Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 7

6 October 2021

CASE I: N17-663 (JPC 4117535)

Signalment:
13-year-old, male, neutered, American Eskimo dog, (*Canis lupus familiaris*)

History:
This dog presented for acute onset of seizures. Magnetic resonance imaging showed a right-sided forebrain mass with associated hemorrhage. Due to poor prognosis and clinical deterioration, the dog was euthanized.

Gross Pathology:
Grossly the right cerebral hemisphere was expanded by a 3 x 3 x 2.5 cm, poorly circumscribed, irregular, multilobulated, white to tan, firm to gelatinous, multifocally hemorrhagic mass that projected into the right lateral ventricle and compressed the thalamus.

Laboratory results:
No laboratory findings reported.

Microscopic Description:
Brain: Effacing and infiltrating the neuropil of the cerebral cortex and spilling into the lateral ventricle, is a poorly demarcated, unencapsulated, densely cellular neoplasm composed of spindle to stellate cells arranged in disorganized streams and bundles that are often arranged perpendicularly (pseudo-palisading) along extensive serpiginous areas of coagulative to liquefactive necrosis that contain abundant karyorrhectic and cellular debris mixed with fibrin and hemorrhage. Neoplastic cells have indistinct cell borders, with scant to moderate amounts of eosinophilic cytoplasm. Nuclei are round to oval, with finely stippled to vesicular chromatin, and 1-3 small nucleoli. Aniso-cytosis and anisokaryosis are moderate.

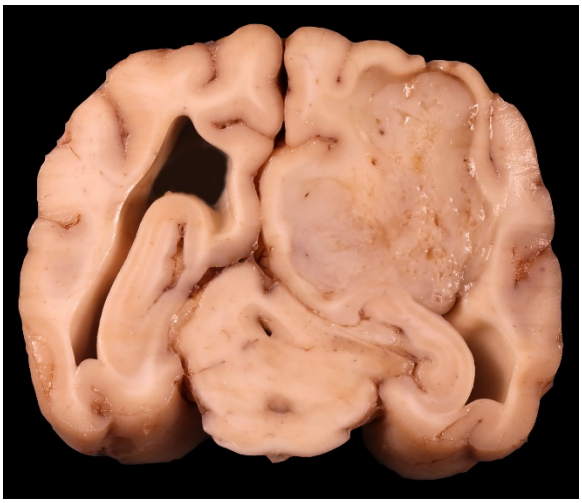

Figure 1-1. Brain, dog. A multilobulated, white to tan, firm to gelatinous mass (astrocytoma, high grade) expands the right cerebral hemisphere, projects into the right lateral ventricle, compresses the thalamus and causes a leftward midline shift. (Photo courtesy of: Department of Biomedical Sciences, Section of Pathology, 200 Westboro Rd., North Grafton, MA. 01536, http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/)

Multifocally, neoplastic cells are arranged in dense sheets supported by a myxoid matrix, have variably distinct cell borders, moderate amounts of vacuolated cytoplasm, and round to oval, uniform nuclei. There are 24 mitotic figures in 10 high power fields. Throughout the neoplasm, there is proliferation of blood vessels with hypertrophied endothelium that frequently form glomeruloid structures.

Contributor's Morphologic Diagnoses:
Brain: astrocytoma, grade IV (glioblastoma)

Contributor's Comment:
Astrocytomas account for approximately 10-13% of primary central nervous system tumors in dogs. [7, 12, 14] The incidence is higher in brachycephalic breeds (approximately 23 times), but they can occur in any breed. [2, 4, 15] Incidence of astrocytic neoplasia in dogs, as in humans, increases with age.[15] Astrocytomas are a diverse group of gliomas that in dogs are subdivided in two distinct subgroups based on their growth patterns: infiltrative or diffuse, and well-demarcated.[4] Diffuse astrocytic tumors are classified into four grades (I to IV), with grade IV being the most malignant form.[2, 4, 12]

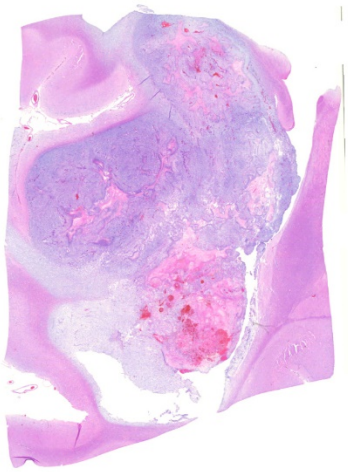

Figure 1-2. Cerebrum, dog. A section of cerebrum that is 80% effaced by an infiltrative neoplasm is submitted for examination. There are serpiginous areas of necrosis and hemorrhage throughout the neoplasm. (HE, 6X)

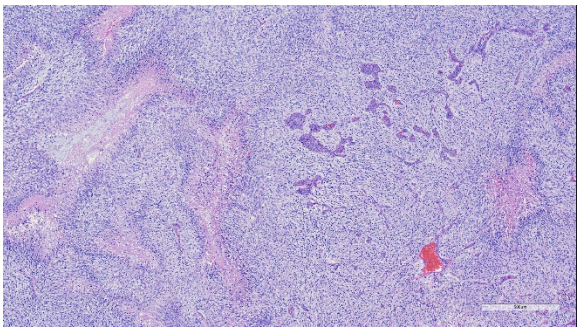

Figure 1-3. Cerebrum, dog. Serpentine areas of necrosis are bordered by elongated, perpendicularly oriented pseudopalisading neoplastic cells. Proliferation of blood vessels with multiple layers of hypertrophied endothelium and pericytes forming tortuous or glomeruloid structures are present. H&E stain. (Photo courtesy of: Department of Biomedical Sciences, Section of Pathology, 200 Westboro Rd., North Grafton, MA. 01536, http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/)

Glioblastoma (grade IV astrocytoma) has been reported to represent 0.5 to 3% of all primary central nervous tumors in the dog.[3,13, 14] Glioblastoma is the most common human brain tumor, and comprises 12-15% of all primary central nervous system tumors and 50-60% of all astrocytomas.[7]

Canine glioblastomas, like their human counterparts, are highly invasive. The infiltrative path within the normal brain parenchyma is not random and often follows white matter tracts, perivascular spaces and the subependyma.[15]

In humans, glioblastomas can be primary (*de novo*) tumors or secondary tumors that develop from a preexisting glial tumor, either diffuse astrocytomas (WHO grade II) or anaplastic astrocytomas (WHO grade III).[15] In veterinary species, there is no evidence that glioblastomas arise from preexisting benign astrocytic/glial tumors.[5]

The most recent update to the WHO classification of Tumors of the Central Nervous

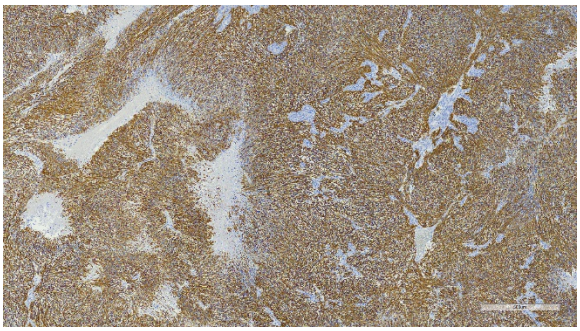

Figure 1-4. Cerebrum, dog. Neoplastic cells have strong cytoplasmic expression of GFAP by immunohistochemistry (glial fibrillary acidic protein). (Photo courtesy of: Department of Biomedical Sciences, Section of Pathology, 200 Westboro Rd., North Grafton, MA. 01536, http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/)

System in humans includes molecular parameters (particularly genotype) in addition to histology to define and characterize many tumors.[1,8,9] Diffuse infiltrative astrocytomas of adults are now subdivided according to the mutational status of isocitrate dehydrogenase-1 or -2 (IDH1/2) genes into IDH-mutant or IDH-wildtype [1, 9], with the large majority of IDH-wildtype astrocytomas (90-95%) being primary glioblastomas (WHO grade IV). These mutations have not been found in canine gliomas.[5, 11]

The main criteria for histological classification and grading of astrocytomas are the cell density, nuclear atypia and mitotic count. Astrocytomas consistently express glial fibrillary acidic protein (GFAP) immunoreactivity, which decreases in the higher grades[4] and is the feature that suggests the glial origin of these tumors.[15] In this dog, most neoplastic cells (>90%) exhibited strong cytoplasmic immunoreactivity for GFAP. Olig2 nuclear immunoreactivity was observed in a large population of the neoplastic cells (approximately 60%). Although this has been used as a marker for cells of oligodendroglial origin, astrocytomas in dogs and cats frequently express Olig2.[5,10, 16] Lack of expression of CNPase by the neoplastic cells makes oligodendroglial origin or differentiation less likely and further supports an astrocytic origin.

Additional criteria for diagnosis of grade IV astrocytoma must include at least one of two essential diagnostic features: necrosis with marked glial pseudopalisading and/or microvascular proliferation.[4] Both of these features were quite prominent in this case.

Canine glioblastomas overexpress EGFR, PDGFR-α, and insulin-like growth factor binding protein 2 (IGFBP-2).[2,3,15,16] Additionally, greater expression of VEGF, VEGFR-1, VEGFR-2, EGFR-1, IL-13RA2 has been described in canine glioblastomas compared to other intracranial brain tumors.[3,7,15] Microvascular proliferation is presumably driven, in part, by this increased expression of VEGF.[7] Proliferative index, measured by Ki67 expression, is described in the literature for glioblastomas and other glial tumors[3,7,16], although its significance or prognostic value has yet to be determined in veterinary species.

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JPC Diagnosis:
Cerebrum: Astrocytoma, high grade (previously known as astrocytoma grade IV or glioblastoma).

JPC Comment:
The contributor provides an outstanding review of the criteria used for the diagnosis of a grade IV astrocytoma. Since 1999,

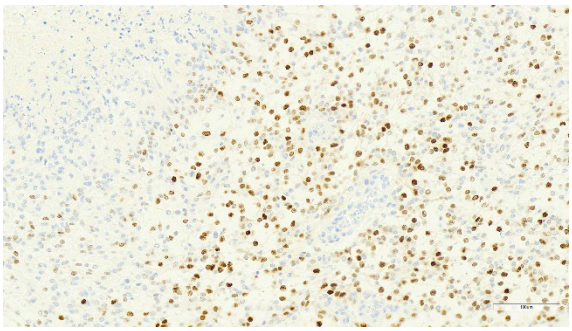

Figure 1-5. Cerebrum, dog. Approximately 60% of neoplastic cells exhibit moderate to strong nuclear expression of Olig2 by immunohistochemistry. (Photo courtesy of: Department of Biomedical Sciences, Section of Pathology, 200 Westboro Rd., North Grafton, MA. 01536, http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/)

diagnostic criteria for canine glial tumors have been derived from the World Health Organization (WHO) Tumor Fascicle Histologic Classification of Tumors of the Nervous System of Domestic Animals, in addition to immunohistochemical markers of glial populations, which have become more readily available.[6]

Following the submission of this case to the WSC, the National Cancer Institute-led multidisciplinary Comparative Brain Tumor Consortium (CBTC) convened a glioma pathology board, composed of both veterinary and physician neuropathologists with the immediate goal of improving existing glioma classification methods, which would in turn would support harmonization of phenotypic characterization between species, serving to bridge veterinary and physician neuropathology and strengthen the validity of the dog as a naturally occurring, translationally relevant model of human glioma. One of several outcomes of the consortium was the creation of a new classification scheme to promote diagnostic consistency across veterinary institutions and to harmonize future comparative research and hypothesis-based investigations designed to aid in clinical management of canine glioma patients, especially the addition of future molecular markers that may stratify canine patients as is used for human patients with gliomas.[6]

Gliomas under the new grading scheme are categorized as an oligodendroglioma, astrocytoma, or undefined glioma. To be classified as an oligodendroglioma >80% of the tumor must meet the one or more of the following criteria: Round nuclei with a coarse chromatin pattern, scant to moderate eosinophilic or lost cytoplasm (artifact), pseudo-rosettes, secondary structures, nuclear rowing, myxoid to mucinous matrix (+/- lakes), branching capillaries, mineralization, and nuclear molding. To be classified as an astrocytoma >80% of the tumor must meet the criteria of one or more of the following criteria: Oval to elongate nuclei (angular) with an open faced chromatin pattern, pleomorphic cells (large nucleoli, multinucleated cells), eosinophilic and abundant cytoplasm (gemistocytic), elongate cells (pilocytic), naked nuclei, random and disorganized patterns, spindle cell morphology, rare mucin microcysts (well-defined), mineralization, and eosinophilic stroma (fibrillary). Gliomas exhibiting a

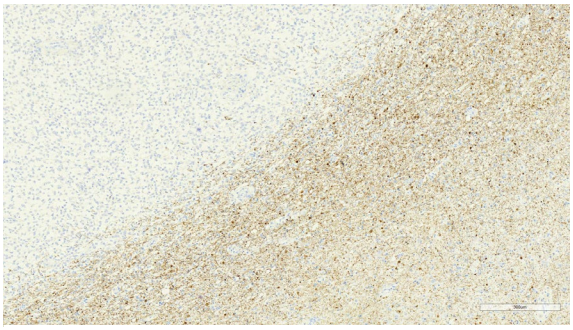

Figure 1-6. Cerebrum, dog. Neoplastic cells (top left) fail to show expression of CNPase by immunohistochemistry. In the neighboring white matter, myelin and oligodendrocytes strongly express CNPase by immunohistochemistry. (Photo courtesy of: Department of Biomedical Sciences, Section of Pathology, 200 Westboro Rd., North Grafton, MA. 01536, http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/)

biphenotypic/biphasic morphology with both phenotypes in high proportions (>30-40% each) or an undifferentiated cellular morphology are classified as an "undefined glioma".[6]

Once the glial tumor type is determined, the neoplasm's infiltration into the adjacent parenchyma is assessed at low magnification and classified as either "none, focal, or diffuse".[6]

The neoplasm is then graded as either "high" or "low" based on multiple criteria. All gliomas with necrosis (excluding single cell) and/or microvascular proliferation +/- pseudopalisading are classified as high grade. Pseudo-palisading necrosis occurs when neoplastic cells line up perpendicularly to areas of geographic necrosis. Microvascular proliferation may be characterized as convoluted and glomeruloid vasculature, multilayered and hypertrophied endothelial cells, and vascular arcades. Microvascular proliferation must be distinguished from reactive vasculature, with multiple layers of cells and high degree of tortuosity being critical distinguishing features. Neoplasms lacking these features are further assessed for any mitoses in ten 400x fields and/or overt features of malignancy (e.g. nuclear pleomorphism, anisokaryosis, anisocytosis, and cellular atypia). The presence of any of these features are consistent with a high grade glioma whereas their absence is consistent with a low grade glioma.[6]

References:

1. Brat DJ, Perry A. Astrocytic and Oligodendroglial Tumors. In: Practical Surgical Neuropathology: A Diagnostic Approach. Elsevier; 2018:91–123.
2. Cantile C, Youssef S. Nervous System. In: Maxie MG, ed. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, Vol. 1. 6th ed. St. Louis, MO: Elsevier, 2016: 250-406.
3. Hicks J, Platt S, Kent M, et al. Canine brain tumours: a model for the human disease?: Canine brain tumours. Veterinary and Comparative Oncology 2017;15:252–272.
4. Higgins RJ, Bollen AW, Dickinson PJ, Siso-Llonch S. Tumors of the Nervous System. In: Meuten DJ, ed. Tumors of Domestic Animals. 5th ed. Ames, IA: Wiley; 2017: 834-891.
5. Johnson GC, Coates JR, Wininger F. Diagnostic Immunohistochemistry of Canine and Feline Intracalvarial Tumors in the Age of Brain Biopsies. Veterinary Pathology 2014;51:146–160.
6. Koehler JW, Miller AD, Miller CR, et al. A Revised Diagnostic Classification of Canine Glioma: Towards Validation of the Canine Glioma Patient as a Naturally Occurring Preclinical Model for Human Glioma. *J Neuropathol Exp Neurol*. 2018;77(11):1039-1054.
7. Lipsitz D, Higgins RJ, Kortz GD, et al. Glioblastoma Multiforme: Clinical Findings, Magnetic Resonance Imaging, and Pathology in Five Dogs. Veterinary Pathology 2003;40:659–669.
8. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathologica 2016;131:803–820.
9. Perry A, Rosenblum MK. Central Nervous System. In: Rosai and Ackerman's Surgical Pathology.11th ed. Philadelphia, PA: Elsevier; 2018: 1948-2085.
10. Ramos-Vara JA, Borst LB. Immunohistochemistry: Fundamentals and Applications in Oncology. In: Meuten DJ, ed. Tumors of Domestic Animals. 5th ed. Ames, IA: Wiley; 2017: 834-891.

11. Reitman ZJ, Olby NJ, Mariani CL, et al. IDH1 and IDH2 hotspot mutations are not found in canine glioma. International Journal of Cancer 2010;127:245–246.
12. Röthlisberger A, Lehmbecker A, Beineke A, et al. Suspected primary glioblastoma multiforme in the canine spinal cord. Journal of Small Animal Practice 2012;53:604–607.
13. Snyder JM, Shofer FS, Winkle TJ, et al. Canine Intracranial Primary Neoplasia: 173 Cases (1986-2003). Journal of Veterinary Internal Medicine 2006;20:669–675.
14. Song RB, Vite CH, Bradley CW, et al. Postmortem Evaluation of 435 Cases of Intracranial Neoplasia in Dogs and Relationship of Neoplasm with Breed, Age, and Body Weight. Journal of Veterinary Internal Medicine 2013;27:1143–1152.
15. Stoica G, Levine J, Wolff J, et al. Canine Astrocytic Tumors: A Comparative Review. Veterinary Pathology 2011;48:266–275.
16. Vandevelde M, Higgins RJ, Oevermann A. Veterinary Neuropathology: Essentials of Theory and Practice. Ames, IA: Wiley-Blackwell; 2012.

CASE II: 19N-0060 (JPC 4136398)

Signalment:
11-year-old castrated male domestic short-haired cat (*Felis catus*)

History:
The patient presented to the veterinary teaching hospital with a 2-3-month history of bumping into things, sitting in corners, vision loss, and behavior changes; he was reported to walk a fixed route in the home. A neurologic exam revealed absent menace responses, a left forelimb conscious pro-prioceptive deficit, and lumbosacral pain. A

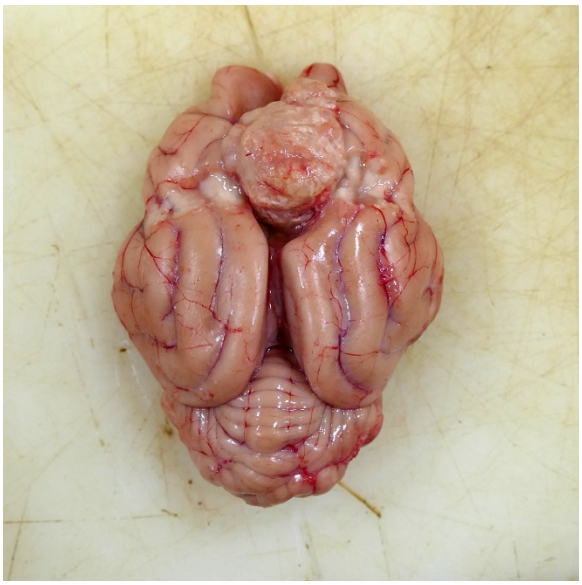

Figure 2-1. Cerebrum, cat. There is a 2 x 1.7 x 1.8 cm, tan to light pink, semi-firm mass occupying the longitudinal fissure and mildly compressing the adjacent frontal cortex (Photo courtesy of: University of Wisconsin School of Veterinary Medicine, Department of Pathobiological Sciences, 2015 Linden Dr., Madison, WI 53706 https://www.vetmed.wisc.edu/departments/pathobiological-sciences/)

fundic examination showed intact retinas. These results did not point toward a clear diagnosis. Due to concerns about quality of life and financial constraints, his owners elected humane euthanasia without additional diagnostics (i.e. no radiographs, CT scan or biopsy were performed). A necropsy was performed to investigate the cause for the patient's clinical signs.

Gross Pathology:
Attached to the surface of the cerebrum and focally expanding the region between the rostral cerebral hemispheres and located 1 cm caudal to the most rostral aspect of the olfactory bulbs was a 2 x 1.7 x 1.8 cm, tan to light pink, semi-firm mass occupying the longitudinal fissure and mildly compressing the adjacent frontal cortex. On the cut surface, the mass was semi-firm, mottled tan and pink and slightly gritty. The primary differential for the well-demarcated, intra-

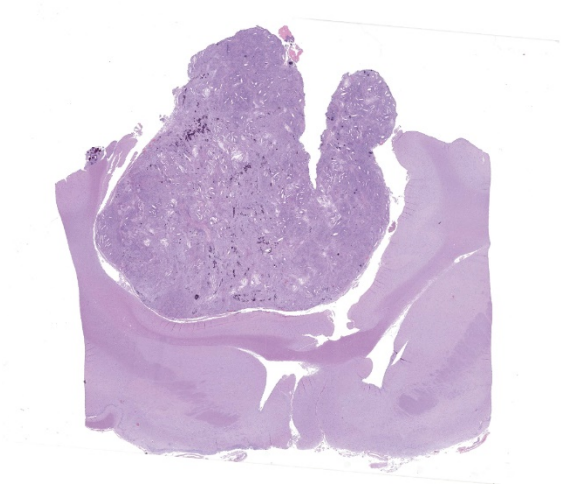

Figure 2-2. Cerebrum, cat. A large neoplasm is present between the lobes of the cerebrum (HE, 5X)

cranial, extra-axial tumor at the time of necropsy was meningioma with the rule-outs of a primary CNS lymphoma, peripheral nerve sheath tumor, and granular cell tumor. No other organ systems demonstrated a neoplastic process.

Laboratory results:
No laboratory findings reported.

Microscopic Description:
Markedly compressing the frontal cortex is an expansile, well-demarcated, non-invasive, unencapsulated, densely cellular neoplasm composed of short interlacing streams, bundles, and whorls of spindle to polygonal cells supported by a variably dense fibrovascular stroma with some stromal collagen bundles being prominent and serpiginous and occasionally mineralized. The neoplastic cells have variably distinct cell borders with small to moderate amounts of finely granular, eosinophilic cytoplasm. Nuclei are oval to elongated with vesiculated to finely stippled chromatin and occasionally have one distinct magenta nucleolus. The mitotic rate is low, with fewer than 1 mitotic figure per ten high powered fields (400x). Scattered and small to large aggregates of foamy macrophages surround acicular cholesterol clefts. Small numbers of lymphocytes, plasma cells, neutrophils, and rare individual macrophages with intracytoplasmic pale brown material (hemosiderin) are scattered throughout the neoplasm. In many areas, there are several variably-sized foci of cellular dropout with replacement by eosinophilic cellular and karyorrhectic debris (necrosis) and individual cellular necrosis characterized by shrunken hypereosinophilic cells with pyknotic nuclei. Multifocally, at the center of a low number of whorls are concretions of lamellated hyaline acellular material that is occasionally mineralized (psammoma bodies). There are also multifocal, irregularly shaped accumulations of mineral (interpreted as dystrophic calcification). The brain parenchyma immediately adjacent to the mass is moderately to markedly compressed and focally concave. In a few regions, the cerebral cortex is hypercellular due to mildly increased numbers of glial cells (gliosis).

Contributor's Morphologic Diagnoses:
1. Intracranial mass: Meningioma with multifocal moderate to marked mineralization and cholesterol clefts; multifocal mild to moderate necrosis, hemosiderosis, and psammoma bodies.
2. Frontal cortex: Multifocal mild subacute to chronic cerebral cortical gliosis.

Contributor's Comment:
Meningiomas are the only tumor arising from meningothelial cells. In most reports, meningiomas have been diagnosed in patients over 7 and 9 years of age for dogs and cats respectively, although they have seldom been reported in young animals (< 6 months of age).[10, 15] In a recent retrospective analyses of canine and feline primary intracranial neoplasia, meningiomas accounted for 22.3% and 59% of canine and feline brain tumors, respectively.[10, 15] They are the most common type of intracranial

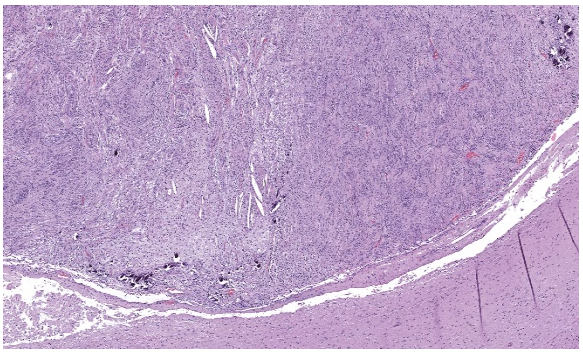

Figure 2-3. Cerebrum, cat: The nodular neoplasm is composed of spindle cells arranged in short streams and bundles with multifocal mineralization and areas of necrosis with cholesterol clefts. (HE, 10X)

neoplasm in the cat in which there may be multiple masses present.[4] Other commonly reported intracranial tumor types in cats are lymphoma, pituitary tumors, and gliomas.[3, 15] The most common neurological signs associated with meningiomas are lethargy, circling, seizures, and central blindness; however, some cats will not have neurologic signs.[1, 15]

Meningiomas form as a result of the neoplastic transformation of meningothelial cells in the arachnoid membrane and pia mater. They are usually discrete variably shaped tumors with smooth surfaces and broad dural attachments.[4, 16] They are solid, gray to tan (when fixed), firm and sometimes gritty on the cut surface. Meningiomas are expansile and compress, but infrequently invade the brain. A significantly decreased incidence in their location occurs from the olfactory bulbs caudally correlating directly with a decrease in the number of arachnoid villi.[9, 16] In dogs, meningiomas are the most common primary CNS tumor in the spinal canal and are most common at the level of C1-4 spinal cord segments; the frequency decreases caudally. [16]

In cats, meningiomas are usually supratentorial, multinodular, well-defined, expansile and locally compressive. They are variably sized and may exist simultaneously in multiple sites; in cats, they may occur in the *tela choroidea* of the third ventricle.[4,6,9, 16] Feline meningiomas are rarely invasive and are more easily separated from the brain parenchyma than the more tightly adherent canine menigiomas.[4,6,9]

Meningiomas in dogs are mostly intracranial but spinal cord, orbital, paranasal, and cutaneous meningiomas also occur.[4,8,9,10,14, 16] While extracranial cutaneous meningiomas are rare in both humans and veterinary species, two cases of canine periocular extracranial cutaneous meningiomas have been recently described.[14] In horses and dogs, the paranasal meningioma is a rare variant that forms from meningeal arachnoid cells either within or outside of skull bones as the cranium develops.[4] Paranasal meningiomas in 10 canine patients were considered to be malignant because of their anaplastic features and aggressive behavior resulting in invasion into the cranial cavity.[11] The clinical presentation of primary orbital (optic nerve) meningiomas includes exophthalmos due to a space-occupying mass behind the globe and blindness due to compression of the optic nerve.[8] Meningiomas are reported sporadically in horses, cattle, pigs, and sheep.[9, 16]

Meningiomas display both mesenchymal and epithelial-like features showing spindle cell morphology with deposition of collagenous stroma or polygonal cell morphology. The following are the most common subtypes of meningiomas in dogs applying the criteria of the latest (2007) human WHO classification system for the histological classification of the subtypes and for the grading of canine meningiomas.[4,6,9, 16]

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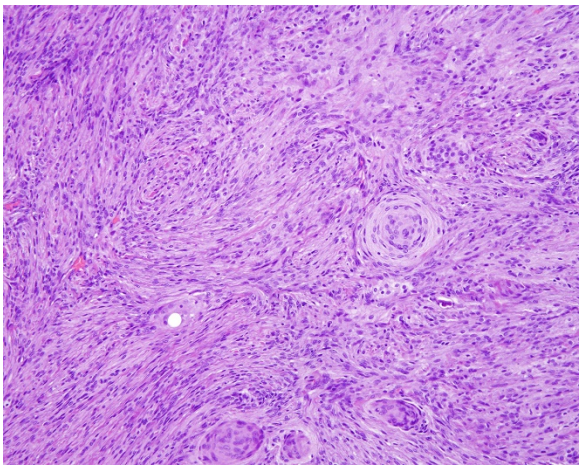

Figure 2-4. Cerebrum, cat. Higher magnification of neoplastic cells demonstrating a storiform pattern of bundles of neoplastic cells as well as several whorls. (Photo courtesy of: University of Wisconsin School of Veterinary Medicine, Department of Pathobiological Sciences, 2015 Linden Dr., Madison, WI 53706 https://www.vetmed.wisc.edu/departments/pathobiological-sciences/)

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Meningioma grade I subtypes:

1. Transitional (mixed):
    a. Features of both meningothelial and fibrous meningiomas.
    b. Syncytial cell clusters or concentric whorls around capillaries or psammoma bodies.
    c. Well-demarcated lobules mixed with meningothelial cells.
    d. The majority of canine meningiomas are of this type.
2. Meningothelial:
    a. Solid, moderately cellular lobules of polygonal cells with ill-defined borders and abundant cytoplasm; frequent cytoplasmic invaginations into the nucleus.
    b. Low mitotic index; giant cells with bizarre nuclei can be seen.
    c. Very common.
3. Psammomatous:
    a. A transitional pattern background
    b. Prominent whorls with abundant, concentric layers of hyaline material that frequently have central aggregates of mineral (psammoma bodies).
4. Angiomatous (angioblastic):
    a. Numerous large or small blood vessels (usually over 50% of the mass)
    b. Rare in domestic animals.
5. Microcystic:
    a. Spindle shaped neoplastic cells with loosely arranged, elongate processes that form small or large, clear, intracytoplasmic and interstitial vacuoles (micro- and macrocysts)
    b. This pattern can have admixed areas of transitional and/or fibrous patterns.

6. Fibrous (fibroblastic):
    a. Spindle shaped neoplastic cells arranged in long interlacing bundles
    b. The neoplastic cells are separated by variable amounts of collagen and reticulin.
    c. These frequently co-exist with the microcystic subtype
    d. Uncommon.
7. Secretory:
    a. Newly recognized.
    b. Epithelial-like, cytokeratin positive glands containing intracytoplasmic, round PAS positive globular structures mixed with areas representing the more common subtypes.
    c. These must be distinguished from adenocarcinoma.

Atypical meningioma grade II subtypes:

Features: Some meningiomas are assigned a grade II status based on their aggressive biological behavior (subtypes listed below). Additionally, a tumor with cellular features consistent with a grade I meningioma that also exhibits brain invasion is re-assigned to a grade II category. Finally, a meningioma is assigned an atypical classification when it has one or more of the common histological patterns but also has features of either:

- A mitotic count of at least 4 mitoses per 10 high power fields –OR-
- At least 3 of the following criteria:
    - Loss of normal architectural pattern replaced by cell sheeting
    - Small cell formation with a high N/C ratio
    - Nuclear atypia or macronuclei
    - Hypercellularity
    - Spontaneous necrosis

Subtypes:

1. Atypical meningiomas (irrespective of the histological subtype):
    a. Hypercellular, moderate to marked cellular atypia,
    b. Small cells with a high N/C ratio
    c. Sheets of neoplastic cells that do not conform to a specific pattern
    d. Areas of necrosis, more than 4 mitoses per 10 high power fields, and brain invasion.
2. Chordoid (myxoid):
    a. Vacuolated neoplastic cells with round nuclei
    b. Cords and trabeculae supported by a myxomatous matrix that is positive-staining for PAS, alcian blue, and mucicarmine.
3. Clear cell:
    a. Sheets of polygonal cells with clear cytoplasm due to glycogen content (non-diastase resistant, PAS-positive cytoplasmic staining).
    b. Rare.

Malignant meningioma Grade III subtypes:

Features: Extreme cellular anaplasia, frequent mitoses (more than 20 mitoses per 10 high power fields), high cellularity, bizarre mitotic figures, extensive necrosis, brain invasion and metastasis.

Histologic subtypes:

1. Papillary:
    1. Neoplastic cells arranged in papillary forms supported by a fibrovascular stroma.
    2. Rare in domestic animals.
2. Rhabdoid:
    1. Non-cohesive cells with a large vesiculate nuclei and intracytoplasmic paranuclear, eosinophilic globular, inclusion-like, bodies.

2. Most cells have features of grade III malignant variants.
3. Rare in dogs.

In canines, approximately half of meningiomas are classified as benign grade I followed by atypical grade II; malignant grade III tumors are rare.[12]

In one study of 38 feline meningiomas, grade III tumors were not identified and the researchers questioned whether current grading systems are relevant for feline meningiomas.[7,10] In further contrast to the dog, cat meningiomas have a remarkably consistent and uniform histological appearance characterized by prominent whorling of elongate cells in a collagen rich matrix in a pattern similar to the classical transitional and fibroblastic subtypes; these tumors usually present as an even mixture of both.[4,9,16] Additionally, repeatable findings are elongate linear foci of mineralization, necrosis, polymorphonuclear infiltrates and clusters of cholesterol crystals, but generally only a few psammoma bodies. A lack of nuclear atypia and mitotic figures, the presence of a relatively uniform cell type, and benign behavior are features most consistent with a grade I tumor.[4,9,16] As a result, it appears the fairly repeatable, though varying, types of neoplastic cells in combination with the cytologically uniform cellular features, create a situation where feline meningiomas do not easily fit into any one existing subtype of the human 2007 WHO classification system.[7,9,10] Furthermore, the morphologic uniformity and overall similarity in biologic behavior among feline meningiomas makes a classification into different morphologic subtypes unnecessary and a grading system biologically meaningless.

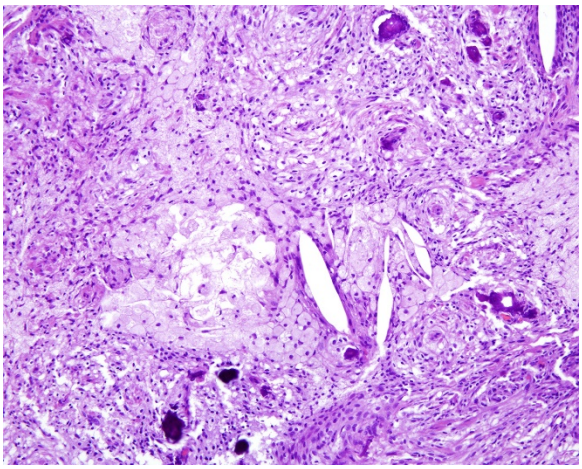

Figure 2-5. Cerebrum, cat. Throughout the mass, there are aggregates of foamy macrophages interspersed with acicular clefts and crystalline mineral. (HE, 400x) (Photo courtesy of: University of Wisconsin School of Veterinary Medicine, Department of Pathobiological Sciences, 2015 Linden Dr., Madison, WI 53706 https://www.vetmed.wisc.edu/departments/pathobiological-sciences/)

Most meningiomas stain positively for vimentin and negatively for GFAP. Staining for cytokeratin, neuron specific enolase and S100 usually yield positive results but with variable (minimal to moderate) expression.[3,4] Frequently, meningiomas are immunohistochemically positive for CD34 and E-cadherin.[4] In canines, the most reliable confirmation of a diagnosis of a canine meningioma still relies on transmission electron microscopy; most are characterized by numerous interdigitating cell processes, cytoplasmic intermediate filaments, desmosomes, and hemidesmosomes.[6]

In this case, the gross findings were highly suggestive of a meningioma due to its location (supratentorial and cerebral convexity). Its characteristic histologic features distinguished it from other intracranial tumors such as peripheral nerve sheath tumor and lymphoma. The variable histologic patterns present within this single neoplasm is typical for feline meningiomas. The neoplastic cells exhibited diffuse strong cytoplasmic positivity for vimentin. They did not express GFAP. Scattered cells exhibited minimal faint cytoplasmic positivity for S100. For this case, the clinical presentation

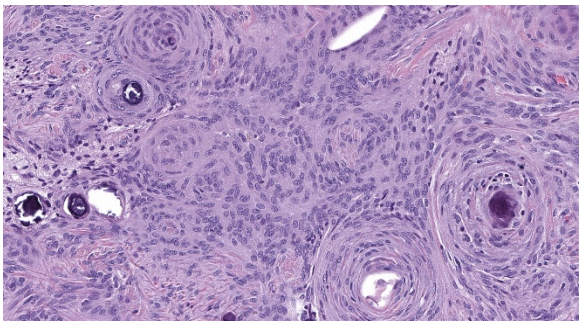

Figure 2-6. Cerebrum, cat. Neoplastic cells often whorl around mineralized psammoma bodies. (HE, 400x) (Photo courtesy of: University of Wisconsin School of Veterinary Medicine, Department of Pathobiological Sciences, 2015 Linden Dr., Madison, WI 53706 https://www.vetmed.wisc.edu/departments/pathobiological-sciences/)

of blindness is within the realm of what has been reported. Despite the dorsal location of the neoplasm, it is suspected that the cause for blindness in this patient is related to the tumor's space occupying effects and subsequent compression of the optic nerves and/or chiasm; however, disruption of pathways to the occipital lobe cannot be ruled out. While meningiomas may enter the optic foramina, this was not observed in this case.

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JPC Diagnosis:
Cerebrum: Meningioma.

JPC Comment:
The contributor provides an outstanding and very thorough overview of meningioma, the most common brain tumor affecting both cats and humans.

Dr. Harvey Cushing first coined the term "meningioma" in 1922; however, Dr. Felix Platter is credited with the oldest written record of what could be a meningioma in the existing literature, first describing this intracranial tumor in 1614. Dr. Platter was a prominent Swiss physician and anatomist who was also the first physician to dissect a human body in Germany. One of his patients, Caspar Bonecurtius, developed a progressively altered mental status, eventually became comatose, and died six months later. Dr. Platter performed an autopsy and described, "*A round fleshy tumor, like an acorn. It was hard and full of holes, and as large as a medium-sized apple. It was covered by its own membrane and entwined with veins. However, it was free of all connections of the matters of the brain so much that when it was removed by hand, it left a remarkable cavity*."[2]

Multiple additional reports describing tumors now known as meningiomas were published in the years that followed. Over 150 years after Dr. Platter, a French surgeon named Antoine Louise published a case series on meningioma entitled "*Fungueuses de la dure –mere"* or "Fungating mass of the dura matter" in 1771. In 1863, Dr. Virchow

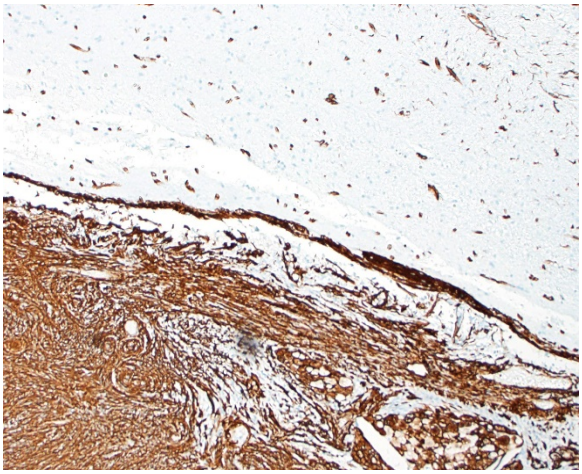

Figure 2-7. Cerebrum, cat. Neoplastic cells exhibit strong cytoplasmic positivity for vimentin. (a, ntio-vimentin, 400x)

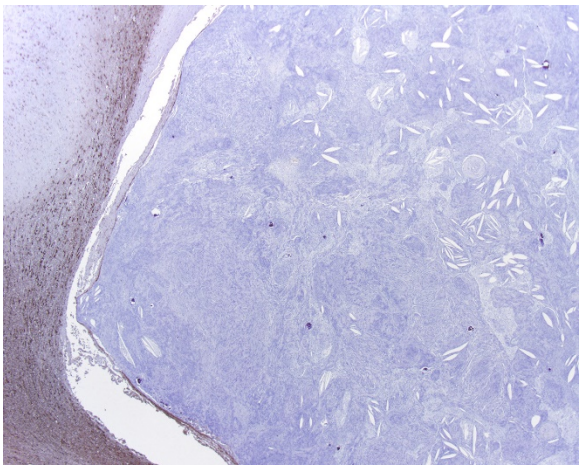

Figure 2-8. Cerebrum, cat. Neoplastic cells are negative for glial fibrillary acidic protein; astrocytic fibers stain strongly positive in the adjacent compressed cerebrum. (anti-GFAP, 4x) (Photo courtesy of: University of Wisconsin School of Veterinary Medicine, Department of Pathobiological Sciences, 2015 Linden Dr., Madison, WI 53706 https://www.vetmed.wisc.edu/departments/pathobiological-sciences/)

identified granules within meningiomas and named the granular bodes "psammomas" (sand-like). In the United States, Dr. William Keen successfully resected a meningioma in 1887.[2]

Today, approximately 33.8% of all reported primary brain and central nervous system tumors in the United States (in humans) are meningiomas. Inherited susceptibility has been suggested based on family histories as well as gene studies in DNA repair genes. People with mutations in the neuro-fibromatosis gene (*NF2*) have a substantially increased risk. High dose ionizing radiation has also been identified as an established risk factor. Because women are twice as likely as men to develop meningioma and these tumors express progesterone receptors, an etiologic role for hormones in human meningiomas has also been proposed. No such female sex predilection exists for dogs or cats.[12, 15]

The primary environmental risk factor for humans is exposure to ionizing radiation, such as demonstrated in data obtained from atomic bomb survivors showing greatly increased risk of meningioma. Evidence also exists for lower dose level exposure, such as patients with a history of full mouth radiographs having significantly increased risk for meningioma.[17]

In humans, meningiomas often have a long latency period (20-30 years or more) and the prevalence of subclinical disease is present in up to 2.8% of women as suggested by autopsy results. In fact, many meningiomas are discovered incidentally via MRIs for conditions such as head trauma and are often managed "conservatively", without surgical removal unless clinically warranted.[17]

Unlike canines, feline meningiomas are not typically subtyped. The features exhibited in this case, including whorls around psammoma bodies and steams of collagenous matrix, would be consistent with a transitional meningioma, the most common subtype affecting canines.

References:

1. Adamo, P. F., Forrest, L., & Dubielzig, R. (2004). Canine and feline meningiomas: diagnosis, treatment, and prognosis. Compendium, 26, 951-965.
2. Bir SC, Maiti TK, Bollam P, Nanda A. Felix Platter and a historical perspective of the meningioma. *Clin Neurol Neurosurg*. 2015;134:75-78.
3. Johnson, G. C., Coates, J. R., & Wininger, F. (2014). Diagnostic immunohistochemistry of canine and feline intracalvarial tumors in the age of brain biopsies. Veterinary pathology, 51(1), 146-160.
4. Kennedy, P. C., & Palmer, N. (2013). Neoplastic Diseases of the Nervous

System. In: Pathology of domestic animals. 396-398 Academic Press.
5. Kishimoto TE, Uchida K, Chambers JK, Kok MK, Son NV, Shiga T, Hirabayashi M, Ushio N, Nakayama H. A retrospective survey on canine intracranial tumors between 2007 and 2017. J Vet Med Sci. 2020 Jan 17;82(1):77-83.
6. Koestner, A., Bilzer, T., Fatzer, R., Schulman, Y., Summers, B.A., & Van Winkle, T.J. Histological classification of tumors of the nervous system of domestic animals. In: WHO International Histological Classification of Tumors of Domestic Animals, Vol. 5. Washington, DC: Armed Forces Institute of Pathology, 1999: 27-29.
7. Mandara, M.T., Pavone, S., Brunetti, B., Mandrioli, L., 2010. A comparative study of canine and feline meningioma classification based on the WHO histological classification system in humans. In: Proceedings of the 22nd Symposium ESVN-ECVN, Bologna, 24–26 September 2009. Journal of Veterinary Internal Medicine 24, 238.
8. Mauldin, E. A., Deehr, A. J., Hertzke, D., & Dubielzig, R. R. (2000). Canine orbital meningiomas: a review of 22 cases. Veterinary ophthalmology, 3(1), 11-16.
9. Meuten, D. J. (Ed.). (2016). Tumors of the Nervous System. In: Tumors in domestic animals. 864-869. John Wiley & Sons.
10. Motta, L., Mandara, M. T., & Skerritt, G. C. (2012). Canine and feline intracranial meningiomas: an updated review. The Veterinary Journal, 192(2), 153-165.
11. Patnaik, A. K., Lieberman, P. H., Erlandson, R. A., Shaker, E., & Hurvitz, A. I. (1986). Paranasal meningioma in the dog: a clinicopathologic study of ten cases. Veterinary pathology, 23(4), 362-368.
12. Saito R, Chambers JK, Kishimoto TE, Uchida K. Pathological and immunohistochemical features of 45 cases of feline meningioma. J Vet Med Sci. 2021 Aug 6;83(8):1219-1224
13. Sturges, B. K., Dickinson, P. J., Bollen, A. W., et al. (2008). Magnetic resonance imaging and histological classification of intracranial meningiomas in 112 dogs. Journal of Veterinary Internal Medicine, 22(3), 586-595.
14. Teixeira, L. B. C., Pinkerton, M. E., & Dubielzig, R. R. (2014). Periocular extracranial cutaneous meningiomas in two dogs. Journal of Veterinary Diagnostic Investigation, 26(4), 575–579.
15. Troxel, M. T., Vite, C. H., Van Winkle, T. J., et al. (2003). Feline intracranial neoplasia: retrospective review of 160 cases (1985–2001). Journal of Veterinary Internal Medicine, 17(6), 850-859.
16. Vandevelde, M., Higgins, R., & Oevermann, A. (2012). Veterinary neuropathology: essentials of theory and practice. 147-150. John Wiley & Sons.
17. Wiemels J, Wrensch M, Claus EB. Epidemiology and etiology of meningioma. *J Neurooncol*. 2010;99(3):307-314.

CASE III: S18-1972 (JPC 4136167)

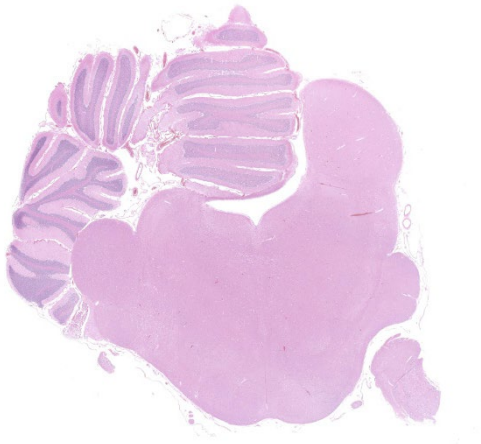

Figure 3-1. Cerebellum brainstem, dog. A section of brainstem with colliculi and cerebellar folia and vermis is submitted; there is no evidence of a lesion at subgross magnification. (HE, 5X)

Signalment:
10-year-old, female, Chihuahua, dog, *Canis lupus familiaris*

History:
History of coughing, suspicion of aspiration pneumonia and megaesophagus. Congenital heart malformation (Ventricular Septal Defect = VSD) without signs of decompensation. Epileptic attacks since three years, under treatment with levetiracetam, imepitoin and phenobarbital.

Gross Pathology:
At necropsy, the dog was in a good body condition with ample body fat. The left cranial lobe of the lung was firm and had a red color. The remaining lung tissue had a red color and was of normal consistency. The macroscopic examination of the heart confirmed the presence of a VSD, which had been previously diagnosed by echocardiography, and measured approximately 1 cm in diameter. Pronounced right atrial myocardial dilation was present mostly likely resulting from a chronic volume overload due to the VSD. The weight of the heart was 44g (relative heart weight: 1.76%, Reference value in dogs: 0.5-1%). Other findings included a moderate dilation of the cranial and middle part of the esophagus. All other organs, in particular the brain, were unremarkable during macroscopic examination.

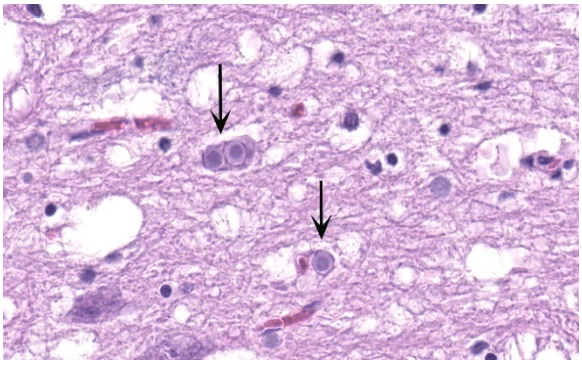

Figure 3-2. Brainstem, dog. Brainstem neurons contain one or lamellated amphophilic to basophilic polyglucosan bodies within their axons or perikarya. (arrows) HE, 900X)

Laboratory results:
No laboratory findings reported.

Microscopic Description:
Cerebellum and brainstem: Multifocal and randomly distributed, there are few to many lightly basophilic inclusions within the white and grey matter, as well as in all layers of the cerebellum, the neuronal perikarya, neuronal processes and scattered within the neuropil. The inclusions are round to globular, range from 2 to 10 µm in diameter and have occasionally a pale red center. The peripheral margins are either smooth or have a faint, perpendicularly oriented striation and most deposits have a thin clear halo. Additionally, there is mild, multifocal satellitosis and mild diffuse increase in microglia.

Contributor's Morphologic Diagnoses:
Cerebellum and brainstem: numerous intraneuronal basophilic inclusions (polyglucosan bodies); mild multifocal satellitosis and microgliosis.

Contributor's Comment:
Significant histologic lesions of the brain involved the cerebrum, the cerebellum, the brainstem including the nuclei of the vagus nerve.

All basophilic inclusions were strongly Periodic Acid-Schiff (PAS) positive and were therefore identified as polyglucosan or Lafora-like bodies. A total of nine histologic sections of brain and vagus nerve were examined.

No other histologic changes other than the presence of these polyglucosan deposits were identified in the brain and vagus nerve. The polyglucosan or Lafora-like bodies were

most numerous in the molecular layer of the cerebellum and in the brainstem, smaller numbers were seen in other brain localizations or in the vagus nerve. No deposits were identified in other tissues such as liver, cardiac and skeletal muscle or esophagus.

The histological examination of the lung within the left cranial lobe revealed that the alveoli were filled with a large number of intact or degenerated neutrophils, accumulations of fine fibrillar eosinophilic material (fibrin) and protein-rich edema fluid and occasional cellular debris (necrosis). Within the alveoli, fine granular, green material was also detected, and interpreted as aspiration of foreign material, resulting from the CNS symptoms. Alveolar septa were moderately congested and frequently necrotic. Interlobular septa were markedly expanded/distended by fibrin, edema fluid, and inflammatory cells (mostly neutrophils).

The histological examination of the heart was unremarkable.

Lafora disease (LD) is an autosomal recessive disorder that causes non-fatal myoclonic epilepsy.[2,6] The disease is characterized by the presence of polyglucosan inclusion bodies (Lafora bodies), predominantly in the central nervous system.

More than 90% of human LD cases are caused by genetic variants in one of two different genes, EPM2A, encoding laforin glucan phosphatase, or EPM2B (also known as NHLRC1) encoding the NHL repeat containing E3 ubiquitin protein ligase 1, also termed malin.[10,11,12]

LD in animals has similar clinical signs as the human disease, including spontaneous and reflex myoclonus, jerks and generalized tonic clonic seizures.

In animals, LD has been reported in the dog, cat, cow, and fennec fox.[7,8,9] In dogs, LD is one of the most commonly recognized structural-metabolic epilepsies. A genetic abnormality has been identified only in canine LD, in association with a repeat expansion in the EPM2B gene[11] causing a functional impairment of the ubiquitin ligase malin, which regulates the glycogen metabolism. Thus, abnormally structured glycogen accumulates and forms poly-glucosan bodies, predominantly in the nervous system.

Miniature wirehaired dachshund is the breed most commonly affected. LD cases with EPM2B repeat expansion have also been reported in the basset hound and beagle. [7,11] The diagnosis of LD is based on functional (clinical sign), genotypic (gene mutation), and phenotypic (histopathology) exam-inations. Histopathologically, LD is char-acterized by the presence of pathognomonic inclusions named Lafora bodies especially in the brain, spinal cord and other tissues such as skin, liver, cardiac and skeletal muscle.

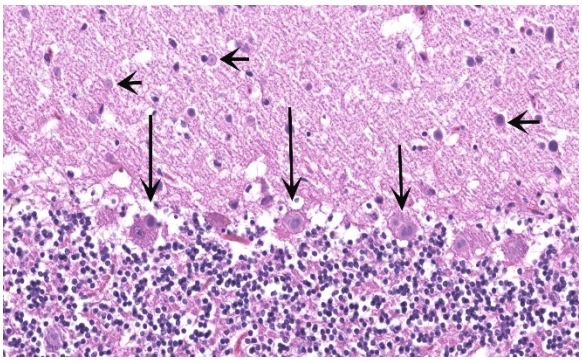

Figure 3-3. Cerebellum, dog. Neurons within the molecular layer (top) and Purkinje cells (bottom) contain one or more lamellated amphophilic to basophilic polyglucosan bodies (arrows) in their axons or perikaryal. HE, 900X)

In this case, no further genetic examination was undertaken.

Contributing Institution:
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JPC Diagnosis:
Brainstem and cerebellum: Intraneuronal Lafora (polyglucosan) bodies, numerous, with mild scattered microgliosis and spongiosis.

JPC Comment:
Lafora disease bears the name of Gonzalo Rodríguez-Lafora, who discovered the characteristic intraneuronal bodies in 1911. Born in Madrid in 1886 and the son of a Spanish army officer, Lafora spent part of his childhood in the Spanish colony of Puerto Rico (before the Spanish-American War of 1898) before attending medical school at the Central University of Madrid and receiving his medical degree at the age of 21. He went on to pursue additional study in the field of neuropsychiatry at multiple prominent Spanish, French, and German institutions in the years that followed. In 1910, he was appointed as the neuropathologist for the Government Hospital for the Insane in Washington D.C., during which time he discovered the intraneuronal bodies that bear his name today while studying familial myoclonic epilepsy. Throughout his career, Lafora published approximately 200 papers, including original works on sleep, neurosyphilis, histological alterations in dementias, and studies on schizphrenia. In addition to his professional life as a neurologist, pathologist, and psychiatrist, Lafora was also an impressionist painter, specializing in cubism.[13]

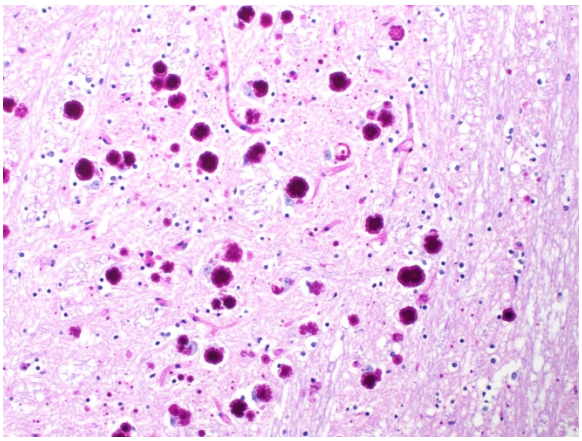

Figure 3-4. Brainstem, dog. Lafora bodies are easily demonstrated with a periodic acid-Schiff stain (PAS, 400X)

Interestingly, the first clinical signs of Lafora disease in both humans and dogs occur at a similar chronological age (in dogs at seven years of age and during late childhood /adolescence in humans). The disease then progresses at a similar rate of the remaining lifetime of the dog and over 10 years in humans. This is unique in that other equivalent neurodegenerative diseases between the two species occur at the same epigenetic age (i.e. a disease affecting an adolescent human will occur in an 8-10 month old puppy).[16]

As noted by the contributor, Lafora disease has no cure and is one of the severest forms of familial myoclonic epilepsies observed in several species, but particularly in humans and dogs. In dogs, Lafora disease is caused by a repeat expansion in the NHL repeat containing the E3 ubiquitin protein ligase 1 (*NHLRC1*) gene. Compared to other species, dogs are predisposed to Lafora disease, with affected individuals carrying 19-26 copies of the repeat sequence rather than the expected 2-3.[16]

Under normal conditions, glucose is stored as soluble glycogen. However, in the absence of *NHLRC1* (*EPM2B*), glycogen is mal-structured and becomes an insoluble complex

polyglucosan.[3,16] Over time, this abnormal glycogen accumulates into Lafora bodies in neurons and astrocytes. This results in progressive neuronal degeneration, which is driven by impaired astrocytic function and neuroinflammation. The mechanism in which Lafora disease results in myoclonic epilepsy is uncertain. However, there is increasing evidence that accumulation of glycogen within astrocytes plays a central role in the pathogenesis. It is hypothesized that inflammation, astrocyte reactivity, and activation of microglia play a major role in the triggering of seizures. Interestingly, blocking brain glycogen synthesis in mouse Lafora disease models prevents disease progression.[16]

In humans, Lafora disease clinically manifests as myoclonus (photomyoclonic response), tonic-clinic seizures, visual hallucinations, and blindness. The disease rapidly progresses, leading to more severe and frequent seizures with increased refractoriness, ataxia, dementia, and eventually a vegetative state. Human patients typically die within 10 years of onset due to status epilepticus. Dogs demonstrate a similar clinical progression, with the addition of aggression and incontinence, and are typically euthanized when quality of life is significantly impacted by the disease.[14,16]

Although Lafora bodies are a characteristic feature of Lafora disease, the presence of these 5-20 μm, periodic acid-Schiff (PAS) positive, basophilic, intracytoplasmic Lafora bodies is not pathognomonic for the disease. In fact, Lafora bodies are most frequently identified as incidental findings in aged animals. However, in cases of Lafora disease they occur in massive numbers, such as in this case.[3]

Lafora disease is an autosomal recessive species wide disorder in canines that will likely continue to spontaneously appear in many breeds and mixed breed dogs. Given this disease has been reported in multiple breeds, genetic testing should be considered when designing breeding strategies in affected breeds, especially those with small genetic pools. This is complicated by the fact Lafora disease has a late onset in dogs and many are bred before developing clinical signs. Muscle biopsy is a useful ancillary test, especially for breeds where genetic tests are available or have not been sufficiently validated. The optimal site for muscle biopsy is the tissue adjacent to the myotendinous insertion and the myofascial union as Lafora bodies are only sparsely scattered in the muscle belly area.[16] Interestingly, a 2011 study evaluating quadriceps biopsies from mice identified Lafora bodies only within type II myofibers, with none found within type I fibers.[15] This finding has since been documented within canine skeletal muscle.[4]

In addition to the mainstay treatment of antiepileptic medications, ketogenic diets have been receiving attention in regard to management of this disease. These diets maintain blood glucose concentrations in the low normal range, resulting in the brain using ketones for energy production. In addition, this type of diet is also antiepileptic due to γ-glutamyl transpeptidase and γ-glutamylation inhibition. A lifelong ketogenic diet in a mouse model of Lafora disease reduced the number of Lafora bodies; however, human studies were less promising but did suggest the disease's progression was delayed if a ketogenic diet was started early in the process. Ketogenic diets have not yet been trialed for dogs with Lafora disease, although their utilization is often used on an empirical basis.[16]

Determination of the morphologic diagnosis elicited spirited and robust discussion amongst participants. Approximately half

favored "degenerative encephalopathy" as the primary lesion being due to the presence of insoluble Lafora bodies within neurons, an inherently degenerative lesion ultimately resulting in neuronal dysfunction. Remaining participants favored the selected morphologic diagnosis of "intraneuronal Lafora bodies" based on the paucity of neurons exhibiting classic characteristics of degeneration, including cell shrinkage, loss of Nissl substance, intensely eosinophilic cytoplasm, and a pyknotic nucleus. Regardless of institutional and training background, there is no debate in regard to the degenerative and progressive nature of Lafora disease as an entity.

References:

1. Andrade DM, Turnbull J, Minassian BA. Lafora disease, seizures and sugars. Acta Myol. 2007;26:83-86.
2. Berendt M, Farquhar RG, Mandigers PJ, et al. International veterinary epilepsy task force consensus report on epilepsy definition, classification and terminology in companion animals. BMC Vet. Res. 2015;11:182.
3. Cantile C, Youssef S. Nervous system. In: Maxie MG, ed. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. Vol 1. 6th ed. Philadelphia, PA: Elsevier Saunders; 2016: 255, 292.
4. Chambers JK, Thongtharb A, Shiga T, et al. Accumulation of Laforin and Other Related Proteins in Canine Lafora Disease With EPM2B Repeat Expansion. *Vet Pathol*. 2018;55(4):543-551.
5. Chan EM, Young EJ, Ianzano L, et al. Mutations in NHLRC1 cause progressive myoclonus epilepsy. Nat Genet. 2003;35:125-127.
6. Delgado-Escueta AV. Advances in Lafora progressive myoclonus epilepsy. Curr Neurol Neurosci Rep. 2007;7(5):428-433.
7. Hajek I, Kettner F, Simerdova V, et al. NHLRC1 repeat expansion in two beagles with Lafora disease. J Small Anim Pract. 2016;57(11):650-652.
8. Hall DG, Steffens WL, Lassiter L. Lafora bodies associated with neurologic sign in a cat. Vet Pathol. 1998;35(3):218-220.
9. Honnold SP, Schulman FY, Bauman K, Nelson K. Lafora's-like disease in a fennec fox (Vulpes zerda). J Zoo Wildl Med. 2010;41(3):530-534.
10. Ianzano L. et al. Lafora progressive myoclonus epilepsy mutation database-EPM2A and NHLRC1 (EMP2B) genes. Human Mutat. 2005;26:397.
11. Lohi H, Young EJ, Fitzmaurice SN, et al. Expanded repeat in canine epilepsy. Science. 2005;307(5706):81.
12. Minassian BA, Ianzano L, Delgado-Escueta AV, et al. Identification of new and common mutations in the EPM2A gene in Lafora disease. Neurology. 2000;54:488-490.
13. Nanduri AS, Kaushal N, Clusmann H, Binder DK. The maestro don Gonzalo Rodríguez-Lafora. Epilepsia. 2008;49(6):943-947.
14. Swain L, Key G, Tauro A, Ahonen S, Wang P, Ackerley C, Minassian BA, Rusbridge C. Lafora disease in miniature Wirehaired Dachshunds. PLoS One. 2017 Aug 2;12(8):e0182024.
15. Turnbull J, Girard JM, Pencea N, et al. Lafora bodies in skeletal muscle are fiber type specific. *Neurology*. 2011;76(19): 1674-1676.
16. von Klopmann T, Ahonen S, Espadas-Santiuste I, et al. Canine Lafora Disease: An Unstable Repeat Expansion Disorder. Life (Basel). 2021;11(7):689. Published 2021 Jul 14.

CASE IV: N9757432 (JPC 4101492)

Signalment:
7 year-old, male neutered, Labrador Retriever dog (*Canis familiaris*)

History:
A 7 year old Labrador Retriever presented to the Emergency Service for cluster seizures. He had a previous history of chronic, intermittent chewing behavior which had not responded to medical management with anticonvulsants, steroids, and pain relievers. On presentation, the patient was experiencing a generalized seizure which was mitigated with diazepam administration, however, focal facial seizures continued to occur. Following diazepam administration, the dog was laterally recumbent, poorly responsive, dehydrated and hyperthermic. He was admitted to the hospital for supportive care, anticonvulsant therapy, and a diagnostic workup. Overnight, he was treated with fluid therapy and long-acting anticonvulsants prior to being transferred to the Neurology service.

An MRI showed diffuse cerebral edema with herniation, and a right-sided mass effect, however, a discrete mass was not visualized with or without contrast enhancement. The dog was discharged, but remained dull with intermittent jaw chomping and required ambulatory support. The jaw chomping increased in frequency over the 6 weeks, after which the owners elected euthanasia.

Gross Pathology:
The brain is removed. The right side is more congested as compared to the left. No obvious masses are found with external evaluation. The occipital lobes are bilaterally mildly indented (presumed subtentorial herniation). Despite the lack of gross evidence of foramen magnum herniation, there is a locally extensive region of lepto-meningeal hemorrhage at the brainstem, adjacent to the cerebellar vermis that measures 2.5 x 0.5 cm.

The fixed brain is sectioned. There is right and left sided expansion of the white matter. On the right side, there is cavitation of the centrum semiovale at the level of the caudate nucleus with hemorrhage and necrosis in the corresponding and contralateral pyriform lobes. On the left side, at the level of the thalamus and midbrain, there is white matter expansion with a mild midline shift and mottling of the cerebral cortex on the right and left sides. These images can be compared with the MRI imaging which shows hyperintensity of the right centrum semiovale and pyriform lobes and enlargement of the hippocampus with cerebrocortical hyper-intensities.

Laboratory results:
History of elevated RMSF titers, antigen test negative.
CSF (6 weeks before necropsy): Neutrophilic pleocytosis

Microscopic Description:
Samples include either one slide containing cerebral cortex and corpus callosum with medulla, cerebellum and caudal colliculus, or cerebral cortex, corpus callosum, hippo-campus, thalamus and pineal gland. Multifocally throughout the samples, in grey and white matter, there are populations of poorly defined, pleomorphic, spindle shaped cells, haphazardly infiltrating the neuro-parenchyma with rare formation of parallel fascicles. These cells are markedly pleomorphic, with scant to moderate amounts of elongate, eosinophilic cytoplasm, and ovoid to slender or twisted nuclei. Nuclei exhibit up to 4-fold anisokaryosis, with coarse chromatin, frequently indistinct nucleoli, and an occasional large, magenta nucleolus. There are 10 mitoses in 10 high power fields, with variation throughout the samples. The spindle cells multifocally form

aggregates in subpial and perivascular regions, with less frequent neuronal satellitosis. In the medulla, the nuclei are more intensely infiltrated by these spindle cell populations. In regions of higher cellularity and edema, there is rarefaction and malacia with gliosis including numerous gemistocytic astrocytes, Gitter cells, myelin vacuolation and axonal swelling (spheroid formation). Neuronal degeneration and loss are observed, most prominently in the hippocampus, with regional gliosis and gemistocytic astrocytosis.

Immunohistochemistry for CD18, GFAP: CD18 staining reveals detectable antigen within cells in between the pleomorphic, spindle shaped cells, but does not stain the atypical cell population. GFAP reveals detectable antigen within astrocytes between pleomorphic spindle cell populations.

Contributor's Morphologic Diagnoses:
Brain (cerebrum, thalamus, midbrain, cerebellum): Gliomatosis cerebri (type I-like) with white matter cavitation, rarefaction, necrosis, gliosis with gemistocytic astrocytosis, spheroid formation, neuronal degeneration and loss.

Contributor's Comment:
The lesion in this case was characterized by widespread infiltration by a population of elongate, hyperchromatic cells, with no distinct mass formation. Multiple portions of the brain were affected, including multiple lobes within the cerebrum, thalamus, midbrain, cerebellum and medulla. These cells were present both on the right and left sides of the brain. The differential diagnoses included diffuse astrocytoma, micro-gliomatosis and more remotely, lymphoma, primitive neuroectodermal tumors (PNETs)

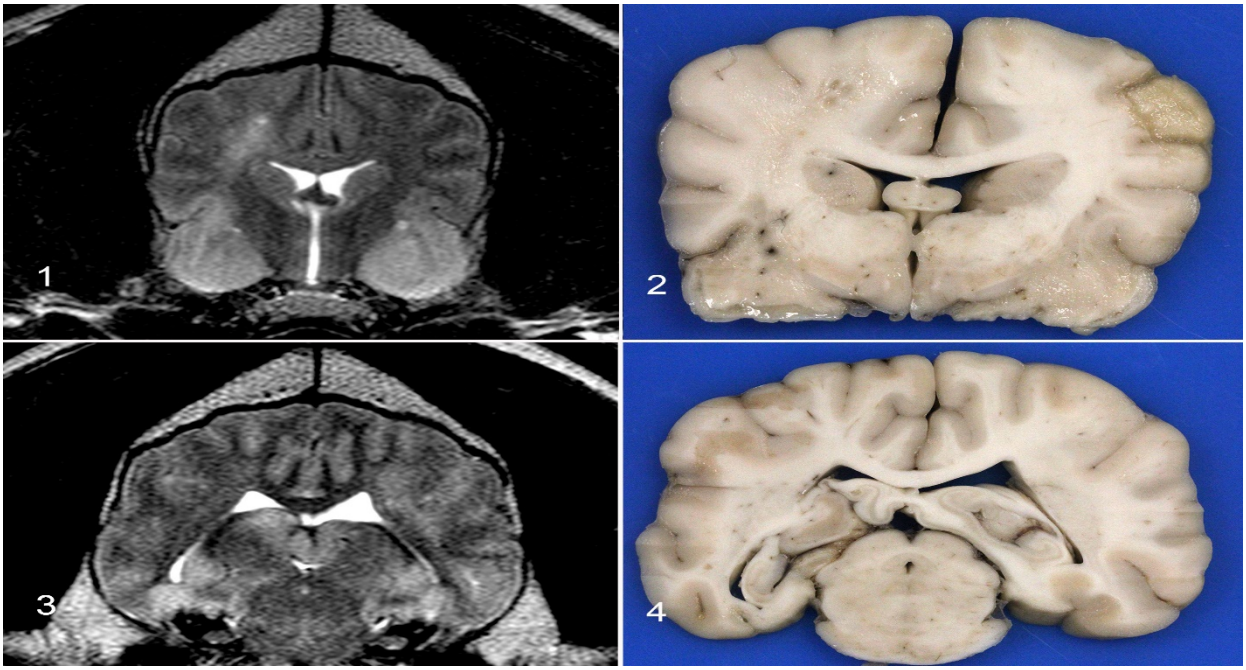

Figure 4-1. Brain, dog. Fig. 1. MRI imaging shows hyperintensity of the right centrum semiovale and pyriform lobes. Fig. 2. The gross specimen demonstrates corresponding cavitation of the centrum semiovale at the level of the caudate nucleus with hemorrhage and necrosis in the corresponding and contralateral pyriform lobes. Fig 3. The MRI shows enlargement of the hippocampus with cerebrocortical hyperintensities. Fig. 4. On the left side, at the level of the thalamus and midbrain, there is white matter expansion with a mild midline shift and mottling of the cerebral cortex on the right and left sides. (Photo courtesy of: The Animal Medical Center, 510 East 62nd St. New York, NY 10065 www.amcny.org.

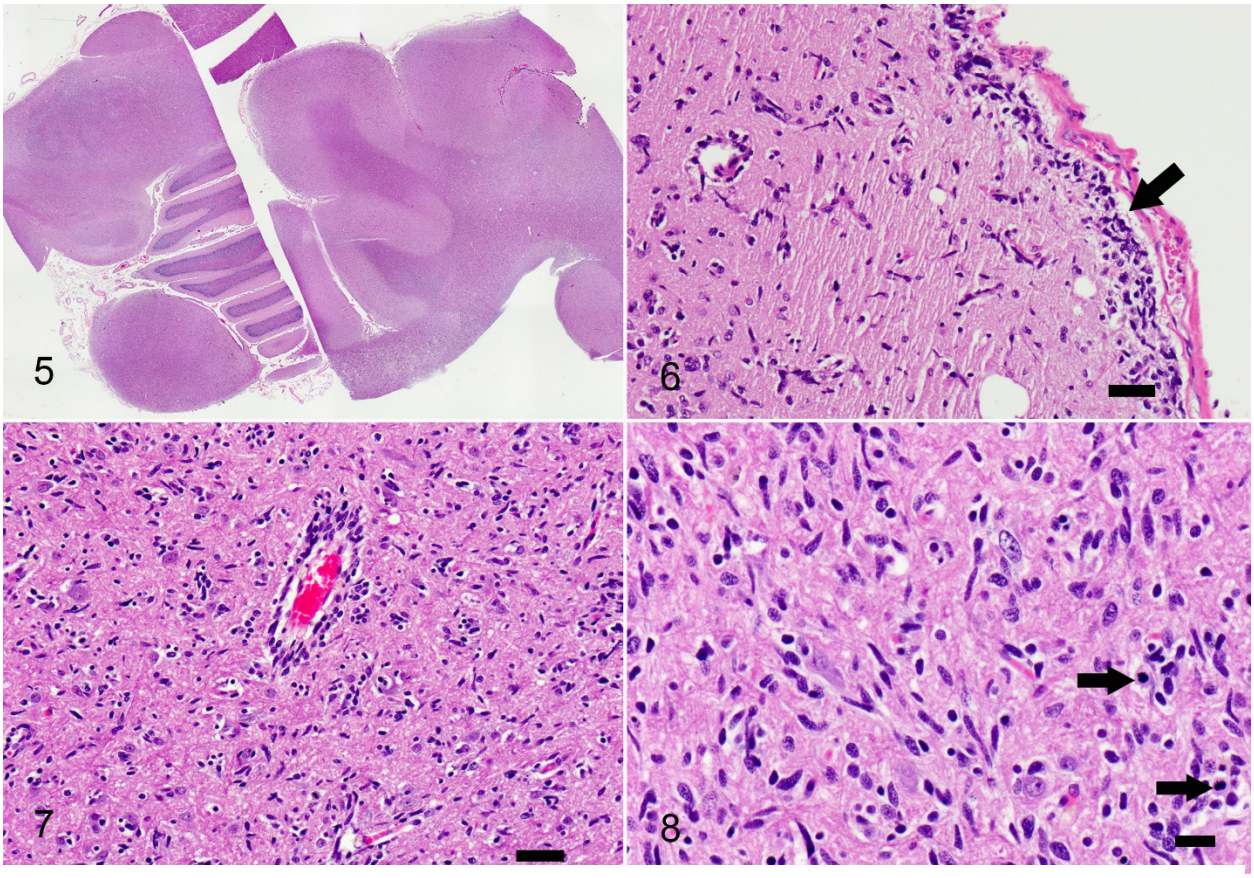

Figure 4-2. Brain, dog. Fig. 5. Subgross view of the slide which contains cerebral cortex and corpus callosum with medulla, cerebellum and caudal colliculus Fig. 6. Neoplastic spindle cells multifocally form aggregates in subpial region. Fig. 7. Neoplastic spindle cells multifocally form aggregates in perivascular areas. Fig 8. Mitoses are common (arrows) (Photo courtesy of: The Animal Medical Center, 510 East 62nd St. New York, NY 10065 www.amcny.org.)

and gliosis.[3] Due to the widespread nature of the infiltration and presence of atypia within this population, gliomatosis cerebri (GC) was given primary consideration. Immunohistochemistry for GFAP and CD18 was negative in the atypical cell populations, which is supportive of GC. The infiltration was accompanied by marked white matter cavitation with malacia, rarefaction, neuronal loss and gliosis including gemistocytic astrocytosis. These findings are interpreted to be secondary.

Gliomatosis cerebri (GC) is a rare neoplasm of unknown cell origin but is classified as a glial cell tumor in the WHO classification of tumors of the nervous system.[5,6,7] GC is a well-recognized entity in human medicine, characterized by widespread infiltration with preservation of the architecture of the brain.[5,7] Gliomatosis in humans is divided into two subtypes. Type I is the most common and presents as a diffuse infiltration of the brain with no mass lesion. With type II gliomatosis, the diffuse infiltrate is accompanied by a mass lesion.[1,6,7] Gliomatosis cerebri in dogs more commonly resembles type I GC,[7] although multiple cases of type II-like GC in dogs were recently reported, and the authors recommended that canine GC should also be subclassified in this manner.[1]

The cell of origin is controversial. In humans, these neoplastic cells are immunoreactive for GFAP, which supports astrocytic differentiation,[6] however, canine cases consistently do not exhibit immunoreactivity

for GFAP, suggesting that these cells are not astrocytes.[6] In one veterinary study, CD18 expression was observed in a subpopulation of neoplastic cells for one case, suggesting divergence along the monocyte/ macrophage/microglial cell line, and a more accurate diagnosis of microgliomatosis.[6] In another study, cases of canine GC were immunoreactive for Olig-2 and negative for GFAP, thus the authors suggested that GC may be a neoplasm of oligodendrocytes.[1] Unfortunately, Olig-2 was not performed in this case.

Dogs with GC have been reported to range from 3 to 11 years of age, with clinical signs including general mentation changes, postural reaction deficits and cranial nerve dysfunction.[6] The duration of these clinical signs is variable, ranging from 1 week to 6 months.[7] In humans, common clinical symptoms include corticospinal tract deficits, dementia, headaches, seizures, mental and behavioral changes.[7] In one study, four of six dogs with GC were males. No sex predilection is reported in people with GC.[6,7] Bearded collies may be genetically predisposed to GC, as a cluster of cases have been observed in young siblings of multiple families.[2]

In reported cases of GC in dogs, there is variation in the area of brain involvement, with the cerebrum most consistently affected, and frequent concurrent brainstem involvement.[7] In one case series, limited disease was reported in two cases; one with caudal brainstem and spinal cord involve-ment, and the other with spinal cord involvement at the level of T13 and L2.[1,6] In humans, neoplastic infiltration typically involves two or more cerebral lobes, the corpus callosum, diencephalon, basal nuclei with variable brainstem involvement.[6] In this case, multiple cerebral lobes were involved, as well as the corpus callosum, diencephalon and basal nuclei. In both humans and dogs, the white matter is typically more affected than the grey matter.[7] A tropism for brain nuclei has been reported in canine cases, but is not recognized in humans.[7] Additional common features include neuronal satellitosis, subpial and subependymal accumulations, perivascular cuffing, and a parallel arrangement of the neoplastic cells within white matter tracts. Subpial and perivascular aggregates were observed in this case.

Imaging findings with GC are nonspecific, making antemortem diagnosis and differentiation from other disease processes difficult.[6] Histopathology is required for definitive diagnosis.[6] In one study of seven dogs with GC, the neuroanatomic location, MRI and gross necropsy findings were suggestive of a focal lesion, however, diffuse CNS infiltration was documented with histologic examination.[1] Minimal findings were observed on the MRI in many of the cases.[1] Interestingly, an MRI performed on this patient 4 months earlier at another institution showed no appreciable abnormalities. An additional characteristic of GC is lack of contrast enhancement with MRI,[1] also observed in this case. In the aforementioned study, neoplastic cells were present bilaterally even in cases with unilateral or no lesions identified with imaging. MRI was unable to predict lesion location in 6 cases, and all 7 cases had histologic lesions in regions that appeared grossly normal.[1]

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JPC Diagnosis:
Cerebrum and brainstem: Astrocytoma, high grade, with diffuse gliomatosis cerebri-like infiltration.

JPC Comment:
This 2017 WSC submission includes an excellent description and overview of gliomatosis cerebri, a rare neoplasm of both canines and humans thought to be of glial cell origin.

The term "gliomatosis cerebri" was first used in human neuropathology in 1938 to describe CNS neoplasms with widespread infiltration of the telencephalic hemispheres. Over the following decades, the WHO Classification of Tumors of the Central Nervous System defined gliomatosis cerebri as a diffusely infiltrative neuroepithelial neoplasm with widespread involvement of more than two cerebral lobes with or without involvement of the deep gray matter structures (such as basal nuclei and thalamus), brainstem, cerebellum, and spinal cord. As discussed in greater detail below, the veterinary community is transitioning away from this grading scheme. Using this scheme, however, most gliomatosis cerebri cases are classified as a grade 2 or 3 glioma, occurring as either a primary or secondary gliomatosis cerebri. Primary gliomatosis cerebri are further classified as type I or II, as previously described by the contributor. Secondary gliomatosis cerebri are characterized by diffuse spread of neoplastic cells arising from a primary glioma. Despite aggressive treatment, including surgery, radiation, and chemotherapy, these neoplasms have a poor prognosis due to their clinical, morphological, and molecular heterogeneity which hinders attempts to assess the efficacy of specific therapies in humans. The vast majority of dogs are euthanized soon after diagnosis.[8]

Following this case's submission, additional tissues were separately evaluated as part of a

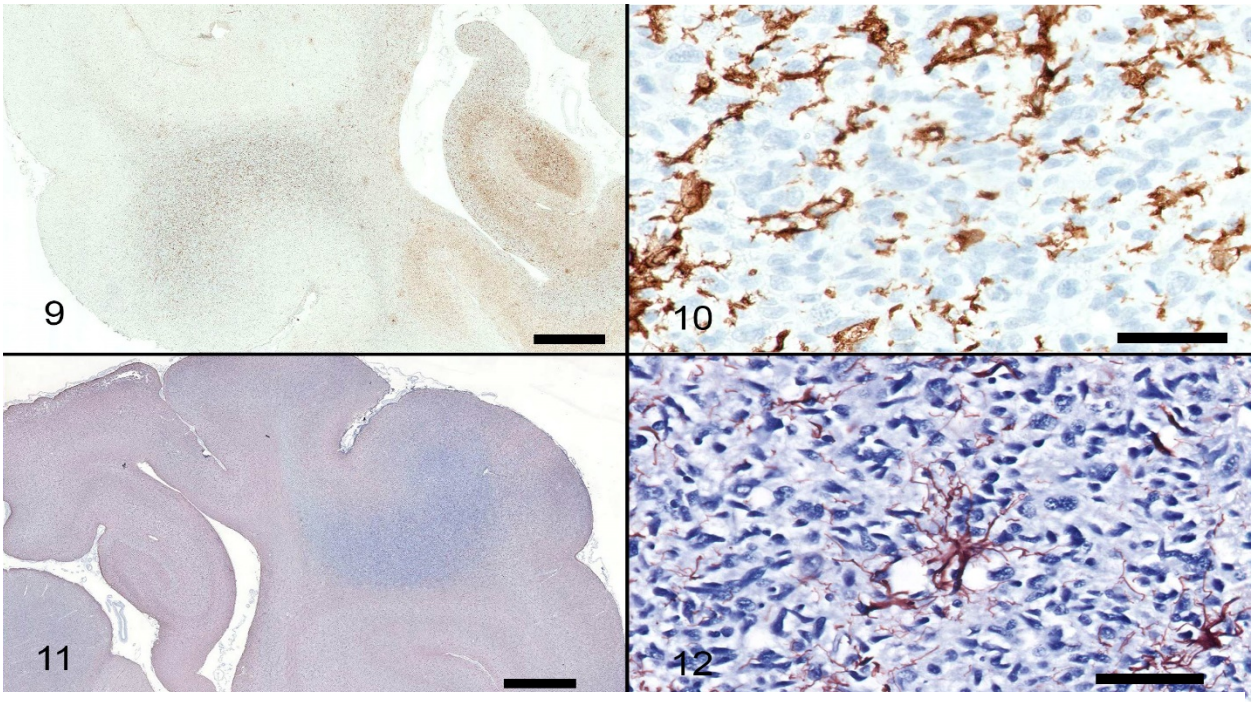

Figure 4-3. Brain, dog. Fig. 9, 10. Neoplastic cells fail to stain with CD18. Figs 11, 12. GFAP stains astrocytes and their processes in the midst of neoplastic cells. (Photo courtesy of: The Animal Medical Center, 510 East 62nd St. New York, NY 10065 www.amcny.org.)

case series evaluating the morphologic and immunohistologic characteristics of 24 cases of canine gliomatosis cerebri.[8] Neoplastic cells from this case were immunoreactive to both GFAP and Olig2, confirming the contributor's suspicion of astrocytic origin based on cellular morphology.

Although a useful diagnostic aid, Olig2 and GFAP immunohistochemical stains are not specific for oligodendrocytes and astrocytes, respectively. However, strong diffuse nuclear immunoreactivity for Olig2 is highly consistent with glial histiogenesis. In the recent case series of 24 gliomas of diffuse growth pattern, neoplasms with oligodendrogial morphology had stronger and more widespread nuclear immunoreactivity to Olig2 associated with a less consistent or absent immunoreactivity for GFAP. In contrast, strong cytoplasmic GFAP immunereactivity was evident and more consistent in neoplasms with astrocytic morphology as well with undefined gliomas. CNPase can be used to confirm oligodendroglial differentiation in cases in which morphology is consistent with glioma but Olig2 and GFAP are inconclusive.[8]

As alluded to above, the veterinary profession is transitioning away from "gliomatosis cerebri" as a distinct neoplastic entity. This shift is linked to the term's elimination from the 2016 WHO CNS tumor classification system due to lack of consensus in terms of definition, morphology, molecular profile, and treatment, as well as the alignment of these cases with existing molecular profiles of astrocytic or oligodendroglial tumors in humans. As a result, "gliomatosis cerebri" is no longer considered a distinct tumor entity but rather a type of growth pattern that is rarely found in humans. These tumors are now categorized as either an astrocytoma, oligodendroglioma, or undefined glioma with a diffuse infiltrative growth pattern.[4,8]

As previously seen with WSC#7 – Case 1, the tumor classification scheme applied to this neoplasm has changed since its submission to the WSC based on the recommendations from the National Cancer Institute-led multidisciplinary Comparative Brain Tumor Consortium (CBTC). These changes simplify the diagnostic grading scheme of glial tumors and align classification schemes between veterinary and physician neuropathology, which will serve to harmonize future comparative research and hypothesis-based investigations designed to aid in clinical management of both canine and human glioma patients.[4]

References:

1. Bentley RT, Burcham GN, Heng HG et al. A comparison of clinical, magnetic resonance imaging and pathological findings in dogs with gliomatosis cerebri, focusing on cases with minimal magnetic resonance imaging changes. Vet Comp Onc 2016;14(3):318-30.
2. Higgins RJ, Bollen AW, Dickinson PJ, Siso-Llonch S. Tumors of the Nervous System. In: Meuten DJ ed. *Tumors in Domestic Animals*, 5th ed. John Wiley and Sons, 2017:839.
3. JPC WSC archives, 2008 Conference 20, Case 1 http://www.askjpc.org/wsco/index.php
4. Koehler JW, Miller AD, Miller CR, et al. A Revised Diagnostic Classification of Canine Glioma: Towards Validation of the Canine Glioma Patient as a Naturally Occurring Preclinical Model for Human Glioma. *J Neuropathol Exp Neurol*. 2018;77(11):1039-1054.

5. Koestner A, Bilzer T, Fatzer R, Schulman FY, Summers BA, Van Winkle TJ. Histological classification of tumors of the nervous system of domestic animals. In: Schulman FY ed. *World Health Organization Histological Classification of Tumors of Domestic Animals*, Second Series, vol. 5. Armed Forces Institute of Pathology, Washington DC, 1999:p 21.
6. Plattner BL, Kent M, Summers B et al. Gliomatosis cerebri in two dogs. J Am Anim Hosp Assoc 2012; 48:359-365.
7. Porter B, DeLaHunta A, Summers B. Gliomatosis cerebri in 6 dogs. Vet Pathol 2003; 40:97-102.
8. Rissi DR, Donovan TA, Porter BF, Frank C, Miller AD. Canine Gliomatosis Cerebri: Morphologic and Immunohistochemical Characterization Is Supportive of Glial Histogenesis. *Vet Pathol*. 2021;58(2):293-304.