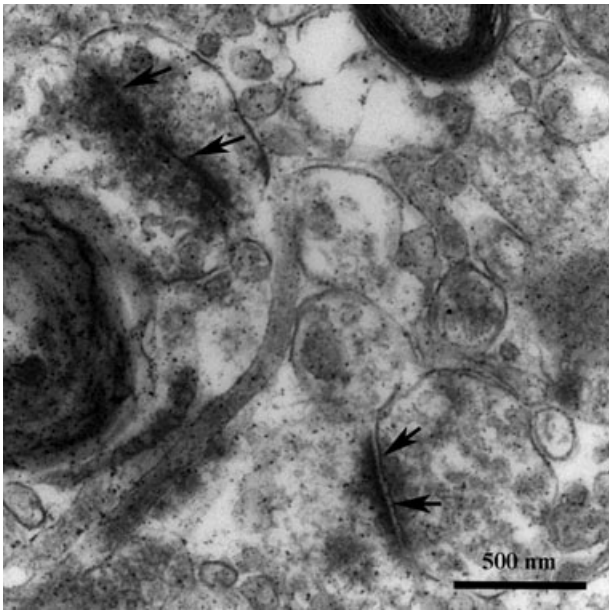

Fig 6. Electron photomicrograph of central neurocytoma from Dog 1 demonstrating multiple, Type I, axo-dendritic synapses (arrows, 40,000×).

Discussion

We describe the clinical, neuroradiologic, and neuropathologic features of intracranial central neurocytomas in dogs, which share many features with their human counterparts.[1–7] These cases also emulate the human experience in that they emphasize the limitations of contemporary imaging and routine histopathological techniques when attempting to accurately classify central nervous system tumors, and particularly central neurocytomas.[1–4] After the description of central neurocytoma as a distinct pathologic entity, retrospective reviews of oligodendrogliomas and ependymomas from multiple institutions have resulted in the reclassification of some of these tumors as central neurocytomas.[1,3]

Both dogs reported here were middle aged at the time of diagnosis, and central neurocytoma is typically a neoplasm of young- to middle-aged adults, with the majority of people diagnosed between 20 and 40 years of age.[1–3] The clinical signs and natural history of disease in these dogs were also similar to that observed in humans, with seizures and visual deficits being frequent clinical complaints.[1–3] Acute clinical decline associated with intracranial hypertension arising from obstructive hydrocephalus is common in humans with central neurocytomas because of their proclivity to arise within the lateral ventricles and impede cerebrospinal fluid flow through the interventricular foramen.[1–4] In this study, indirect antemortem clinical and radiological evidence of intracranial hypertension existed in both dogs, with this hypertension having catastrophic and terminal clinical consequences as evidenced by the brain herniations.

The MRI characteristics of intraventricular human central neurocytoma resemble those seen in Dog 1, including T1 isointensity, heterogeneous signal intensity on T2-weighted sequences, marked and heterogeneous contrast enhancement, and intraventricular extension.[2,3] The mixed signal intensity seen in some central neurocytomas can arise from intratumoral hemorrhage and mineralization, which were present in the tumors in dogs of this study, as well as from cystic changes.[2,3] However, as also illustrated by the MRI of Dog 1, the imaging features of the central neurocytoma are not specific in humans or dogs, and can specifically mimic the appearance of choroid plexus tumors (CPT) and intraventricular meningiomas.[2,3,8]

The differential diagnostic considerations for lateral ventricular brain neoplasms include CPT, ependymoma, meningioma, oligodendroglioma, numerous rare primitive neuroectodermal tumors (PNET), or neuronal or neuronal-glial tumor variants, such as central neurocytoma. The age of onset of clinical signs in these dogs were supportive of a diagnosis of CPT or PNET.[8] Although the MRI features of the tumor from Dog 1 were consistent with CPT or meningioma,[8] these diagnoses were readily excluded based on microscopic tumor morphology. Even though pseudorosettes were observed in these tumors, ependymoma and PNET/neuroblastoma were considered unlikely in these dogs given the absence of true rosette formation and vimentin immunoreactivity or Homer-Wright rosettes and NF or chromogranin A immunoreactivity, repectively.[5,7] Central neurocytoma can also resemble pineocytoma, but this diagnosis was not considered in these cases because of the intraventricular location of the tumors and absence of chromogranin A expression.[5]

Despite their phenotypic resemblance to oligodendrogliomas, tumors from both dogs expressed multiple neuronal-associated proteins, including NSE, SYN, N-CAM, and NeuN, and demonstrated ultrastuctural neuronal features, which allowed for their identification as central neurocytomas.[1–7] In addition to their expression of numerous neuronal antigens, neither tumor displayed immunoreactivity to MBP, CNP-ase, or Olig 2, so they were considered unlikely to be oligodendrogliomas with glioneuronal differentiation.[9] Both tumors in this study also expressed the 140- and 180-kDa neuronal isoforms of N-CAM, although the 120-kDa glial isoform was also detected in Dog 1. However, N-CAM expression is highly variable in human brain tumors, and not specific for neural histiogenesis.[10] The sparse NeuN immunoreactivity within canine neurocytomas may be explained by the fact that NeuN is expressed after terminal differentiation of neurons, which, as supported by the IHC and microscopic findings in these tumors, may not be present or synchronized. In addition, NeuN is not ubiquitously expressed in all neuronal populations, including some photoreceptor lineages, and central neurocytomas may display photoreceptor differentiation.[11] Perivascular GFAP immunoreactivity was observed in 1 dog, which can be a feature of central neurocytoma, and is thought to represent trapped, reactive astrocytes within the tumor.[1] The distribution and appearance of GFAP positive cytoplasmic processes observed in this study further support that theory.

In conclusion, we believe these cases are novel and representative of intracranial central neurocytomas in dogs based on cumulative morphological evidence and neuronal-associated protein expression profiles consistent with the reported human features of this neoplasm.[1–6] Central neurocytoma should be considered a differential diagnosis for dogs with intraventricular brain masses. Definitive diagnosis of central neurocytoma requires immunohistochemical or ultrastructural demonstration of neuronal differentiation, as these tumors often morphologically mimic other intraventricular neoplasms.

Footnotes

[a] Dako, Carpinteria, CA
[b] Millipore, Billerica, MA
[c] Benchmark XT, Ventana Medical Systems, Tucson, AZ
[d] Abcam, Cambridge, MA
[e] EMC-10 CA, Carl Zeiss SMT, Inc, Peabody, MA

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