Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

Conference 6

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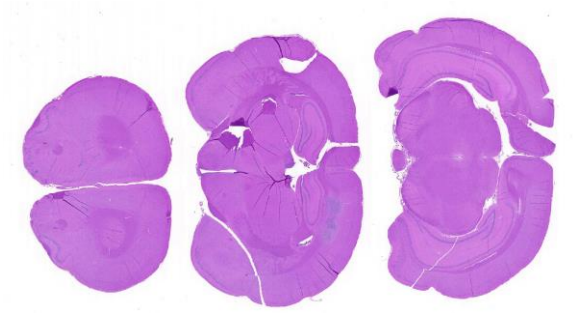
CASE I: 21608 (JPC 4089879-00).

Signalment: 22 week old female Crl:WI(Han) rat (*Rattus norvegicus*)

History: Tissues were routinely collected from this control female rat at the terminal sacrifice of a 13 week oral gavage study of a drug candidate. No clinical observations were noted prior to sacrifice.

Gross Pathology: No macroscopic observations were noted.

Laboratory results: None given.

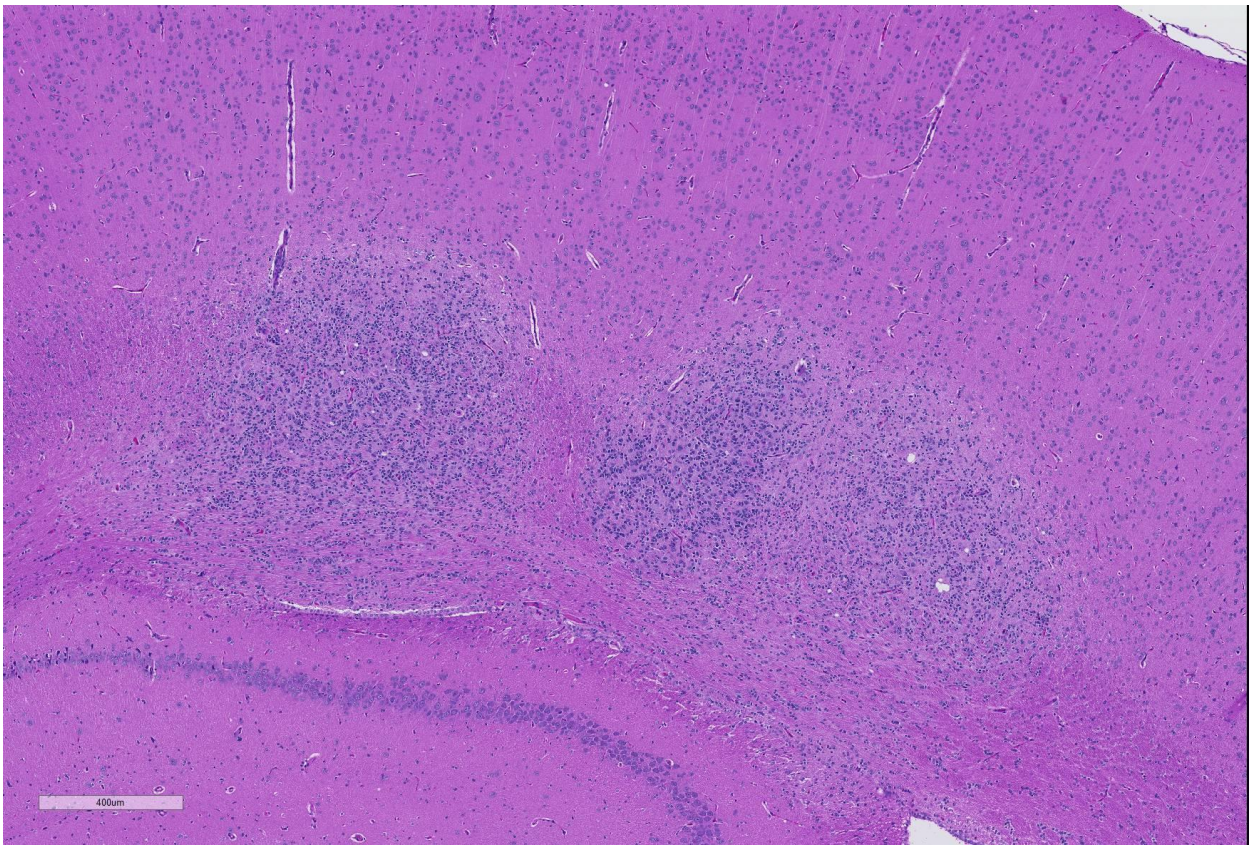


Cerebrum, rat. Three section of brain from telencephalon (left) to diencephalon (right) are submitted for examination. The middle section has a focal unilateral hypercellular mass. (HE, 5X)

Microscopic Description: Brain: In the cerebrum, subcortical white matter in one hemisphere is focally replaced by a poorly demarcated mass composed of densely packed round to fusiform cells. These cells are often arranged in small palisades, rarely form perivascular whorls, and have indistinct cytoplasmic margins, scant to abundant abundant pale amphophilic fibrillar cytoplasm, and round to oval euchromatic nuclei that often contain one or two nucleoli.

There is marked anisokaryosis and there are rare mitotic figures. Interspersed among these neoplastic cells are cells with large nuclei and abundant eosinophilic cytoplasm (interpreted as gemistocytic astrocytes).

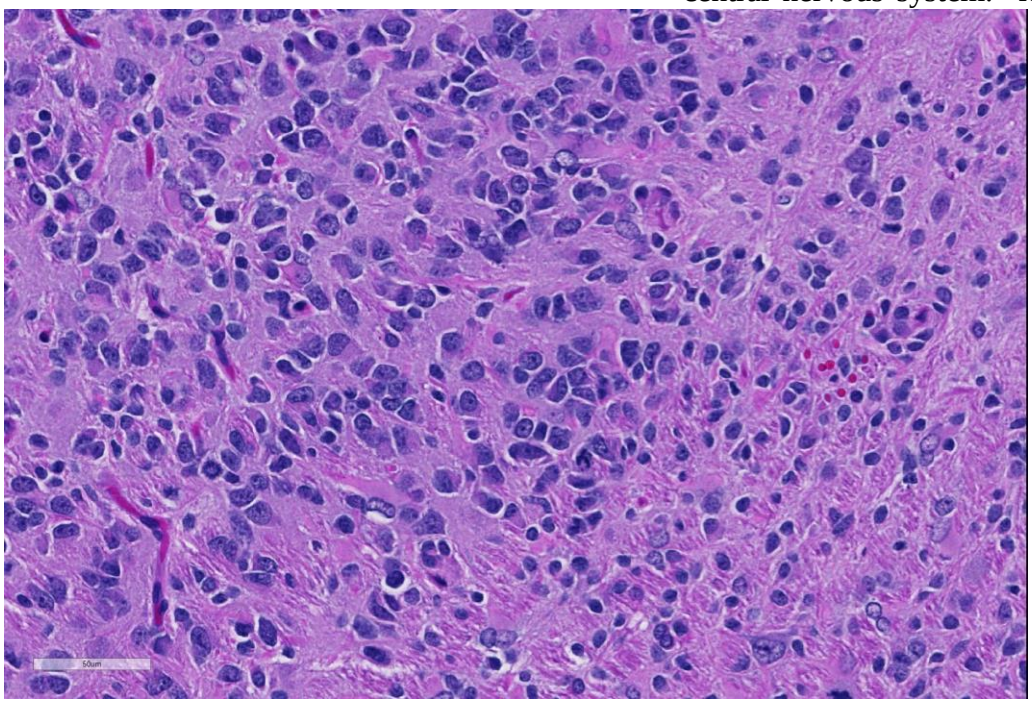
Contributor's Morphologic Diagnoses: Glioma, brain.



Cerebrum, rat. There is a focal, unencapsulated, minimally infiltrative neoplasm within the deep cortex immediately above the hippocampus. (HE, 67X)

Contributor's Comment: Glial neoplasms are the most commonly occurring spontaneous brain neoplasms in Sprague Dawley (SD) rats, with a reported incidence of approximately 1.5 – 2.0% in control rats in carcinogenicity studies, and are generally more common in males. Most of these tumors have been classified as malignant astrocytomas.[1,6] Although rarely fatal during the first year of life, spontaneous glial neoplasms have been reported in SD rats as young as 21 or 32 weeks of age.[3,8]

Recent immunohistochemical studies of rat glial neoplasms have demonstrated that the vast majority of spontaneous and chemically induced tumors traditionally termed "astrocytomas" do not express detectable glial fibrillary acidic protein (GFAP) or other astrocyte markers,[2,4,5] although GFAP positive neoplastic astrocytes have been demonstrated in oligodendrogliomas and mixed gliomas of rats.[7] In contrast, most rat "astrocytomas" are immunopositive for markers of microglia and monocytes/macrophages such as ionized calcium-binding adapter molecule-1 (Iba-1) and bind the lectin *Ricinus communis* agglutinin type 1 (RCA-1).[2,4,5] Based on these studies, the majority of rat "astrocytomas" are more appropriately termed malignant microglial tumors. It has been recommended that these tumors be diagnosed as "gliomas" in routine toxicology or carcinogenicity studies, and that more specific diagnosis as to cell of origin should be based on immunohistochemical findings.[2]



Cerebrum, rat. Neoplastic cells resemble both astrocytes (large open-raced nuclei with moderate amounts of granular eosinophilic cytoplasm), oligodendroglia (scant cytoplasm and round or deeply cleaved nuclei) and are admixed with few reactive astrocytes (marginated chromatin, ovoid nuclei) and activated microglia (small cells with minimal cytoplasm and triangular hyperchromatic nuclei. (HE, 400X)

Covance Laboratories, Inc, Madison, Wisconsin,

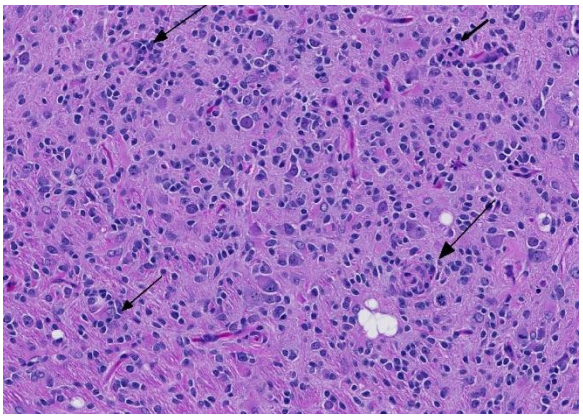
USA.http://www.covance.com/industry-solutions/drug-development/services/safety-assessment/nonclinical-pathology-services.html

JPC Diagnosis: Cerebrum: Glioma.

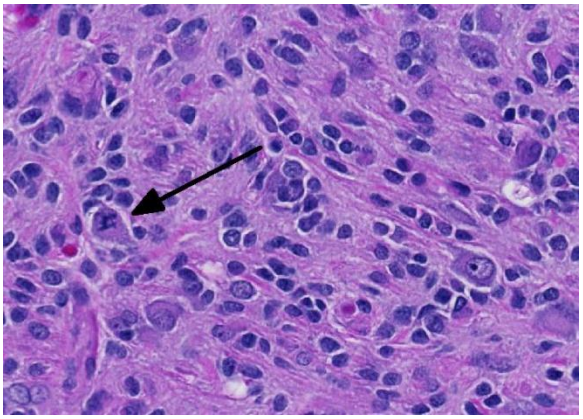
JPC Comment: As recently as the early 2000's, texts referred to primary tumors of the central nervous system in rats as "uncommon" neoplasms.[2] This somewhat questionable statement may have resulted from the fact that 1) many of these tumors are not grossly visible, 2) brain sampling protocols generally only require three coronal sections of grossly normal brains, and 3) the relative resistance of post-natal rats to chemical induction of tumors in the central nervous system. Most brain cancer models have traditionally required intrauterine exposure at critical stages of development to carcinogenic substances.[2]

In the last decade, a growing body of literature, improved sampling techniques as promoted by the Society for Toxicologic Pathologists, and the publication of historical control databases has shed more light on CNS tumors in rats.[3] In general, the incidence of spontaneous CNS tumors is higher than in mice and spontaneous tumors are found more frequently in males than in females. Incidence does not differ significantly between strains. Tumors of with glial and meningeal differentiation are more commonly seen than those with

neuronal



Cerebrum, rat. Smaller cells form clusters/ pseudorosettes in perivascular areas. (HE, 216X)



Cerebrum, rat: An atypical mitotic figure is present. (HE, 400X)

or primitive neuroepithelial differentiation.[3]

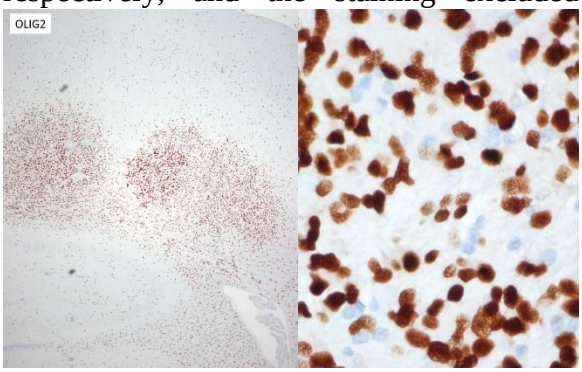
Glial cell tumors are believed to arise from neoplastic radial glial cells (RGCs). [3] These neoplasms may move within the CSF into the spinal cord but metastasis outside the nervous system is has not yet been reported. For this reason, the terms "benign" and "malignant" have largely been replaced in the toxicologic pathology community with the terms "low-grade" and "high-grade".[3]

Before the advent of immunohistochemistry and other advanced diagnostics that allowed for more precise identification of the cell of origin, most glial tumors were identified as "glioma".[2] Today, immunohistochemical protocols are allowing pathologist to sort glial tumors into more specific categories with precision. Immunohistochemical and lectin profiles exist for astrocytomas and oligodendrogliomas. The term "glioma" is largely reserved for mixed tumors in which cells fitting the morphology and the immunohistochemical and lectin profiles of both astrocytoma and oligodendroglioma are present in the neoplasm. These neoplasms may be either low- or high grade tumors. High-grade gliomas are characterized by infiltration into multiple areas of the brain, cellular atypia and pleomorphism, necrosis, and rarely giant cell formation (most likely of astrocytic origin). If these changes are present and each glial cell provides at least 20% of the neoplasm, the tumor is diagnosed as a high-grade mixed glioma. Interestingly, experimental studies have indicated that gliomas an adult rats are initially composed of either differentiated astrocytes or oligodendrocytes. As these neoplasms increase in size, cellular composition becomes mixed and anaplastic over time.[3] These high-grade mixed gliomas share some histologic features with the so-called glioblastoma multiforme of humans.[3]

The conference moderator commented on the heterogeneous nature of the neoplastic cells, with cells resembling both astrocytes and oligodendrocytes in the neoplasm, as well as accompanying activated microglia and reactive astrocytes, ultimately forming a complicated mix of morphologies. The JPC diagnosis of glioma in this case is largely based on the HE appearance.

The majority of neoplastic cells were strongly immunopositive for an OLIG2 stain run at the JPC and is consistent with the diagnosis of glioma. (OLIG2, counterintuitively to its name, will stain both oligodendrocytes and astrocytes.) While there was extensive staining with the JPC-run GFAP and Iba1 within the neoplasm, the

moderator believes that this reflects reactive astrocytes and infiltrating microglia respectively, and the staining excluded



Cerebrum, rat: Neoplastic cells are strongly positive for OLIG2, denoting a glial origin.

neoplastic cells. Based on this additional data, the moderator believes that a diagnosis of glioma is appropriate for the HE appearance of this neoplasm, and that the immunohistochemical findings are consistent with a primitive glial tumor, but does not enable a more specific diagnosis.

References:

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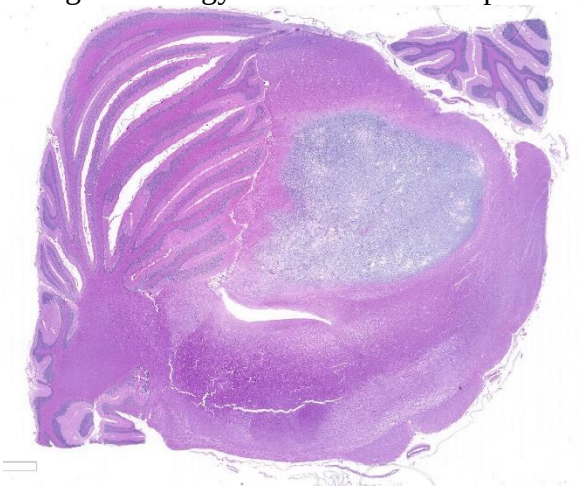
CASE II: A16-38521 (JPC 4102645-00).

Signalment: Seven-year-old neutered male mixed breed dog, *Canis familiaris*

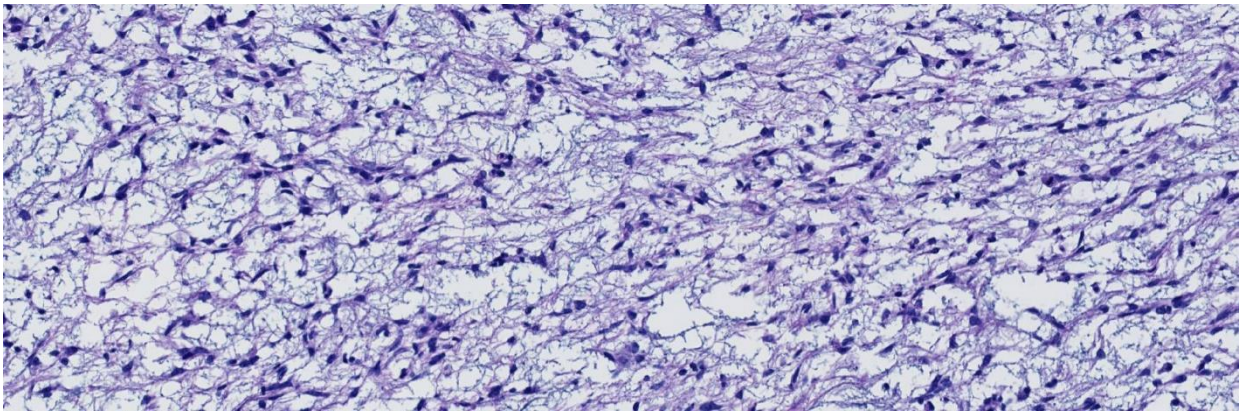
History: The dog presented to a veterinary college neurology service one month prior to necropsy following a 3-week history of circling toward the left and altered mentation. On neurologic examination, the dog had inappropriate, dull mentation and a left head turn. He was ambulatory with no weakness or ataxia but circled to the left with right-sided postural reaction deficits in the thoracic and pelvic limbs. There was an inconsistent menace response on the right side. His neuroanatomic localization was to the left prosencephalon. Magnetic resonance imaging (MRI) revealed a T2-weighted homogenously hyperintense, non-contrast enhancing mass lesion within the left thalamus and midbrain with secondary obstructive hydrocephalus, characterized by distension of the lateral and third ventricles. The dog was started on corticosteroid therapy, but gradually declined until the owners elected to euthanize.



Brainstem, dog: A large well-demarcated, non-infiltrative neoplasm within the brainstem compresses the fourth ventricle and extends across the midline. (HE, 5X)

Gross Pathology: The brain was mildly edematous and there was thinning of the cerebral cortex bilaterally with distension of the lateral ventricles. Serial sectioning revealed a well-circumscribed, well demarcated, pale, gelatinous mass on the left side effacing the mesencephalon with compression of the third ventricle and mesencephalic aqueduct.

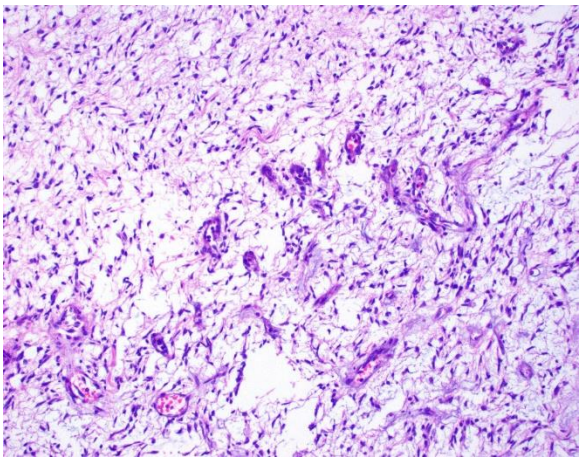
Laboratory results: None given.



Brainstem, dog. The neoplasm is primarily composed of streams of bipolar elongate spindle cells which are widely separated by a granular amphophilic myxoid matrix. (HE, 207X)

Microscopic Description: Extending from the thalamus caudally to the cerebellar peduncles caudally and expanding the mesencephalon with compression of the lateral ventricles and mesencephalic aqueduct was a well demarcated, unencapsulated, expansile, moderately cellular mass. There were two morphologically distinct cellular populations within the mass: bipolar, spindloid cells with

small ovoid nuclei and long thin wispy cytoplasmic processes, and proliferative, small, round, individualized cells with round to indented, condensed nuclei and scant, granular cytoplasm. Cells were embedded within an abundant loose myxoid matrix that was positive on Alcian blue pH 2.5. Throughout the mass, there were scattered proliferations of hypertrophied endothelial cells, frequently forming glomeruloid capillary loops. There was pallor and mild vacuolation of the surrounding neuroparenchyma. Most of the large, bipolar cells and their associated spindloid processes were strongly positive for GFAP on immunohistochemistry. The nuclei of the smaller round cells stained positively for Olig2, but did not stain with GFAP.



Brainstem, dog. There is robust neovascularization in some areas of the tumor. (HE, 200X)

Contributor's Morphologic Diagnoses: Neoplasia, oligoastrocytoma, thalamus to brain stem, left side with bilateral hydrocephalus

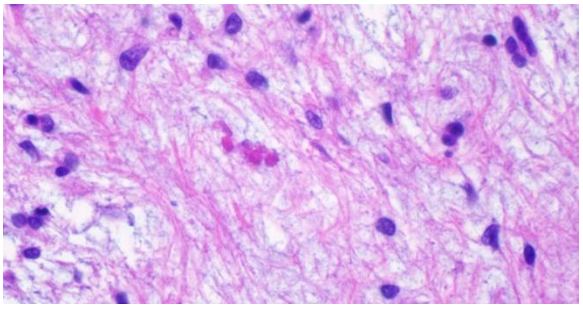
Contributor's Comment: There is a diffuse mixture of GFAP-positive and Olig2-positive cells within the mass. The Olig2-positive cells, interpreted as oligodendrocytes, are too proliferative to be considered reactive cells and therefore are likely neoplastic. There is also proliferation of GFAP-positive astrocytic cells, but it is less clear whether these are reactive or neoplastic. Astrocytes will commonly proliferate into and around oligodendrogliomas in response to the neuroparenchymal destruction[5]. This reactive response should comprise no more than 30% of the mass, which is far less than the proportion of astrocytic, GFAP-positive cells observed in this mass, which led us to the diagnosis of oligoastrocytoma. Oligoastrocytomas, or mixed gliomas, have been described previously in dogs, where both glial lineages have neoplastic features. These two populations are often separated and distinguishable within the mass, but more heterogeneous and mixed masses are reported.[2,5,6]It has also been proposed gliomas originating from more primitive cell lines may be dually positive for astrocytic and oligodendroglial markers.[5] In this case, however, there appear to be two distinct populations.

Contributing Institution:
University of Georgia, College of Veterinary Medicine, Department of Pathology, (www.vet.uga.edu/VPP)

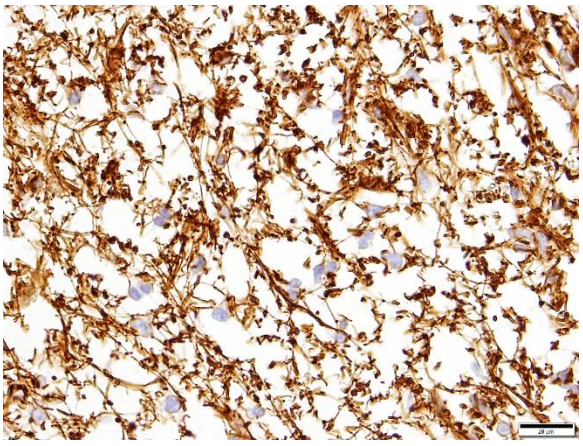
JPC Diagnosis: Brainstem, adjacent to 4th ventricle: Astrocytoma, noninfiltrative, low grade.

JPC Comment: A 2018 publication by the Comparative Brain Tumor Consortium[4] has laid the groundwork for a sweeping reclassification of canine glial tumors. Currently the World Health Organization (WHO) Tumor Fascicle Histological Classification of Tumors of the Nervous

System of Domestic Animals, published in 1999, still serves as the gold standard for diagnosis of canine glial tumors; however, its diagnostic content is based largely on morphology alone (with a small amount of immunohistochemical data– then in its infancy – on some of the more common neoplasms). The human side of the WHO has published not one, but two editions in their



Brainstem, dog. Neoplastic cells for small Rosenthal fibers. (HE, 400X)



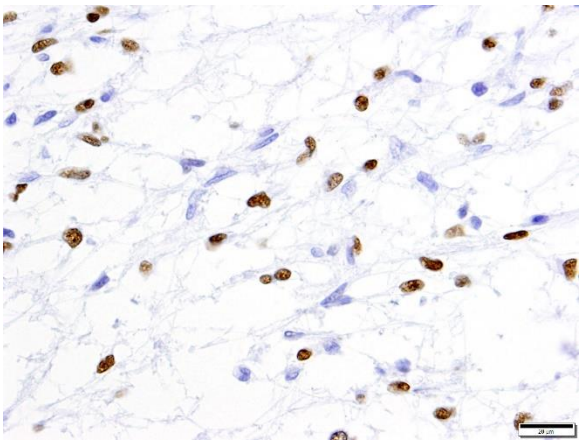
Brainstem, dog. Bipolar spindle cells and multipolar cells stain strongly positive for glial fibrillary acidic protein, a marker of astrocytes. (GFAP, 400X) (Photo courtesy of: University of Georgia, College of Veterinary Medicine, Department of Pathology, (www.vet.uga.edu/VPP)

series on Tumors of the CNS, grouping these neoplasms into expected outcomes based on morphologic features, but also incorporating molecular data as part of the diagnostic and prognostic algorithms. This new publication on canine tumors lays the important groundwork for similar advances when they become more widely available.

The new publication also has an immediate impact on the current methods of diagnosis of canine glial tumors, simplifying the classification of these tumors into three groups: astrocytoma, oligodendroglioma, and "undefined glioma". A takeaway message that will immediately simplify classification is the recommendation that any tumor in which greater than 80% of the neoplastic cells exhibit morphology and immunoreactivity of either astrocytic or oligodendroglial differentiation, that the tumor be classified into one of those two groups. "Undefined" gliomas are characterized as glial neoplasms which do not have predominant features of either an oligodendroglioma or an astrocytoma; the geographic distribution of the neoplastic cells in these tumors might be either segregated or intermingled.[4]

A number of excellent tips regarding the interpretation of immunohistochemistry on canine gliomas is also presented in this article. The authors recommend the following panel in the diagnosis of canine gliomas: GFAP, Olig2, CNPase (a component of myelin expressed exclusively by oligodendroglia in the CNS), and Ki67. It should be noted that despite its name, Olig2 is not specific for oligodendroglia and may be expressed by neoplastic astrocytes as well. A lack of Olig2 staining is of diagnostic importance as well as a result of its specificity for oligodendro- and astroglia, and a lack of staining should lead the pathologist to strongly consider non-glial tumors at that point in the diagnostic process. The article also comments on the interpretation of immunohistochemical results, with decreased staining of GFAP, CNPase and Ki67 all being negatively impacted by time to fixation (which may be important in the processing of necropsy

samples) as well as time in fixation, and fixation buffering.[4]



Brainstem, dog. Scattered neoplastic cells stain strongly positive with OLIG-2 (a non-specific glial marker). (Olig-2, 400X) (Photo courtesy of: University of Georgia, College of Veterinary Medicine, Department of Pathology, (www.vet.uga.edu/VPP)

Finally, the paper suggests morphologic criteria (albeit subjective) that may be useful in classifying gliomas into low- or high-grade, to include necrosis, pseudo-palisading of neoplastic cells along areas of necrosis, mitotic activity, microvascular proliferation, and cellular features of malignancy (atypia, anisocytosis, anisokaryosis, and nuclear pleomorphism). Interestingly, in this study, oligodendrogliomas had an overall increased rate of high-grade tumors than astrocytomas, and in keeping with anecdotal observations by many veterinary neuropathologists, oligodendroglial tumors in general far outnumbered astrocytic tumors.[4]

A number of features in this particular neoplasm are key to its classification as an astrocytoma (notwithstanding the diffuse immunoreactivity for GFAP, as this information was not provided to attendees.). The minimal amount of cytoplasm of the neoplastic cells, elongate nuclei, bipolar cell morphology, lack of necrosis, and lack of any apparent nuclear rowing (aka "interfascicular queuing") were considered more appropriate for the diagnosis of astrocytoma in this case. It is important to note that in pilocytic astrocytomas in humans (which this neoplasm strongly resembles in appearance and location), smaller Olig2 cells can be interspersed with the bipolar astrocyte population and do not confer a diagnosis of oligoastrocytoma. The presence of microcysts and formation of Rosenthal fibers by some of the neoplastic cells was also considered of diagnostic importance (a feature that has been well established in human astrocytomas)

During the discussion of this case, the moderator acknowledged the difficulty of identifying true edema in neurologic sections and the lack of well-defined critieria for it, but believes that the area of rarefaction surrounding the neoplasm and extending into the adjacent parenchyma in this case is a good example of spongiosis.

References:

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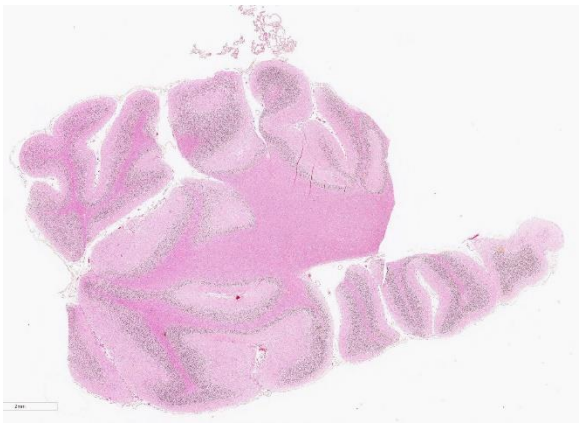
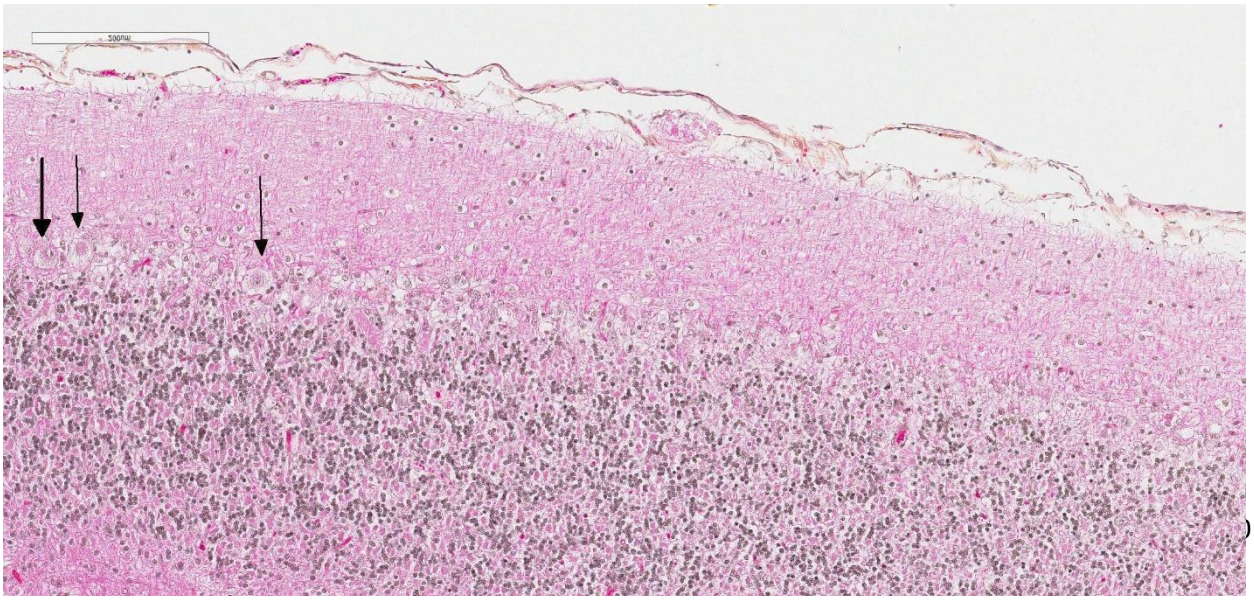


Cerebellum, cat. At subgross magnification, there is mild thinning of the cerebellar cortex. (HE, 12X)

CASE III: P3213-13 (JPC 4048997-00).

Signalment: 1½ year-old, male neutered, Domestic shorthair, (Felis catus), cat

History: This cat was reported to be normal at birth. At 7 months of age, it started showing progressive signs of cerebellar disturbance, i.e. abnormal gait and balance. Another cat from the same litter developed similar symptoms. Two MRI examinations of the head/brain were performed. The first one, on April 2013, only showed a slightly smaller than normal cerebellar size with a small suspicion of cerebellar abiotrophy. The second one, on December 2013, revealed a reduced cerebellar size with moderate diffuse widening of the cerebellar sulci, consistent with a diagnosis of cerebellar abiotrophy. The neurological examination also supported a disease process affecting all portions of the cerebellum. Due to progression of the illness, the cat was euthanized and a full

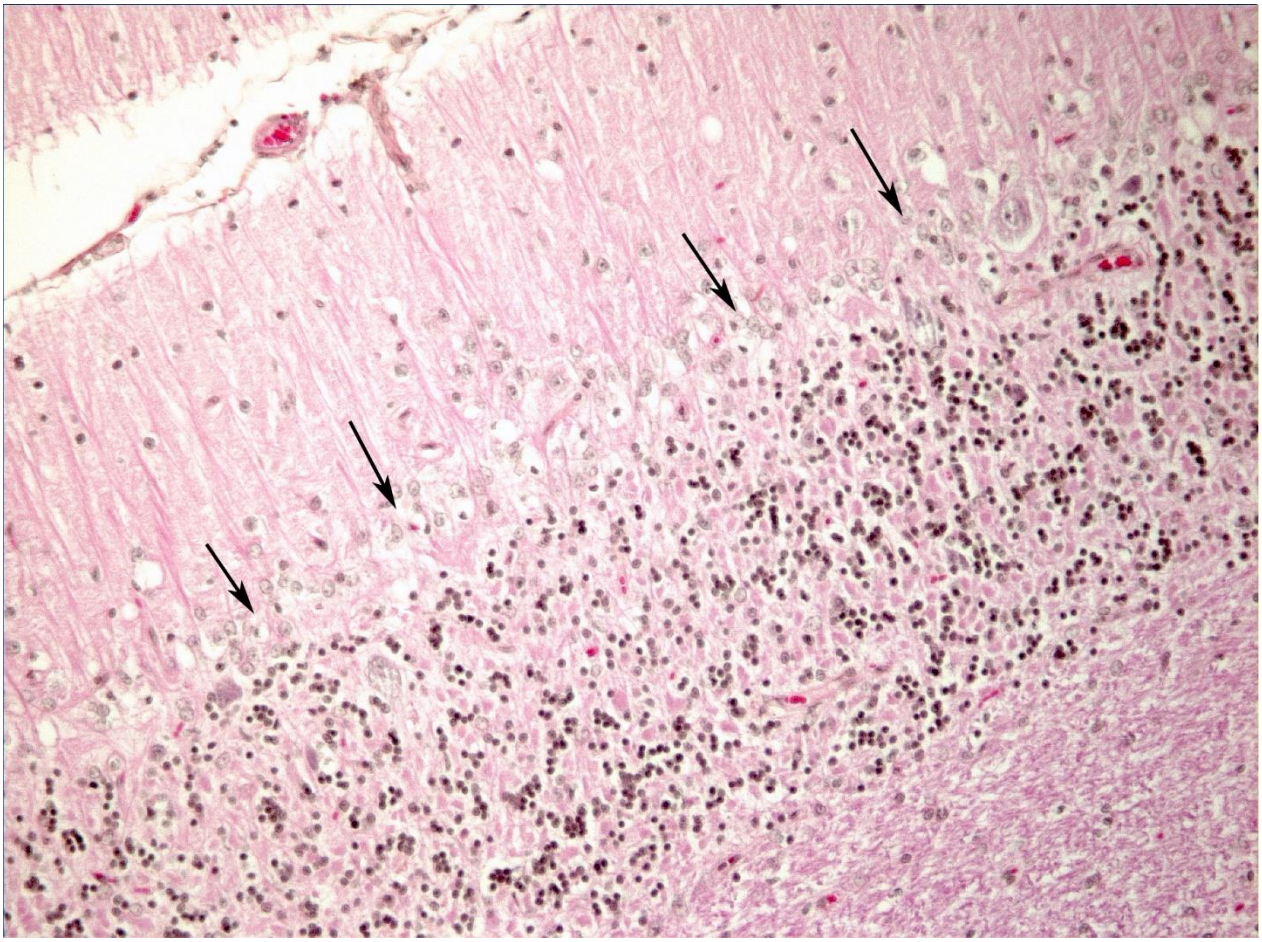


Cerebellum, cat. There is significant segmental loss of Purkinje cells (note three at left and none to the right). Remaining Purkinje cells are swollen and vacuolated. (HE, 163X)

necropsy was performed.

Gross Pathology: The only significant change was observed in the cerebellum which had diffusely widened sulci (more conspicuous when compared with an age-matched control), consistent with atrophy of folia.

Laboratory results: None given.

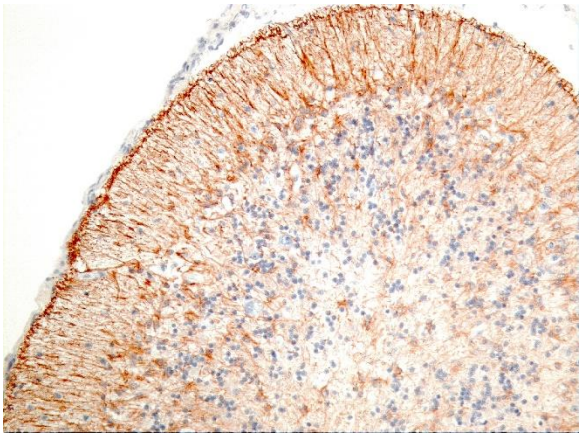


Cerebellum, cat. There is a proliferation of Bergmann's glia at the level previously occupied by Purkinje cells. Their prominent processes run perpendicularly to the overlying pia mater. (HE, 400X) (Photo courtesy of: Université de Montréal, Faculty of veterinary medicine, St-Hyacinthe, Quebec, Canada. http://www.medvet.umontreal.ca)

Microscopic Description: Diffusely in the cerebellar cortex, there is a severe loss of Purkinje cells, leaving multiple "empty baskets"; most of the persistent Purkinje cells have a pale and vacuolar cytoplasm (degeneration). There is an associated proliferation of Bergmann astrocytes (astrogliosis), often with prominent processes extending radially in the molecular layer to the pia mater (isomorphic gliosis); this was confirmed by GFAP immunohistochemistry. The molecular and granular layers are irregularly thin; the granular layer has a variably decreased density (loss of granule cells). Rare torpedoes can sometimes be seen. In some sections, there is Wallerian degeneration (mostly digestion chambers) in the cerebellar white matter; this change was also present bilaterally and symmetrically in the lateral and superficial portions of the medulla (not submitted). In the cerebellar nuclei (present in some sections), there is mild diffuse gliosis with some neuropil vacuolation, but no obvious neuronal

changes. These lesions were present in all portions of the cerebellum.

Spinal cord (not submitted): There is bilateral and symmetrical Wallerian degeneration affecting all portions (cervical, thoracic and lumbar) of the spinal cord, with variation in the affected tracts/fasciculi depending on the level examined. In some dorsal spinal ganglia, a few neurons were chromatolytic.



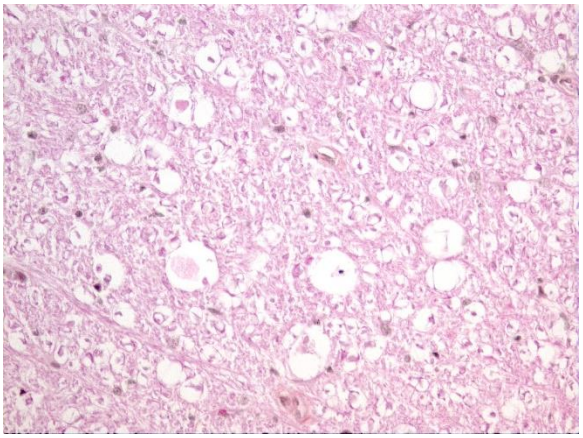
Cerebellum, cat. A GFAP stain demonstrates the dense parallel network of fibers of the Bergmann's glia (i.e., Bergmann's astrocytes) (isomorphic gliosis). (anti GFAP, 100X). (Photo courtesy of: Université de Montréal, Faculty of veterinary medicine, St-Hyacinthe, Quebec, Canada. http://www.medvet.umontreal.ca)

Contributor's Morphologic Diagnoses: Diffuse, severe loss of Purkinje cells (cerebellar abiotrophy)

Bilateral and symmetrical degenerative myeloencephalopathy (spinal cord and medulla; not submitted)

Contributor's Comment: Based on the lesions in the cerebellum, medulla and spinal cord, this case was diagnosed as cerebellar abiotrophy and degenerative myeloencephalopathy, consistent with spinocerebellar ataxia; a genetic component is strongly suspected as one littermate had similar clinical signs. The cerebellar lesion is characteristic of cerebellar abiotrophy or cerebellar cortical abiotrophy. In veterinary medicine, cerebellar abiotrophy is a rare condition described in most domestic animals, non-human primates and a few rodent species.[1-12] Contrary to cerebellar hypoplasia – a condition targeting the cerebellar outer germinal layer and resulting in failure to reach normal organ size (e.g. *in utero* infection with feline panleukopenia parvovirus or BVDV) – cerebellar abiotrophy is characterized by the loss of neuronal populations after full development of the organ. Thus, a hallmark of this condition is normal function at birth, followed by progressive signs of cerebellar disease of variable onset.[10]

Cerebellar abiotrophy is well recognized in dogs, with reports in several different breeds (e.g. English bulldog, American Staffordshire terrier, Border collie),[2,3,5,7,10] and often a suspicion of primary genetic origin. Affected animals usually show progressive neurological signs including cerebellar ataxia, head tremors and symmetrical hypermetria. At necropsy, the cerebellum is usually of normal size but shrinkage of cereberellar folia and broadening of sulci may be present in chronically affected dogs. Microscopic examination reveals an ongoing loss of neurons associated with reactive gliosis. Purkinje cells are severely depleted, leaving clear space ("empty baskets"); Bergmann astrogliosis is a common associated finding. Depletion of the different neuronal populations eventually leads to shrinkage of the molecular and granular layers.[10]



Spinal cord, cat. Mild Wallerian degeneration was present throughout the spinal cord. (HE, 400X). (Photo courtesy of: Université de Montréal, Faculty of veterinary medicine, St-Hyacinthe, Quebec, Canada. http://www.medvet.umontreal.ca)

In cats, cerebellar abiotrophy is uncommon, with only a few cases published[1,6,9,10,11,12]. However, the present feline case is to our knowledge unique as reported feline cases did not describe spinal cord lesions.

Contributing Institution:
Université de Montréal, Faculty of veterinary medicine, St-Hyacinthe, Quebec, Canada. **http://www.medvet.umontreal.ca**

JPC Diagnosis: Cerebellum: Purkinje cell degeneration and loss, diffuse, severe, with marked granular cell loss and Bergmann gliosis.

JPC Comment: Cerebellar cortical degeneration, also referred to as cerebellar abiotrophy, encompasses a group of diseases characterized by degeneration of formed neurons within the cerebellar cortex. As opposed to cerebellar hypoplasia, in which viral infection in utero damages the external granular cell layer (the source of formation of Purkinje cells and neurons that popular the granule cell layer), the cerebellar cortex is grossly and histologically normal at birth, and progressively degenerates over time. While many of the mutations associated with both autosomal dominant and recessive forms of cerebellar cortical degeneration (CCD) have been elucidated in humans, this work is yet in its early stages in veterinary medicine. Mutations in the SEL1L gene have been associated with early onset progressive cerebellar ataxia in Finnish Hounds, and PARK2 gene mutations are common to hereditary striatonigral and cerebello-olivary degeneration in Kerry Blue Terriers and humans.

In the absence of such genetic information, most forms of CCD in veterinary medicine continue to be classified largely based on the locations in which degenerative changes are noted. Most cases of cerebellar cortical degeneration involve Purkinje cells, and the spontaneous depletion of granule cells (neurons populating the adjacent granular cell layer) is often a concurrent, although less obvious finding. Primary degeneration of neurons of the granule cell layer (also referred to as cerebellar granuloprival degeneration) has been reported in a number of canine breeds, including the Coton de Tulear (see WSC 2016-2017, Conference 12, Case 1). A number of syndromes, such as hereditary striatonigral and cerebello-olivary degeneration, as mentioned above, involve neuronal degeneration outside of the cerebellar cortex as well.

While well-documented in a wide variety of dog breeds, far fewer cases of cerebellar abiotrophy have been identified in cats. The disease has been reported both in kittens and in adult cats, with adult-onset “late” onset disease appearing in animals from 1.5 to 9 years. (Biolatti) A syndrome of olivopontocerebellar atrophy in two possibly related cats involved loss of neurons in the cerebellar cortex, olivary complex, and the pontine nuclei in an unusual orderly and progressive fashion. Subtle differences

between the human and the feline disease exist, to include depletion of the granular cell layer in the feline variant and an absence of ubiquitinated intranuclear inclusions within degenerating Purkinje cells. A report of three sibling kittens described severe Purkinje cell loss as well as vacuolation of the neuropil in the cervical spinal cord (as described by the contributor in this case.)

Prominent in this section is the proliferation of Bergmann's glia (often referred to as Bergmann's astrocytes) in the areas of Purkinje cell loss. The cell bodies of Bergmann's glia are found in the Purkinje cell layer, and their processes extend through the molecular layer with endfeet located on the pia mater. In additional to traditional tasks of gray matter protoplasmic astrocytes following injury to Purkinje cells, they have numerous other functions. In the developing cerebellum, they contribute to the layering of the cerebellar cortex - proneurons migrate along their processes from the external germinal layer to populate the granule cell layer. At the late stage of Purkinje cell development, Bergmann's glia form an intricate interwoven network to provide physical and trophic support to the developing Purkinje dendrite tree. Bergmann's glia even assist in information processing in the mature molecular layer – clearing neurotransmitters from synaptic cleft (particularly excitotoxic glutamates), buffering potassium, and even coupling neuronal activity with energy supply and blood flow.

The moderator noted the marked depletion of granular cells as well as that of Purkinje cells, but identified the Purkinje cell loss as the primary lesion, both in terms of importance to the clinical picture as well as the first of the two lesions to occur temporally.

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CASE II: V15-09895-X (JPC 4068932-00).

Signalment: 1 day old, male, Maine Anjou mixed breed, *Bos taurus taurus*, cattle

A plant submitted was identified as Astragalus species by the faculty in New Mexico State University Range Sciences. (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services; www.nmda.nmsu.edu)

History: The owner stated that a large percentage of the spring calf crop was born three to four weeks prematurely. Approximately one half of the calves that lived were lethargic at birth and die. A few of the cows had lost weight and thin despite of eating well.

Gross Pathology: The only significant gross lesion was the thyroid gland was equivocally enlarged.

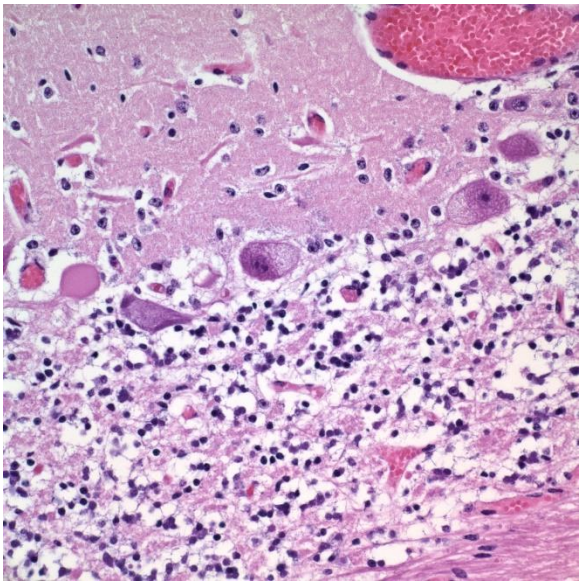
Laboratory results: A plant submitted was identified as *Astragulus* species by faculty in New Mexico State University Range Sciences.

Microscopic Description: The Purkinje cells of the cerebellum are swollen by clear cytoplasmic vacuoles that are most prevalent at axon hillocks (Figure 2). There are rare

necrotic Purkinje cells. There are rare affected neurons in the granule cell layer.

Contributor's Morphologic Diagnoses: Diffuse, severe loss of Purkinje cells (cerebellar abiotrophy)

Contributor's Comment: Locoism in livestock is caused by the chronic ingestion of legumes of the genera *Astragulus* and *Oxytropis*.[7,11] There are over 2000 species of *Astragulus* and *Oxytropis* worldwide with 370 known species of these legumes occurring in North America.[7] Most livestock poisonings caused by these two genera of plants in the United States occur in the western United States.[11] Common names for plants of *Astragulus* and *Oxytropis* genera are locoweeds, milk vetches or vetches. Not all species of *Astragulus* and *Oxytropis* cause locoism. However, these plant species can cause other toxicities as they can contain toxic nitro compounds and can be selenium accumulators.[7,11] Identification of a specific species of locoweed is difficult and is best performed by experienced botanists after the plant has flowered and/or produced seed pods.[11]

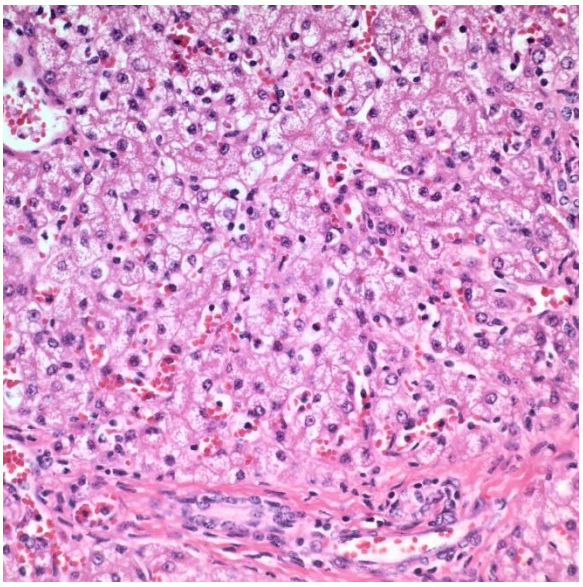


Cerebellum, calf. Purkinje cells are swollen by numerous discrete cytoplasmic vacuoles, most predominantly seen at the axon hillock. (HE, 400X) (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services; www.nmda.nmsu.edu)

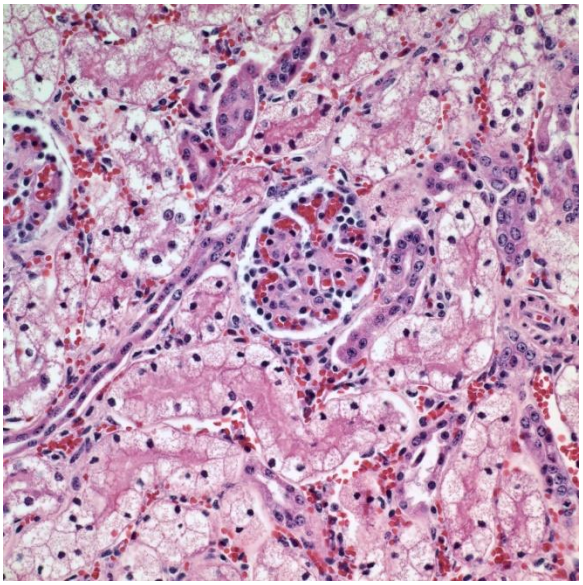
The locoweed population is cyclical with the highest number of locoweeds occurring in wet years such as the fall of 2014 and the spring of 2015 in New Mexico.[11] Contrary to prior belief, locoweeds are palatable and some animals will preferentially graze locoweeds in the spring over dormant warm season grasses due to the nutritional value of locoweeds, which in some species is comparable to alfalfa.[7,11] Animals grazing locoweeds can become habituated to locoweeds, but do not become addicted.[7,11] Toxicity occurs when the level of the locoweed toxin reaches the toxic threshold.

The toxic principle of locoweeds is the indolizidine alkaloid swainsonine.[7,11] Swainsonine can be found in all parts of the plant with higher concentrations in the above ground portion of the plants particularly the flowers and seeds.[5,7] Production of swainsonine by the plant is dependent on infection of the plant by commensal endophyte fungi of the genera *Undifilum*.[3-6] Swainsonine inhibits α-D-mannosidase and Golgi mannosidase II causing cells to accumulate oligosaccharides.[7,11] The result is a systemic lysosomal storage disease with cytoplasmic accumulations of oligosaccharides manifesting microscopically as clear cytoplasmic vacuoles in multiple organs including but not limited to the neurons of the brain mainly in the axon hillock, liver, kidney and thyroid gland.[7,8,11] In fetuses, the accumulation of oligosaccharides can also occur in trophoblasts of the placenta.[8] If the cellular damage is not severe, then preventing livestock from ingesting locoweeds can result in recovery of the animal with cellular changes remaining the longest in the liver and neurons.[7] The cytoplasmic vacuoles can persist for one year in the Purkinje cells of the cerebellum.[7]

That being said, it is recommended to not ever return horses with locoism to work such as riding due to the potential dangers associated with residual neurological effects.[7]



Liver, calf. Hepatocytes are diffusely expanded by similar clear cytoplasmic vacuoles, obscuring normal sinusoidal architecture. (HE, 400X) (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services; www.nmda.nmsu.edu)



Kidney, calf. Proximal convoluted tubular epithelium is markedly swollen due to numerous clear cytoplasmic vacuoles. (HE, 400X) (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services; www.nmda.nmsu.edu)

The most widely known manifestation of locoweed toxicity in livestock is neurological disease (locoism). However, locoism in livestock can manifest as chronic weight loss/ill thrift, reproductive losses and worsening the cardiac disease associated with high-mountain disease in cattle.[7,11] The reproductive losses can be devastating to a producer with losses of close to fifty percent of offspring occurring in some cases. Chronic ingestion of locoweeds has been known to prolong the estrus cycle, result in the failure to conceive and cause early embryonic death.[2,7-9] Some pregnant cows with locoism develop hydrops.[7] Abortion may occur at all stages of gestation with cytoplasmic vacuoles present in fetal tissues such as in this case.[2,7,8] In addition, neonates may be born small and weak. Congenital abnormalities such as limb and head deformities can occur in affected fetuses.[2,7,8,9] Testicular atrophy and decreased spermatogenesis can occur in adult males with locoism.[7]

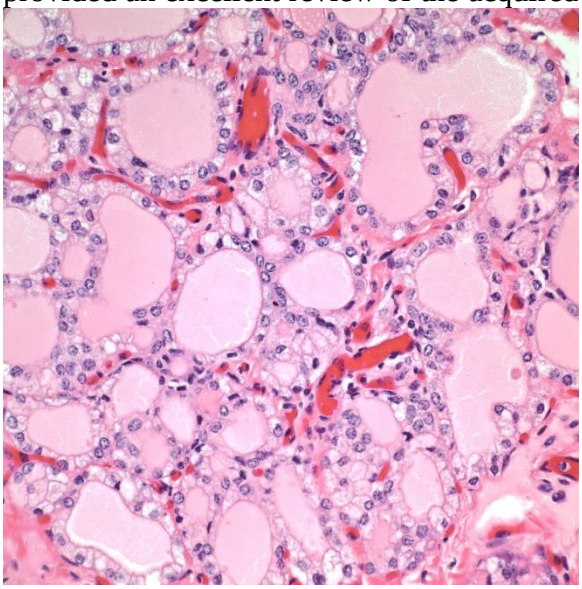
The moderator pointed out that the cytoplasmic vacuolation is especially prominent in the area of the axon hillock of Purkinje cells in this particular case, a feature often described in this particular entity. Some participant noted the relative paucity of nuclei within the granular layer; the cause of this finding was ascribed by some to the young age of the animal (one day, and with a prominent external granular cell layer, and likely continued maturation of this region), and some preferred to ascribe it to poor tissue preservation.

Contributing Institution:
New Mexico Department of Agriculture Veterinary Diagnostic Services

www.nmda.nmsu.edu

JPC Diagnosis: Cerebellum, Purkinje and granular cells: Cytoplasmic vacuolation, diffuse, marked.

JPC Comment: The contributor has provided an excellent review of the acquired lysosomal storage disease known as locoism in cattle.



Thyroid, calf. Follicular epithelium is swollen from numerous clear cytoplasmic vacuoles. (HE, 400X) (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services; www.nmda.nmsu.edu)

Lysosomal storage diseases are a group of inherited an acquired disorders. Over 50 genetically-determined lysosomal storage diseases have been identified in veterinary medicine.[1] The majority of these diseases result from mutations of genes coding specific lysosomal hydrolases which contribute to the activation of lysosomal enzymes. Moreover, infantile and adult types of inherited diseases may result from different mutations of the same gene. Infantile disease results in early death due to a total absence of enzymatic activity, while adult disease may have late presentation and mild-to-moderate manifestations as partial enzyme activity is present. Rare cases of congenital disease may results in the absence of hydroxylase activator proteins, recognition markers (which results in lysosomal enzymes being aberrantly secreted into the extracellular milieu), or protector proteins which results in rapid degradation of hydrolytic enzymes.[1] A list of the more common inherited lysosomal storage disease is present Table 1. A more complete review is available in the excellent review by Alroy and Lyons.[1]

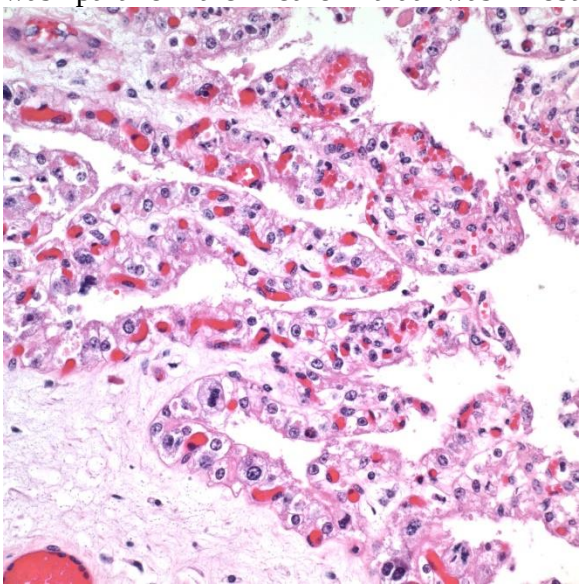
Acquired lysosomal storage disease share a common mechanism in the inhibition of alpha-manosidase II by ingestion of a number of plants from the genera *Astragalus*, *Swainsona*, *Oxytropis*, and *Ipomoae* as well as a number of amphophilic cationic drugs, including amiodarone and chloroquine. [1]

As a general rule, the accumulation of undigested substrate in cells occurs when residual activity of deficient enzymes is less than 10 to 15% of normal levels. There is wide variation in the cell types and organs affected by different types of lysosomal storage diseases; neuronal vacuolation, (as seen in this case), is seen in approximately two thirds of all lysosomal storage diseases.[1]

Histochemical stains have traditionally been involved in the differentiation of many types of lysosomal storage diseases. Periodic acid-Schiff stains (with and without diastase digestion), Alcian blue, and colloidal iron (with and without hyaluronidase treatment) are useful for identifying oligosaccharide and glycolipids. Sudan Black, oil red O, and Luxol fast blue stains are helpful in the characterization of abnormally stored lipids. Normal ceroid lipofuscinosis is diagnosed by autofluorescence and rarely requires ultrastructure. Definitive identification of enzyme deficiencies is most commonly

performed on white blood cells or cultured fibroblasts by measuring activity of specific enzymes.[1]

The moderator noted that the axon hillock was part of the neuron that was most



Placenta, ox. Trophoblasts contain numerous intracytoplasmic vacuoles. (HE, 400X) (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services; www.nmda.nmsu.edu)

Table 1: Select inherited lysosomal storage diseases[8]

Condition	Enzyme Defect	Storage Material	Inheritance/species
GM1 gangliosidosis	β-galactosidase	GM_1 ganglioside in lysosomes of neurons, glial cells, macrophages	- autosomal recessive - dogs, cats, Friesian cattle; - suffolk sheep - deficiencies in β1-galactosidase AND α-neuraminidase.
GM_2 gangliosidosis (Tay-Sachs and Sandhoff diseases)	-hexosaminidase (αβ- or ββ-dimer) -activator protein	GM_2 ganglioside in lysosomes of neurons, glial cells, macrophages	- autosomal recessive 1. domestic and Korat cats, German shorthaired pointers, golden retrievers: β-subunit deficiency 2. Japanese spaniel dogs, Yorkshire pigs: activator protein deficiency
Sphingomyelinosis (Niemann-Pick disease)	sphingomyelinase	sphingomyelin in lysosomes of neurons and macrophages	- autosomal recessive in cat and dog
Globoid cell leukodystrophy (glactocerebrosidosis)	galactocerebrosidase	galactocerebrosides in oligodendrocytes/Schwann cells, globoid cell macrophages (NOT in neurons) demyelination, axonal loss	- autosomal recessive - dogs, cats and polled Dorset sheep
Glucocerebrosidosis (Gaucher disease)	glucocerebrosidase	glucocerebroside in lysosomes of hepatic/lymph node sinusoidal macrophages, some neurons (NOT in Purkinje cells or the spinal cord)	- Sydney Silky Terriers
α-Mannosidosis	α-mannosidase	mannose/N-acetylglucosamine oligosaccharide in lysosomes of neurons, macrophages, secretory epithelial cells	- Angus cattle
β-Mannosidosis	β-mannosidase	oligosaccharides in lysosomes of neurons, macrophages, secretory epithelial cells	- Salers cattle and Nubian goats
α-L-fucosidosis	α-L-fucosidase	- fucose containing glycoconjugates in lysosomes of neurons - similar appearance to α/β-Mannosidosis	- autosomal recessive - English springer spaniels
MPS I	α-L-iduronidase	mucopolysaccharide storage in mesoderm derived cells	- domestic shorthair cats and Plott hounds
MPS III	N-acetylglucosamine-6-sulfatase	heparan sulfate in mesoderm-derived cells; neurons contain gangliosides	- Nubian goats
MPS VI	arylsulfatase-B	mucopolysaccharide storage in mesoderm derived cells; neuronal storage does not occur	- Siamese and domestic shorthair cats
MPS VII	β-glucuronidase	widespread neurovisceral storage	- dogs and cats
Glycogenosis (type II in humans)	α-1,4-glucosidase	widespread glycogen storage within lysosomes and intracytoplasmically; including neurons	- autosomal recessive in shorthorn and Brahman beef cattle

obviously vacuolated and this was very apparent in this particular specimen. The participants also discussed the possibility of decreased numbers of nuclei within the granular cell layer in this individual and its potential causes, to include the possibility of incomplete maturation in a 1 day old calf (with a very visible external granular cell layer in this case) as well as the possibility of autolysis in the particular specimen.

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