Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #17 — 24 January 2024

CASE I:

Signalment:
1-year-old, male neutered mixed-breed dog (*Canis familiaris*)

History:
This dog presented for severe cervical pain progressing to C6-T1 myelopathy with paraplegia. An MRI of the cervical region and a CSF analysis were within normal limits. The patient was not responsive to antibiotics, prednisone, or cytarabine treatment. The owner elected euthanasia, and the body was submitted for necropsy.

Gross Pathology:
The gross examination was unremarkable except for a 12 x 6 x 6 cm, firm, tan, multilobulated mass in the posterior mediastinum adjacent to the bodies of vertebrae T5-T7. The mass extended through the intervertebral foramen into the vertebral canal, compressed the spinal cord at the level of T6, and infiltrated the adjacent epaxial skeletal muscle.

Microscopic Description:
The mass is composed of lobules of round to polygonal neoplastic cells separated by thick bands of connective tissue. The cells show considerable variation in size, ranging from 15µm to over 100µm in diameter. The small cells are round with scant eosinophilic cytoplasm and round nuclei with coarsely clumped

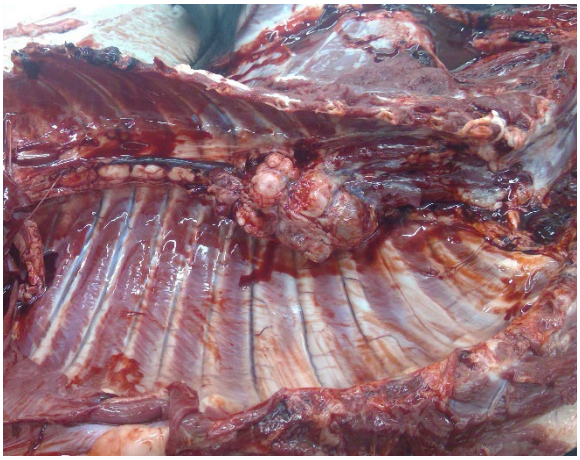

Figure 1-1. Posterior mediastinum, dog. There is a 12 x 6 x 6 cm, firm, tan, multilobulated mass in the posterior mediastinum adjacent to the bodies of vertebrae T5-T7. (*Photo courtesy of:* Virginia Tech College of Veterinary Medicine, https://vetmed.vt.edu/)

chromatin and indistinct nucleoli (neuroblasts). The larger cells have abundant granular cytoplasm which is eosinophilic around the nucleus and basophilic at the periphery. Nuclei in these cells are round and usually eccentric with vesicular chromatin and single, large, central nucleoli (ganglion cells). The larger cells are often dissected by streams of spindle cells and wavy fibrillar to vacuolated eosinophilic material (Schwannian stroma). Anisocytosis and anisokaryosis are marked and the mitotic rate is higher in the smaller cells (15 in 2.37 mm^2) than in the larger cells (2 in 2.37 mm^2). In approximately 25% of the mass, the

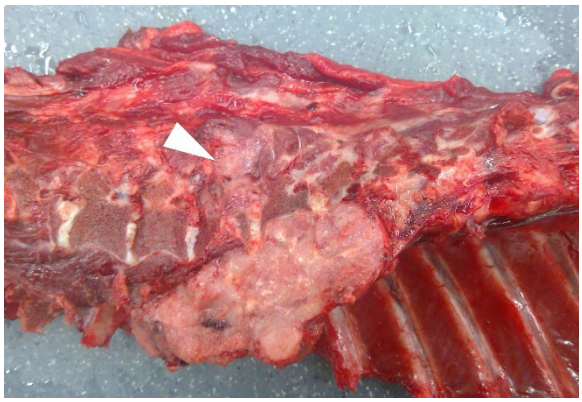

Figure 1-2. Posterior mediastinum, dog. The mass extends through the intervertebral foramen into the vertebral canal, compresses the spinal cord at the level of T6, and infiltrates the adjacent epaxial skeletal muscle. (*Photo courtesy of:* Virginia Tech College of Veterinary Medicine)

cells are hypereosinophilic with loss of cellular detail (coagulation necrosis), and a focal area is replaced by granular basophilic material (mineralization). At the periphery of the mass, the neoplastic cells infiltrate the adjacent adipose tissue.

Contributor's Morphologic Diagnosis:
Posterior mediastinal mass: Ganglioneuroblastoma.

Contributor's Comment:
Peripheral neuroblastic tumors arise from immature cells in the ganglia of the cranial and spinal nerves and the sympathetic nervous system. Locations include the adrenal gland, neck, mediastinum, retroperitoneal space, and pelvis. These tumors are classified based on the degree of differentiation: neuroblastomas are composed of small, undifferentiated cells (neuroblasts); ganglioneuroblastomas also contain neuroblasts, along with maturing and mature ganglion cells, nerve fibers, Schwann cells and stroma (Schwannian stroma); and ganglioneuromas, the most differentiated of the three, are composed of mature ganglion cells, nerve fibers, Schwann cells, and Schwannian stroma. Ganglioneuroblastomas are further classified, based on the amount of Schwannian stroma, into intermixed (Schwannian stroma-rich) and nodular (Schwannian-stroma rich and Schwannian stroma-poor areas) subtypes. The presence of neuroblasts, gangliocytes, nerve fibers and Schwannian stroma in this case is consistent with a ganglioneuroblastoma.[20] Based on the human classification system, this tumor could be further characterized as a nodular subtype.

Neuroblastomas, ganglioneuroblastomas, and ganglioneuromas can also arise from neuronal progenitors in the central nervous system (central neuroblastic tumors) and the olfactory epithelium (olfactory neuroblastic tumors). Olfactory neuroblastomas are also known as esthesioneuroblastomas. Ganglioneuromatosis is a condition characterized by nodular proliferation of neural tissue in the intestine with a segmental or diffuse distribution.

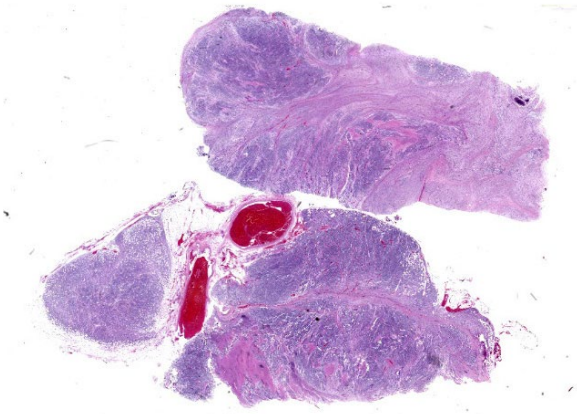

Figure 1-3. Posterior mediastinum, dog. Two sections of a multilobulated mass infiltrating the mediastinal fat are submitted for examination. (HE, 5X)

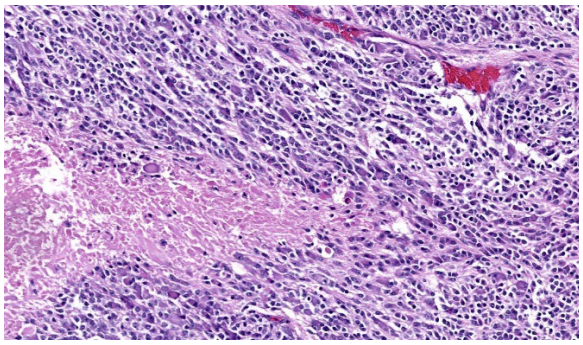

Figure 1-4. Posterior mediastinum, dog. The neoplasm is composed of two cell types arranged in poorly defined streams and areas of coagulative necrosis. (HE, 230X)

Ganglioneuromatosis is classified as a hamartomatous rather than neoplastic process and is associated with genetic syndromes, such as phosphatase and tensin homolog hamartoma (PTEN) syndrome, neurofibromatosis type 1 or 2, and multiple endocrine neoplasia type IIb.[9] By immunohistochemistry, neuroblasts are positive for synaptophysin, neuron specific enolase (NSE), and chromogranin A, although immunoreactivity in this population may be weak or variable; ganglion cells and neural processes are positive for neurofilament and NSE; and Schwann cells are positive for S100, NSE, and GFAP.[3,7,10] In humans, neuroblastic tumors comprise 80% of neoplasms in children under 5 years old and are rare after the age of 10.[1] Prognosis depends on tumor type, and cellular features (degree of differentiation, mitotic rate, karyorrhexis).[19]

In general, ganglioneuromas develop in older children and adults and are considered benign with a favorable prognosis. The prognosis for neuroblastomas depends on the age of the patient, the degree of differentiation of the neuroblasts, and the mitosis-karyorrhexis index. Intermixed ganglioneuroblastomas are associated with a favorable prognosis while the prognosis of the nodular subtype depends of the degree of differentiation of the neuroblastic component.

Peripheral neuroblastomas have been documented in dogs[3] and cattle[11,22] and peripheral ganglioneuromas are described in dogs,[5] horses,[2] cats,[8] and pigs.[7] Ganglioneuromatosis has also been reported in a horse,[16] dogs,[12] and cattle.[4] Previous reports of veterinary ganglioneuroblastomas are listed in Table 1.

Contributing Institution:
Virginia-Maryland
College of Veterinary Medicine
205 Duck Pond Dr.
Blacksburg VA 24061
https://vetmed.vt.edu/

JPC Diagnosis:
Fibroadipose tissue: Ganglioneuroblastoma.

JPC Comment:
As the contributor notes, peripheral neuroblastic tumors arise from sympathetic nervous system progenitor cells. These progenitor cells migrate from the neural crest to form, among other tissues, sympathetic ganglia and the chromaffin cells of the adrenal medulla.[3]

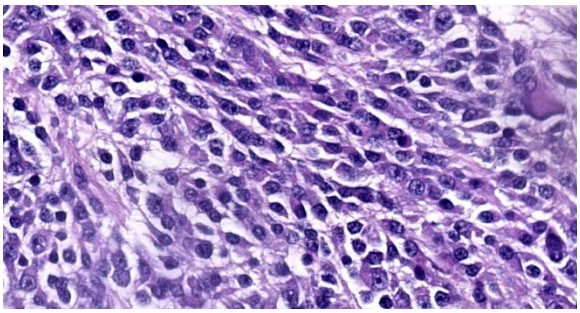

Figure 1-5. Posterior mediastinum, dog. The primary cell type is a small polygonal cell (neuroblast). (HE, 808X)

Species (breed)	Age/Sex	Location	Clinical Outcome
Dog (Bichon-frise)[10]	13 years/MN	Oral cavity	Died of heart failure 2 months after surgery
Dog (Lab)[18]	18 months/M	Cranial mediastinum	Euthanized 1 week after diagnosis
Dog (German Shepherd)[17]	8 years/M	Footpad	Amputation with no recurrence for over 1 year
Cat (mixed)[15]	11 month/MN	Facial nerve	Died 30 days after onset of neurologic signs
Cat (DSH)[21]	8 years/MN	Footpad	Treated with electrochemotherapy; remission for more than 1 year
Sheep (Akkaraman)[23]	18 months/not specified	Retroperitoneum	Diagnosed at slaughter
Calf (Japanese Black)[13]	Newborn/M	Cervical	Euthanized

Table 1. Reported peripheral ganglioneuroblastomas in veterinary species.

The factors that lead to the persistence and subsequent neoplastic transformation of these progenitor cells are incompletely understood in both human and veterinary medicine.

In humans, most neuroblastic tumors carry an excellent prognosis if detected early in life; however, an unpredictable subset of human neuroblastic tumors carries increased risk and a poorer prognosis. The most well-characterized marker of these high-risk tumors is increased expression of MYCN, a transcription factor in the MYC family, which occurs in approximately 25% of pediatric neuroblastomas.[6] Rodent studies have determined that MYCN, like MYC, can induce cellular proliferation and cell cycle progression in quiescent cells; however, there is a spatiotemporal difference between the two transcription factors. MYC is expressed in a broad spectrum of tissues and gradually subsides over time in adult mice.[6] By contrast, MYCN is found only during early developmental stages and is expressed most substantially in the forebrain, hindbrain, and kidneys of newborn mice.[6]

Given its role in early development, it is perhaps unsurprising that the degree of differentiation in neuroblastoma cells is negatively correlated with MYCN expression, with more differentiated tumors characterized by lower levels of MYCN than less differentiated tumors.[6] MYCN is also involved in maintaining the multipotency and capacity for self-renewal characteristic of the neural crest cells from which neuroblastic tumors arise, and mouse models have induced neuroblastomas by targeted overexpression of MYCN in migrating neural crest cells.[6]

MYCN's contribution to the stem cell-like state of neural crest and neoplastic cells is multifactorial. MYCN increases expression of proliferation-permissive proteins such as CDK1 and CDK4, promotes angiogenesis by stimulating VEGF production, and facilitates cell survival through the p53-blocking protein MDM2.[6] MYCN also inhibits the production of cell cycle arrest proteins and transcription factors that promote differentiation, down regulates immune surveillance by MCP-1, and creates a pro-metastasis environment through,

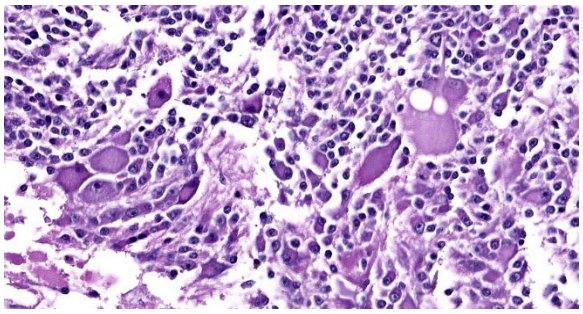

Figure 1-6. Posterior mediastinum, dog. The second cell type strongly resembles a ganglion cell. (HE, 483X)

among other actions, blocking production of E-cadherin and certain integrins.[6]

In dogs and humans, clinical signs vary widely depending on tumor localization and extent, and on the presence or absence of any paraneoplastic syndromes.[3,14] In humans, 65% of peripheral neuroblastic tumors arise in the abdomen, with half of these originating in the adrenal medulla.[14] With local or regional disease, patients may be asymptomatic, while more extensive disease may cause abdominal distention and pain, Horner's syndrome (for cervical tumors), and episodic secretory diarrhea due to tumor production of vasoactive intestinal peptides.[3,14]

Due to the paucity of published case reports, the prognoses and biologic behaviors of canine peripheral neuroblastic tumors are hard to predict. In a recent case study of canine neuroblastomas, the majority of the dogs were either euthanized at the time of diagnosis or lost to follow up; of those submitted for necropsy, metastatic disease was discovered in 4/9 dogs, most commonly in the liver.[3]

This week's moderator, Dr. Andrew Miller, Associate Professor and Section Chief of the Department of Population Medicine and Diagnostic Sciences at the Cornell University College of Veterinary Medicine, began discussion by reviewing the characteristics of various embryonal tumors. This heterogenous group of tumors includes central and peripheral neuroblastoma, ganglioneuroblastoma, medulloblastoma, and primitive neuroectodermal tumors, all of which can occur as paraspinal masses that compress the spinal cord. As a definitional matter, Dr. Miller noted that the term "primitive neuroectodermal tumor," or PNET, is no longer used in human neuropathology due to the World Health Organization's reclassification of nervous system tumors based on genetic and molecular markers. In veterinary medicine, the umbrella term "PNET" persists as the genetic mutations underlying nervous system tumors and the molecular methods used to diagnose them are less fully developed.

Ganglioneuroblastomas are generally rare in veterinary medicine, and diagnosis requires clear, obvious ganglion cell differentiation, neuroblasts, and some amount of Schwann cells and Schwannian stroma. Dr. Miller noted these elements in the examined slide and called attention to the large areas of confluent necrosis, an indication that the neoplasm was aggressive and rapidly growing.

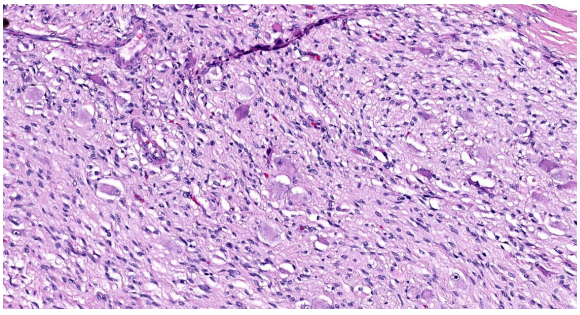

Figure 1-7. Posterior mediastinum, dog. Neoplastic cells are embedded in stroma that strongly resembles a peripheral nerve ("Schwannian stroma"). (HE, 250X)

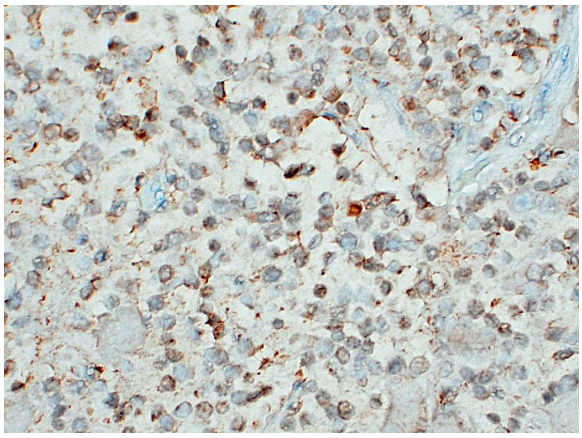

Figure 1-8. Posterior mediastinum, dog. Neuroblasts demonstrate strong nuclear immunopositivity for synaptophysin. (Anti-synaptophysin, 250X)

Discussion of the morphologic diagnosis was blissfully straightforward, with only momentary debate about tissue localization. Conference participants were not able to detect the tissue's mediastinal origin from the H&E section alone and thus chose to omit any reference to a specific anatomic location.

References:

1. Alessi S, Grignani M, Carone L. Ganglioneuroblastoma: Case report and review of the literature. *J Ultrasound*. 2011; 14(2):84-88.
2. Allen D, Swayne D, Belknap JK. Ganglioneuroma as a cause of small intestinal obstruction in the horse: a case report. *Cornell Vet*. 1989;79(2):133-141.
3. Arenas-Gamboa AM, Tanabe M, Edwards J, Storts R. Peripheral neuroblastomas in dogs: a case series. *J Comp Pathol*. 2014; 150(4):361-365.
4. Cole DE, Migaki G, Leipold HW. Colonic Ganglioneuromatosis in a Steer. *Vet Pathol*. 1990;27(6):461-462.
5. Goto M, Yonemaru K, Hirata A, Furuhashi H, Yanai T, Sakai H. Lingual ganglioneuroma in a dog. *J Vet Med Sci*. 2018; 80(3):488-491.
6. Huang M, Weiss WA. Neuroblastoma and MYCN. *Cold Spring Harbor Perspect Med*. 2013;3(10):a014415.
7. Inoue R, et al. Cardiac ganglioneuroma in a juvenile pig. *J Vet Med Sci*. 2016;78(1): 117-119.
8. Kobayashi R, Ohsaki Y, Yasuno K, et al. A malignant and metastasizing feline cardiac ganglioneuroma. *J Vet Diagn Invest*. 2012;24(2):412-417.
9. Koullouros M, Candler S, Smith C, Olakkengil S. Appendicitis and ganglioneuroma-an unusual co-existence. *J Surg Case Rep*. 2022;2022(1):rjab632.
10. Nakamura K, Ochiai K, Kadosawa T, Kimura T, Umemura T. Canine ganglioneuroblastoma in the oral mucosa. *J Comp Pathol*. 2004;130(2):205-208.
11. Omi K, Kitano Y, Agawa H, Kadota K. An immunohistochemical study of peripheral neuroblastoma, ganglioneuroblastoma, anaplastic ganglioglioma, schwannoma and neurofibroma in cattle. *J Comp Pathol*. 1994;111(1):1-14.
12. Paris JK, McCandlish IAP, Schwarz T, Simpson JW, Smith SH. Small intestinal ganglioneuromatosis in a dog. *J Comp Pathol*. 2013;148(4):323–328.
13. Park CH, Shiwa N, Kimitsuki K, Kakizaki T, Watanabe D. Cervical ganglioneuroblastoma in a new born Japanese Black calf. *J Vet Med Sci*. 2018;80(5):755-759.
14. Park JR, Eggert A, Caron H. Neuroblastoma: biology, prognosis, and treatment. *Pediatr Clin North Am*. 2008;55(1):97-120.
15. Pereira PR, Tagliari NJ, Leite-Filho RV, Schaefer G da C, Costa FVA da, Pavarini SP. Facial nerve ganglioneuroblastoma in a feline leukemia virus-positive cat. *Ciênc Rural*. 2017;47(5)1-5.

16. Porter BF, Storts RW, Payne HR, Edwards JF. Colonic ganglioneuromatosis in a horse. *Vet Pathol*. 2007;44(2):207–210.
17. Salvadori C, Cantile C, Massari F, Chiti L, Colombo S, Abramo F. Footpad peripheral ganglioneuroblastoma in a dog. *Vet Dermatol*. 2019;30(4):346-e100.
18. Schulz KS, Steele KE, Saunders GK, Smith MM, Moon ML. Thoracic ganglioneuroblastoma in a dog. *Vet Pathol*. 1994;31(6):716–718.
19. Shimada H, Chatten J, Newton WA, et al. Histopathologic prognostic factors in neuroblastic tumors: definition of subtypes of ganglioneuroblastoma and an age-linked classification of neuroblastomas. *J Natl Cancer Inst*. 1984;73(2):405-416.
20. Shimada H, Umehara S, Monobe Y, et al. International neuroblastoma pathology classification for prognostic evaluation of patients with peripheral neuroblastic tumors. *Cancer*. 2001;92(9):2451-2461.
21. Spugnini EP, Citro G, Dotsinsky I, Mudrov N, Mellone P, Baldi A. Ganglioneuroblastoma in a cat: a rare neoplasm treated with electrochemotherapy. *Vet J Lond Engl 1997*. 2008;178(2):291-293.
22. Uchida K, Murakami T, Tometsuka T, Iwakiri A, Yamaguchi R, Tateyama S. Peripheral neuroblastoma and primitive neuroectodermal tumor in Japanese black cattle. *J Vet Med Sci*. 1998;60(7):871–875.
23. Yener Z, Kiran MM. Undifferentiated Ganglioneuroblastoma in a Sheep. *J Comp Pathol*. 2002;126(2):216-219.

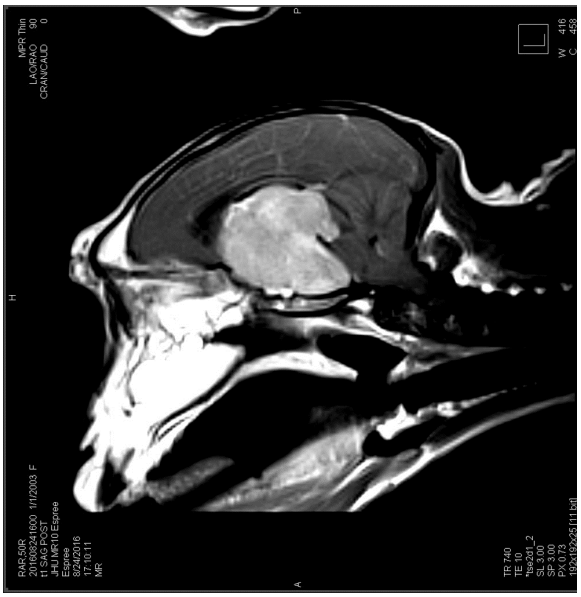

Figure 2-1. Pituitary gland, rhesus macaque. MRI examination revealed a large (3.2 x 2.9 x 2.5 cm) contrast-enhancing hypophyseal mass protruding from the pituitary fossa with associated marked compression of the adjacent brain parenchyma. (*Photo courtesy of:* Johns Hopkins University School of Medicine, Department of Molecular and Comparative Pathobiology, http://www.hopkinsmedicine.org/mcp)

CASE II:

Signalment:

13-year old, intact female Indian origin Rhesus macaque (*Macaca mulatta*)

History:

This female Rhesus macaque presented with progressive weight loss, vision loss, and wide circling to the right over the course of one year. She also exhibited galactorrhea for approximately 4 years, despite not having given birth in over two years. Ophthalmologic exam revealed inability to fix or follow an object, searching behavior, and subjective bilateral optic nerve pallor on fundic exam. Subsequent MRI revealed a large (3.2 x 2.9 x 2.5 cm) contrast-enhancing hypophyseal mass protruding

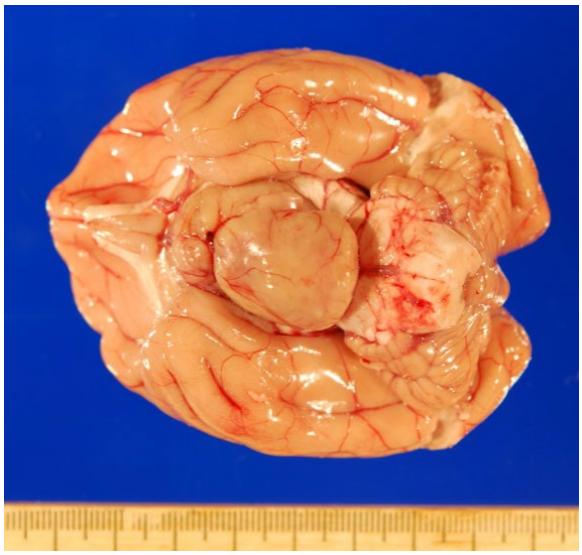

Figure 2-2. Pituitary gland, rhesus macaque. There is a 2.4 x 2.0 x 1.4 cm, well-circumscribed, tan, firm mass attached to the mid-ventral aspect of the brain at the level of the pituitary gland dorsal to the sella turcica. (*Photo courtesy of:* Johns Hopkins University School of Medicine, Department of Molecular and Comparative Pathobiology)

from the pituitary fossa with associated marked compression of the adjacent brain parechyma. Due to poor prognosis, humane euthanasia was elected.

Gross Pathology:
Gross necropsy revealed a 2.4 x 2.0 x 1.4 cm, well-circumscribed, tan, firm mass attached to the mid-ventral aspect of the brain at the level of the pituitary gland dorsal to the sella turcica. The mass compressed adjacent neural tissue and the optic nerve at the level of the optic chiasm. On cut section, the mass extended as far rostrally as the basal ganglia and as far caudally as the pons. The mass also asymmetrically compressed the lateral ventricles, causing mild to moderate hydrocephalus.

Laboratory Results:
Significant clinical pathology results are listed below:

- Complete blood count (CBC): Stress leukogram
- Chemistry panel: Mild hypokalemia
- Thiamine: WNL
- Estrogen (E2): WNL (<5 pg/ml)
- Progesterone (P4): WNL (0.10 ng/ml)
- Prolactin: Elevated (147.6 ng/ml - expected value 20-50 ng/ml)

Immunohistochemistry:

- Synaptophysin (+)
- ACTH (-)
- FSH (-)
- Growth hormone (-)
- MSH (-)
- Prolactin (-)

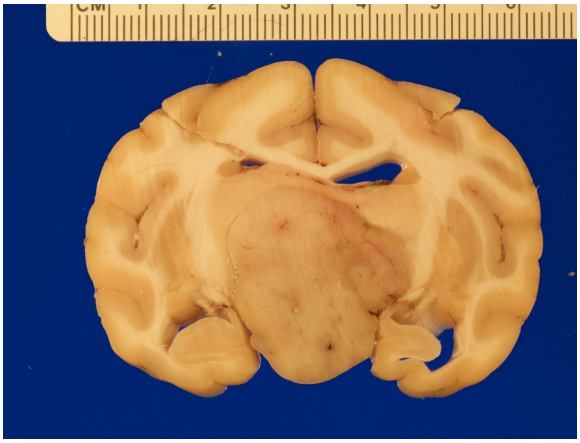

Figure 2-3. Pituitary gland, rhesus macaque: The mass extended as far rostrally as the basal ganglia and as far caudally as the pons. The mass also asymmetrically compressed the lateral ventricles. (*Photo courtesy of:* Johns Hopkins University School of Medicine, Department of Molecular and Comparative Pathobiology)

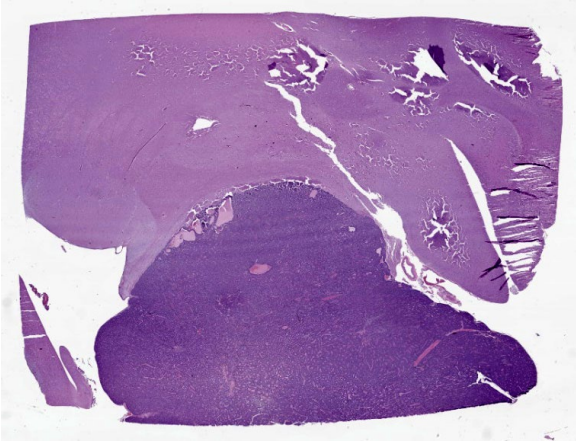

Figure 2-4. Pituitary gland, rhesus macaque. A pituitary neoplasm effaces the normal glandular architecture and compresses the overlying hypothalamic neuropil. (HE, 5X)

Microscopic Description:
Cerebrum: Expanding and compressing surrounding neuroparenchyma is a densely cellular, well-demarcated, nodular, unencapsulated neoplasm. Neoplastic cells are closely packed and are arranged in nests/packets supported on a fine fibrovascular stroma. Neoplastic cells frequently palisade around blood vessels (pseudorosettes) or a central area of fiber-rich neuropil (Homer-Wright rosettes). Cells are columnar to polygonal with indistinct cell borders and a scant to moderate amount of eosinophilic granular cytoplasm. Nuclei are round and centrally located with coarsely stippled chromatin and 1-2 variably prominent nucleoli. Anisocytosis and anisokaryosis are mild. Mitoses are rare (avg <1 per 10 high powered fields). There are multifocal areas of necrosis within the center of the neoplasm consisting of pyknotic and karyorrhectic cellular debris admixed with concretions of mineral, fibrosis, and rare cholesterol clefts. There is no evidence of vascular or lymphatic invasion. There is diffuse compression and variable rarefaction of the adjacent neuropil. Within the normal adjacent section of brain, there are numerous mineralized blood vessels.

Transmission electron microscopy: Neoplastic cells have indistinct borders. Within the cytoplasm are multiple, approximately 100 nm, electron dense, spherical granules. The Golgi apparatus and endoplasmic reticulum are indistinct and often poorly formed.

Contributor's Morphologic Diagnosis:
Cerebrum: Pituitary null-cell adenoma.

Contributor's Comment:
This aged female rhesus macaque presented with progressive weight loss, hyperprolactinemia and galactorrhea without recent pregnancy, and neurologic signs including vision loss and circling. The most significant lesion on necropsy was a large, compressive pituitary adenoma, which is believed to be the main contributing factor for all clinical findings. Immunohistochemistry of the tumor is diffusely negative for hormone production and electron microscopy revealed multiple small, approximately 100 nm granules. Combined, these results highly suggest that this tumor is an endocrinologically inactive (aka, non-functional or null) adenoma.

In veterinary species, non-functional pituitary adenomas occur most commonly in dogs, cats, and parakeets, with the most common type being chromophobe adenomas arising from the pars distalis.[12,13] Reports of pituitary adenomas in non-human primates are scattered, but have been noted in both Old World and New World primates.[4,5,11] The majority of these tumors are described as lactotroph or corticotroph adenomas.[5,11] However, there is one case report of a suspected non-functional adenoma with galactorrhea in a male Rhesus macaque, which describes a similar entity to the present case, both clinically and histologically.[4]

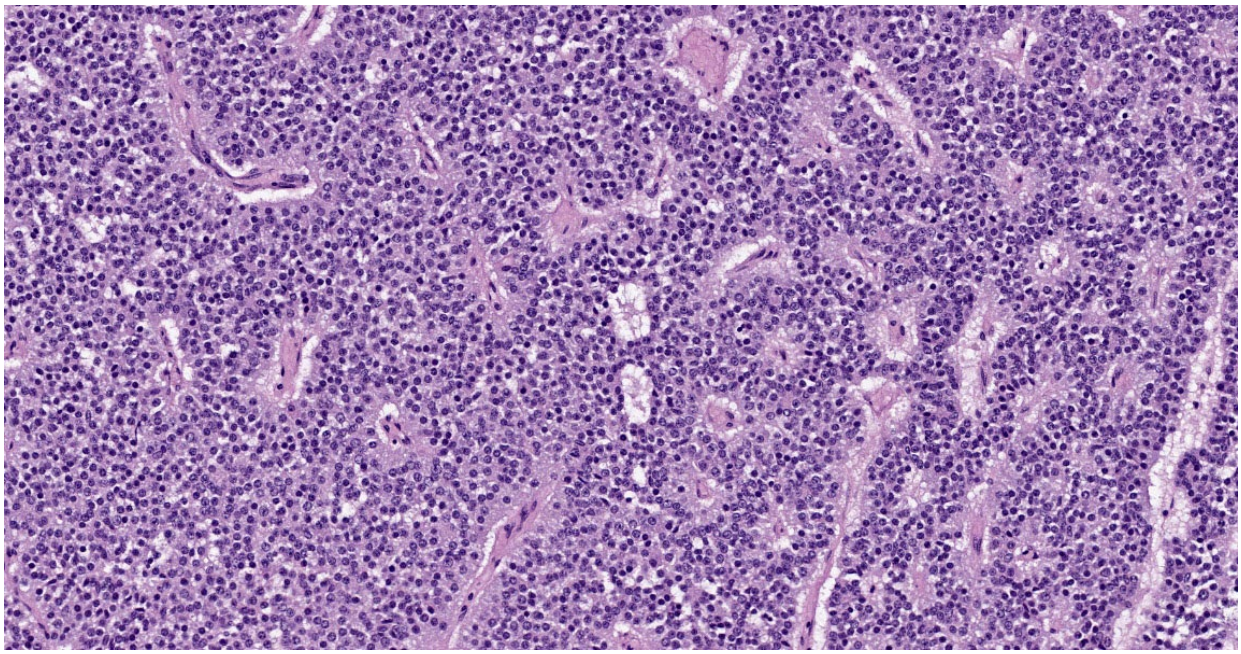

Figure 2-5. Pituitary gland, rhesus macaque. Neoplastic cells are arranged in nests and packets with frequent pseudorosette and rosette formation. (HE, 199X)

As these tumors are often behaviorally benign, clinical signs of nonfunctional adenomas are typically due to expansion of the tumor into the unaffected pituitary and adjacent CNS, leading to decreased secretion of pituitary hormones and/or CNS dysfunction.[12,13] For instance, progressive weight loss and muscle atrophy occurs due to decreased growth hormone; gonadal atrophy due to decreased gonadotropic hormones; and dilute urine with low specific gravity in the face of dehydration due to decreased antidiuretic hormone. Thus, panhypopituitarism caused by endocrinologically inactive pituitary adenomas should be a differential in older animals with incoordination, depression, polyuria, blindness, and sudden behavioral changes. Additionally, as in this case, animals may develop blindness with fixed, dilated pupils due to dorsal extension of the tumor and compression of the optic nerves. Ophthalmic exam is typically otherwise unremarkable because dysfunction is originating from the CNS.

Typical gross lesions of null cell pituitary adenomas can include the following:

- Large tumor (>1cm) with compression or replacement of the remaining adenohypophysis, infundibular stalk, and hypothalamus;
- Small thyroid glands;
- Small adrenal glands due to cortical atrophy;
- Small gonads due to atrophic seminiferous tubules with little active spermatogenesis; and
- Atrophy of skin and muscle.[12,13]

Histopathologic and electron microscopic features characteristic of null cell adenomas in humans are elongated small cells forming pseudorosettes around dilated capillaries; modest cytoplasm; poorly developed rough endoplasmic reticulum and Golgi apparatus; and rare, round, small secretory granules (up to 250 nm).[7,9,10]

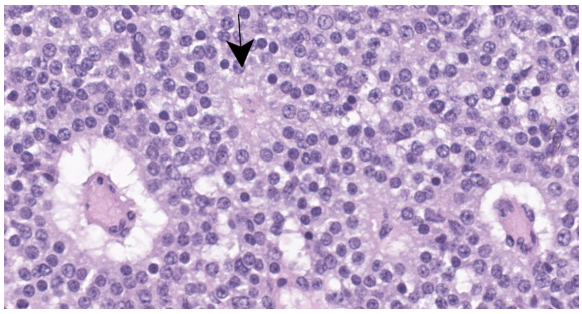

Figure 2-6. Pituitary gland, rhesus macaque. High magnification of neoplastic glands with two pseudorosettes and one rosette (arrow) in this field. (HE, 689X)

Ancillary diagnostics, including immunohistochemistry (IHC) and electron microscopy, are key to diagnosing null cell adenomas. The pituitary gland is made up of at least six different cell types that can give rise to tumors that are clinically functioning or silent. Each of these cell types produce one or more specific hormones that can be targeted via immunohistochemistry (See Table 2).[9,10] Moreover, plurihormonal adenomas can also occur. Furthermore, neuroendocrine tumors from other regions of the body may metastasize to the pituitary and thus can be positive for both synaptophysin and chromogranin; therefore, other markers may be necessary for differentiation.[2]

Electron microscopy can provide important information regarding the intracellular structure and hormonal activity of the cells.[9] The size and number of secretory granules as well as the degree of development of organelles such as rough endoplalsmic reticulum (RER) and Golgi apparatus can help indicate the secretory status of tumor cells.[8,9] In the present case, the poorly-formed RER and Golgi as well as scant, small granules suggest a non-functional secretory status.[8,10]

Interestingly, this animal was galactorrheac with elevated serum prolactin levels despite diffuse negative immunoreactivity for prolactin in the tumor. We propose that hyperprolactinemia in this case is due to "stalk syndrome" or "pituitary stalk compression syndrome," a phenomenon in which non-secretory suprasellar tumors induce hyperprolactinemia by inhibiting dopamine delivery to lactotrophs.[1-3] Since dopamine is a prolactin-inhibiting factor, reduction of dopamine levels leads to increased prolactin output. It is hypothesized that dopamine levels are reduced by one of two mechanisms: 1) physical compression of the dopaminergic neurons of the infundibular stalk or 2) disruption of hypophyseal portal blood flow delivering dopamine to lactotrophs.[3,7] However, increased compression caused by continued growth of the tumor may eventually lead to lactotroph insufficiency and failure.[3]

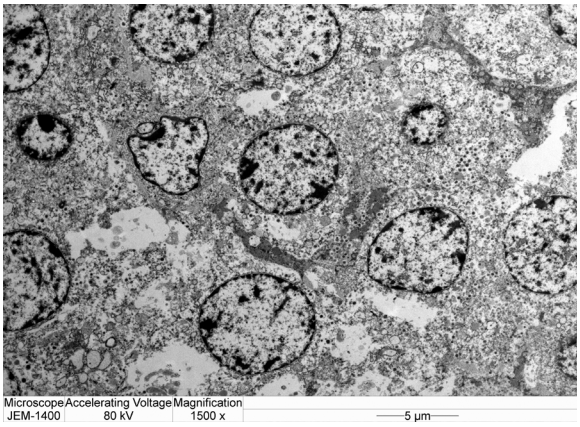

Figure 2-7. Pituitary gland, rhesus macaque. Neoplastic cells have indistinct borders. The Golgi apparatus and endoplasmic reticulum are indistinct. (*Photo courtesy of:* Johns Hopkins University School of Medicine, Department of Molecular and Comparative Pathobiology)

Marker	Tumor Type
GH	Somatotroph adenomas Mammosomatotroph adenomas
PRL	Lactotroph adenomas Lactotroph ademonas with GH reactivity
TSH	Thyrotroph adenomas
ACTH	Corticotroph ademonas
FSH	Gonadotroph adenomas
MSH	Melanotroph adenomas

Table 2. IHC hormone markers for pituitary adenomas.
GH: Growth Hormone; PRL: Prolactin; TSH: Thyroid Stimulating Hormone; ACTH: Adrenocorticotropin Hormone; FSH: Follicle Stimulating Hormone; MSH: Melanocyte Stimulating Hormone

In humans, the degree of prolactinemia can provide some clues to help differentiate a prolactinoma from a large tumor with "stalk syndrome." Serum prolactinemia over 200 ng/mL in humans is almost always due to a hormone-producing prolactinoma, while less than 200 ng/mL can be associated with stalk effect.[6] In the present case, serum prolactin levels measured at 147.6 ng/ml (expected value 20-50 ng/ml). Unfortunately, normal ranges are less established in rhesus macaques, and the relationship of degree of elevation to tumor type remains unclear.

Contributing Institution:
Johns Hopkins University School of Medicine
Department of Molecular and Comparative Pathobiology
http://www.hopkinsmedicine.org/mcp

JPC Diagnosis:
Pituitary gland: Pituitary adenoma.

JPC Comment:
As noted by the contributor, the pituitary gland is classically described as having six different cell types, each of which can become neoplastic. The clinical and biochemical signatures of pituitary adenomas differ depending on the cell or cells of origin and on whether these cells produce their native hormone products.

Hormone production is rarely an all-or-nothing affair in pituitary tumors, and clinical signs referable to these neoplasms therefore exist on a continuum.[6] In humans, a large number of pituitary adenomas, 22% - 54% depending on the study, present with signs related to the mass effect of the tumor rather than excess hormone secretion; these tumors are referred to as "clinically nonfunctioning pituitary adenomas," or NFPAs.[6] A related term, the "silent pituitary adenoma," or SPA, refers to tumors that express transcription factors or their hormone products at a level detectable by immunohistochemistry, but not at a level detected clinically.[6] A NFPA can be converted to an SPA if a a clinically silent pituitary adenoma nonetheless, on investigation, shows positive IHC immunoreactivity to relevant hormones or transcription factors. In between these lie the "whispering adenomas," characterized by elevated serum hormone levels, but borderline, often overlooked clinical symptoms.[6]

At the extreme end of this secretory spectrum is the rarest pituitary tumor type of them all, the null cell adenoma. Null cell adenoma is a

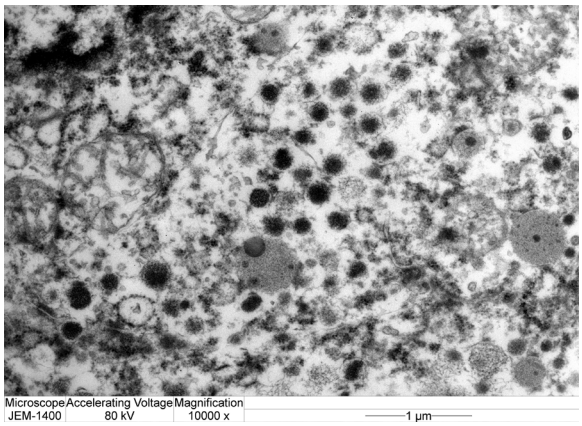

Figure 2-8. Pituitary gland, rhesus macaque. Within the cytoplasm are multiple, approximately 100 nm, electron dense, spherical granules. (*Photo courtesy of:* Johns Hopkins University School of Medicine, Department of Molecular and Comparative Pathobiology)

diagnosis of exclusion and requires immunonegativity of all adenohypophyseal hormones and, in humans, a lack of cell lineage-specific transcription factors.[6] The distinction is important in humans as null cell adenomas generally have a more aggressive course and more unfavorable outcomes compared with SPAs, including those who produce no hormones, but still express lineage-specific transcription factors.[6]

Conference discussion of this deceptively complex case began with a review of the anatomy and histology of the pituitary gland and its consistuent parts—the pars distalis, pars intermedia, and pars nervosa—along with an overview of pituitary neoplasia generally. Dr. Miller noted that several pituitary neoplasias have species predilections, including corticotroph adenoma/carcinoma (most common in dogs); pars intermedia adenoma (most common in horses and associated with pituitary pars intermedia dysfunction, or PPID); somatotroph adenoma (most common in cats and budgerigars); and lactotroph adenomas (most common in macaques, rats, and rabbits). Particular attention was given to the equine pars intermedia adenoma as its pathogenesis is unique and results from loss of dopaminergic inhibitory innervation from the hypothalamus, leading to unchecked proliferation of the pars intermedia.

With this background knowledge firmly in hand, conference participants moved to the first hurdle: tissue identification. While most conference participants were able to identify the tissue confidently, a significant minority initially identified this tumor as an embryonal tumor such as medulloblastoma. Dr. Miller noted that a firm grasp of neuroanatomy is key to tissue identification in this slide. Evaluation of the adjacent neuroparenchyma contains a haphazard arrangement of scattered neuronal cell bodies that is characteristic of the thalamus. This location, along with the rich vascularization of and the multifocal aggregates of eosinophilic fluid within the tumor, all suggest pituitary origin.

With the neoplasm localized to the pituitary, Dr. Miller emphasized its well-demarcated, expansile, non-invasive nature, which is apparent on subgross examination. As invasion is the most important histologic feature that differentiates pituitary adenoma from carcinoma, the lack of invasion gives this tumor a fairly classic pituitary adenoma appearance. Finally, Dr. Miller noted the multifocal mineralization in vascular walls throughout the neuroparenchyma in the examined section. This is a common age-related lesion in many animals and, in aged macaques, the thalamus is a common location to find this incidental change.

References:

1. Al-Brahim NYY, Asa SL. My approach to pathology of the pituitary gland. *J Clin Pathol*. 2006;59:1245-1253.
2. Asa SL. Practical pituitary pathology: what does the pathologist need to know? *Arch Path Lab Med*. 2008;132(8):1231-1240.
3. Bergsneider M, et al. The pituitary stalk effect: is it a passing phenomenon? *J Neurooncol*. 2014;117(3):477-84.
4. Chalifoux LV, MacKey JJ, King NW. A sparsely granulated, nonsecreting adenoma of the pars intermedia associated with galactorrhea in a male Rhesus Monkey (Macaca mulatta). *Vet Pathol*. 1983; 20:541-547.
5. Daviau JS, Trupkiewicz JG. Pituitary adenoma with galactorrhea in an adult male Cynomologus Macaque (Macaca fascicularis). *JAALAS*. 2001;40(5):57-59.
6. Drummond J, Roncaroli F, Grossman AB, Korbonits M. Clinical and pathological aspects of silent pituitary adenomas. *J Clin Endocrinol Metab*. 2019;104(7):2473-2489.
7. Dulai M, Vogel H. Pituitary adenomas. In: *Nervous System*. New York, NY: Cambridge University Press; 2009:262-284.
8. Horvath E, Kovacs K. Fine structural cytology of the adenohypophysis in rat and man. *J Electron Microsc Tech*. 1998;8(4): 401-432.
9. Kovacs K, Horvath E, Vidal S. Classification of pituitary adenomas. *J Neurooncol*. 2001;54:121-127.
10. Osamura RY, et al. Pathology of the human pituitary adenomas. *Histochem Cell Biol*. 2008;130:495-507.
11. Remick AK, et al. Histologic and immunohistochemical characterization of spontaneous pituitary adenomas in fourteen Cynomolgus Macaques (Macaca fascicularis). *Vet Pathol*. 2006;43:484-493.
12. Rosol TJ, Grone A. Pituitary gland. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. Volume 3. Elsevier;2016:276-291.
13. Rosol TJ, Meuten DJ. Tumors of the pituitary gland. In: *Tumors in Domestic Animals*. 5th ed. Wiley-Blackwell;2017:768-782.

CASE III:

Signalment:

3-year-old, male castrated mixed breed dog (*Canis lupus familiaris*)

History:

This dog initially presented to the submitting veterinarian for significant anxiety and urinating and defecating in the home.

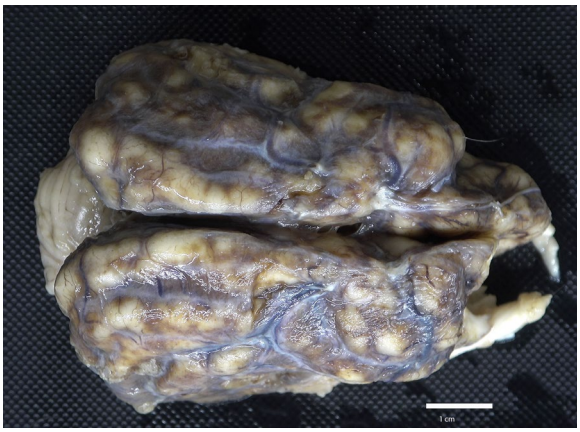

Figure 3-1. Cerebrum, dog. The cerebrum was hardened, lacked distinct sulci, and meninges were diffusely expanded by tan to dark brown exudate. (*Photo courtesy of* Michigan State University Veterinary Diagnostic Laboratory, 4125 Beaumont Rd, Lansing, MI 48910 https://cvm.msu.edu/vdl).

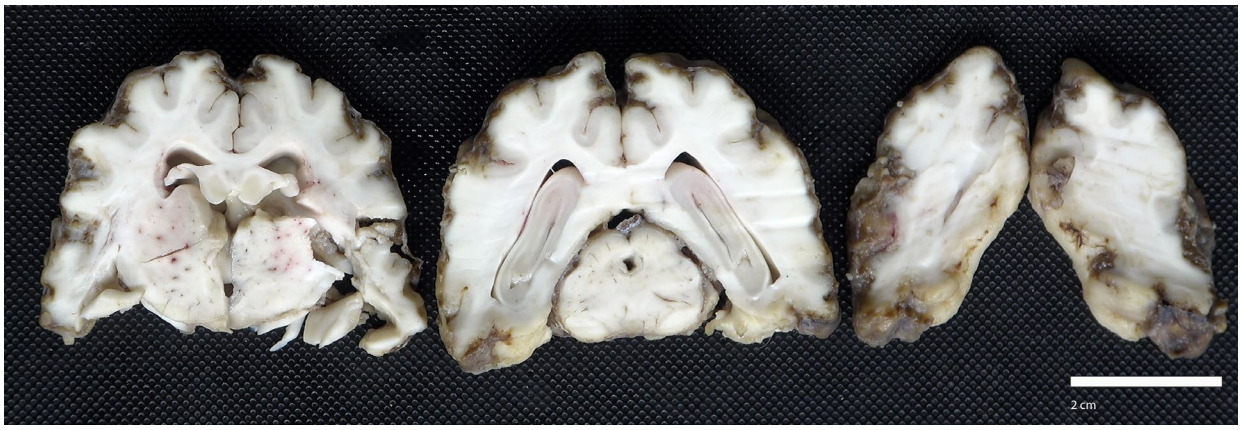

Figure 3-2. Cerebrum, dog. On cut surface, the exudate extended into and replaced the outer layers of the cerebral cortex. (*Photo courtesy of:* Michigan State University Veterinary Diagnostic Laboratory, 4125 Beaumont Rd, Lansing, MI 48910, https://cvm.msu.edu/vdl).

Bloodwork was performed and revealed a marked eosinophilia (4,341 cells/µL, RR: 70-1,490 cells/ µL). Idexx SNAP test was negative for heartworm antigen, *Borrelia burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp. No additional diagnostics were pursued. At that time, the animal was started on Prozac for generalized anxiety; however, his condition continued to progress, and the owner began noticing additional sensory deficits (sight and smell) and incoordination. Approximately six months later, humane euthanasia was elected due to poor quality of life and increasing aggression toward the owner. The owner was bitten by the dog, resulting in rabies testing and a full necropsy.

Gross Pathology:
The cerebrum was semi-firm, lacked distinct sulci, and meninges were diffusely expanded by tan to dark brown exudate. On cut surface, the exudate extended into and replaced the outer layers of the cerebral cortex.

Laboratory Results:
Direct fluorescent antibody for rabies virus, brain: Negative
Neospora caninum PCR, brain: Not detected
Toxoplasma gondii PCR, brain: Not detected
Qualitative fecal analysis: Negative

Microscopic Description:
Brain: Meninges were markedly expanded by numerous eosinophils, hemosiderin-laden macrophages, fewer plasma cells, and rare multinucleated giant cells. This infiltrate extended into the cerebral gray matter and surrounded large regions of necrosis and rarefaction. The adjacent white matter contained low numbers of scattered spheroids and Virchow-Robin spaces were expanded by low numbers of lymphocytes and plasma cells. No infectious agents, infarction, or neoplastic cells were observed on hematoxylin and eosin, periodic acid-Schiff reaction, or Gram-stained sections.

Contributor's Morphologic Diagnosis:
Brain: Severe, chronic, diffuse, eosinophilic and granulomatous to necrotizing meningoencephalitis with perivascular cuffing.

Contributor's Comment:
This case is consistent with eosinophilic meningoencephalitis (EME), which is an uncommon to rare neurologic disease in humans and animals.[2,3,8,12] In humans, EME is often asso-

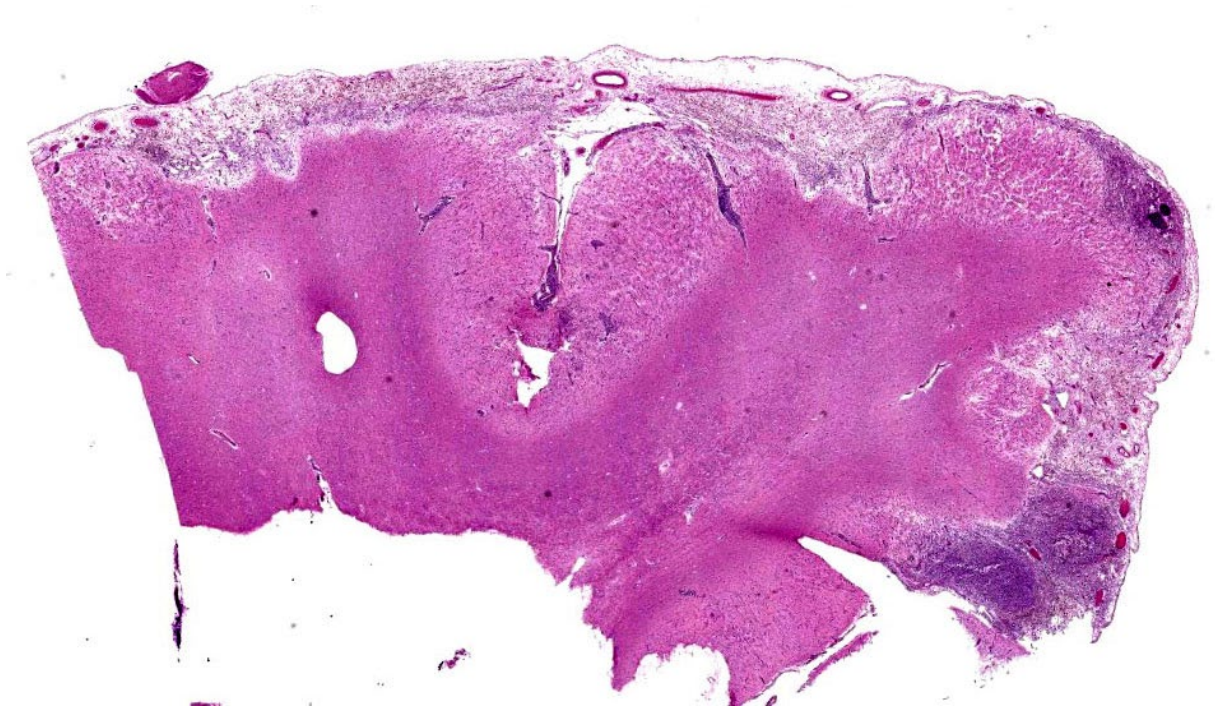

Figure 3-3. Cerebrum, dog. There is marked expansion, hypercellularity, and edema of the meninges with a loss of demarcation with the underlying submeningeal neuropil. (HE, 9X)

ciated with parasitic infections (such as *Angiostrongylus cantonensis*, *Gnanthostoma spinigerum*, cysticercosis (*Taenia* spp.), schistosomiasis, paragonimiasis, fascioliasis, and *Toxocara canis*) and occasionally tuberculosis, syphilis, coccidiodomycosis, and meningeal lymphoma.[8,9,12]

In veterinary medicine, EME is most often reported in dogs and has been associated with the following etiologies: *Toxoplasma gondii*, *Neospora caninum*, *Cryptococcus* spp., *Prototheca* spp., canine distemper virus, rabies virus, bacterial encephalitis, and aberrant migration of parasites (including *Angiostrongylus* spp. and *Cuterebra* spp).[3,6,8,9,12,13] Other non-infectious causes of EME include infarction, trauma, or neoplasia.[8,12] Additionally, if an underlying disease process cannot be identified, it is classified as idiopathic EME.[3,12] Within the literature, idiopathic EME is predominantly noted in dogs, but has rarely been reported in cats, cattle, and sheep.[2,3,6-13] Clinically, these dogs often present with mentation or behavioral changes, seizures, ataxia, blindness, and pain.[3,6,8-10,12,13] On a routine diagnostic work-up, the only major abnormality may be peripheral eosinophilia, as seen in this case. Only about half of these cases have peripheral eosinophilia; therefore, cerebrospinal fluid analysis is considered more sensitive.[3,12,13] On MRI, these cases have variable findings but often have bilateral, symmetrical lesions limited to the cerebral cortex that are associated with diffuse meningeal contrast uptake.[3,13] In the reported case, neither CSF evaluation nor MRI was performed.

On postmortem examination, the meninges are often reported to appear thickened and green.[12] However, this was not observed in our case, likely due to the abundant amount of hemosiderin within the macrophages. Microscopically, the leptomeninges of the brain and

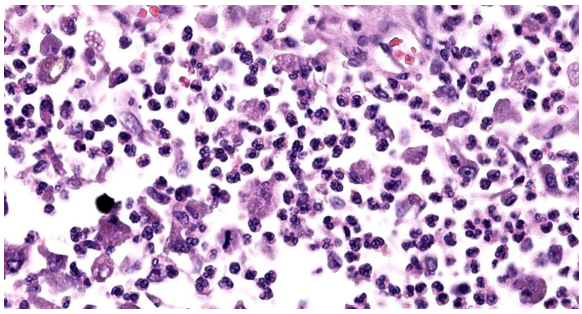

Figure 3-4. Cerebrum, dog. The meninges are infiltrated by large numbers of eosinophils, debris-laden macrophages and fewer lymphocytes and plasma cells. (HE, 736X)

spinal cord in cases of EME are frequently infiltrated by eosinophils, macrophages, and other mononuclear cells, and there is a variable amount of parenchymal loss, degeneration, and necrosis within the underlying cortical grey matter with multifocal regions of perivascular cuffing.[8-10,12]

The CNS, particularly neurons and myelinated axons, are highly susceptible to eosinophilic-induced neurotoxicity.[8,12] Eosinophils are often activated in association with chronic inflammatory conditions (asthma, chronic allergen, etc.) by helper T (Th2) cells releasing IL-4, IL-5, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF).[5,6,8,12] Once activated, eosinophils release major basic protein, eosinophil cationic protein, eosinophil peroxidase, and oxygen free radicals which aid in killing parasites.[2,5,6,10,12] However, they are also neurotoxic and can cause severe tissue damage within the CNS.[12] In the reported case, the absence of overt infectious agents, parasites, infarction, trauma, or neoplasia, combined with the ancillary testing, make a diagnosis of idiopathic EME most likely.

The exact pathogenesis of idiopathic EME is unknown. Idiopathic EME is reported more often in young to middle-aged, large breed dogs.[2,8,10,12,13] Additionally, rottweilers and golden retrievers appear to be over-represented in the population of reported cases, suggesting a possible breed predisposition.[2,6,9,10,12,13]

Many of the reported cases of idiopathic EME had minor to drastic improvement in the patient's condition when treated with corticosteroids, which has led to the hypothesis that there is an underlying immune-mediated or hypersensitivity process.[2,3,6-9,12] It is important to note that this improvement could be due to the normal pathophysiological interactions of steroids in the body rather than treating a specific underlying disease process, as steroids can induce eosinophil apoptosis and reduce the number within the blood.[5] Mayhew IG, et al. discussed a case of EME in a dog with suspected underlying hypersensitivity or drug-induced (firocoxib) reaction.[7] Additional cases with extensive clinical and pathological evaluation are necessary to further understand the underlying etiology and prognosis of idiopathic EME.

Contributing Institution:
Michigan State University
Veterinary Diagnostic Laboratory
4125 Beaumont Rd, Lansing, MI 48910
https://cvm.msu.edu/vdl

JPC Diagnosis:
Cerebrum: Meningoencephalitis, eosinophilic, superficial, marked, with cortical necrosis.

JPC Comment:
Dogs play host to a wide range of diseases, including EME, for which the contributor provides an excellent summary, that are characterized histologically by the accumulation of eosinophils. Canine eosinophilic pulmonary

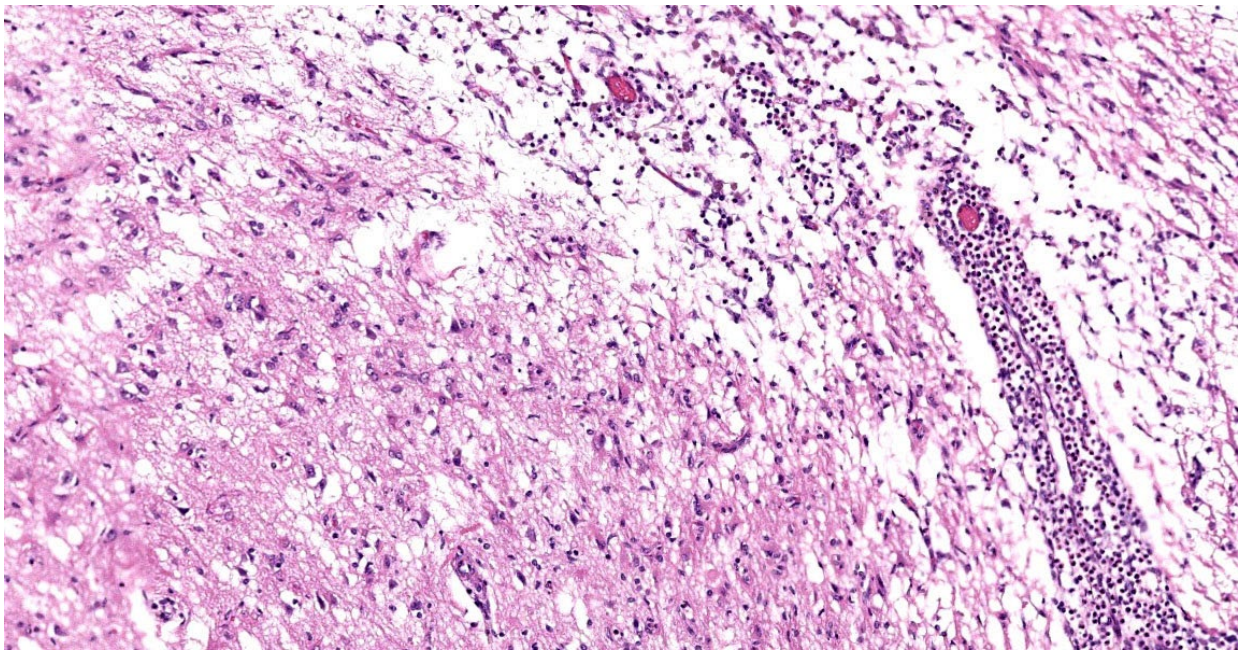

Figure 3-5. Cerebrum, dog. There is marked rarefaction of the neuropil of the submeningeal cortex and expansion of Virchow Robin spaces by numerous inflammatory cells. (HE, 149X)

granulomatosis and eosinophilic bronchopneumopathy were discussed earlier this conference year, and insights on these conditions can be found in WSC #9, case 2. Other canine eosinophilic diseases include eosinophilic granuloma; eosinophilic furunculosis; eosinophilic esophagitis, gastritis, and gastroenteritis; eosinophilic cystitis; and hypereosinophilic syndrome.

Canine eosinophilic granuloma is an uncommon skin disease that frequently presents as papules, nodules, or plaques, most frequently in the oral cavity.[4] Canine eosinophilic granuloma is attributed to hypersensitivity reactions to insect bites or environmental and food allergens, though some bacterial and fungal agents have been implicated.[4] There is a pronounced breed predilection in Siberian husky dogs and Cavalier King Charles Spaniels, suggesting a genetic basis in at least some cases.[4]

Eosinophilic gastrointestinal disease (EGID) is an umbrella terms encompassing a spectrum of disorders characterized by eosinophilic inflammation in one or more sites such as the esophagus, stomach, intestine, and colon.[1] Diagnosis of EGID requires abnormal numbers and distributions of eosinophils in the gastrointestinal tract in the absence of other underlying etiologies such as parasitism.[1] Some studies have noted that the number of identifiable eosinophils in tissue section is likely an undercount, as degranulated eosinophils are easily overlooked or confused with other granulocytes. Immunohistochemical staining for eosinophil peroxidase, which highlights both viable and degranulated eosinophils, have revealed a four to 40-fold increase in eosinophil number over H&E evaluation alone, depending on the examined tissue.[1] As in the rest of the eosinophilic entities, EGID is thought to be a Th-2 driven hypersensitivity reaction likely, in the case of EGID, due to food allergens.[1]

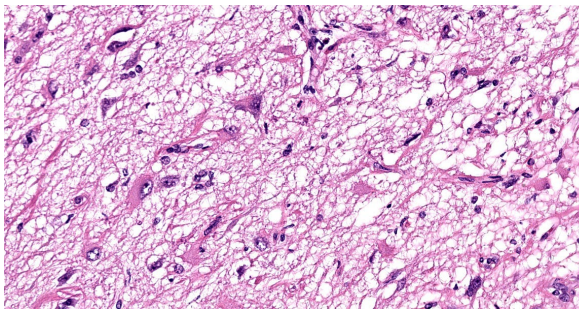

Figure 3-6. Cerebrum, dog. There are numerous gemistocytic astrocytes within rarefied areas of neuropil. (HE, 381X)

The moderator began discussion with a broad overview of possible causes for eosinophilic encephalitis and meningoencephalitis, including parasitic involvement, protozoal infection, hypersensitivity reactions, and the breed specific presentations noted by the contributor. Dr. Miller noted the striking subgross histologic appearance of this slide, with the eosinophil-induced neuroparenchymal necrosis giving the cerebral profile an unusual, scalloped appearance. Dr. Miller noted that the tissue loss focally extends into the white matter in the examined section and, as such, the term neuropil, which refers exclusively to gray matter, would be an inappropriate descriptor here; neuroparenchyma, encompassing both gray and white matter, would be the correct term to refer to the extent of the tissue loss.

The neuroparenchymal loss and eosinophilic inflammation present in section are both more extensive than typically seen with EME and more closely resemble lesions associated with parasitism. Dr. Miller noted that *Cuterebra* larval migration, relatively common in cats and occasionally occurring in dogs, has a very similar histologic presentation. Neuroparenchymal loss caused by *Cuterebra* is due to an exotoxin secreted by the larvae that leads to necrosis of adjacent neuroparenchyma. The lesion is both necrotizing and eosinophil-rich, often with concurrent vasculitis, and larvae are frequently not identified in histologic section. Dr. Miller provided examples of the gross and histologic appearance of feline ischemic encaphlopathy caused by *Cutererba* larval migration and reminded participants to keep this differential in mind when presented with this histologic picture.

Consideration of the morphologic diagnosis for this case sparked robust discussion. Participants noted the numerous histiocytes within the examined section and considered whether to include "granulomatous" or "histiocytic" to characterize the inflammation. Participants decided to omit the histiocytic component as the pathogenesis here is likely eosinophil-driven, with histiocytes called in secondarily to repair the damage. Participants also struggled to capture the extent and distribution of the striking lesions. After much discussion, participants felt that "superficial" best conveyed the location of the inflammation within the meninges and in the outermost portion of the cortex. A few participants doggedly argued for the term "cortical loss" to capture the unique appearance of the cerebral contour; however, as the loss was caused by necrosis, participants felt that "cortical necrosis," while perhaps not as punchy, was most appropriate.

References:

1. Bastan I, Rendahl AK, Seelig D, et al. Assessment of eosinophils in gastrointestinal inflammatory disease in dogs. *J Vet Intern Med*.
2. Bennett PF, Allan FJ, Guilford WG, et al. Idiopathic eosinophilic meningoencephalitis in Rottweiler dogs: three cases (1992-1997). *Aust Vet J.* 1997;75:786-789.
3. Cardy TJA, Cornelis I. Clinical presentation and magnetic resonance imagining

findings in 11 dogs with eosinophilic meningoencephalitis of unknown aetiology. *J Small Anim Pract*. 2018;59(7):422-431.
4. Knight EV, Shipstone MA. Canine eosinophilic granuloma of the digits treated with prednisolone and chlorambucil. *Vet Dermatol*. 2016;27(5):446-e119.
5. Kumar V, Abbas AK, Aster JC. *Robbins & Cotran Pathologic Basis of Disease.* 10th ed. Elsevier;2020.
6. Lillihook I, Gunnarsson L, Zakrisson G, Tvedten H. Disease associated with pronounced eosinophilia: a study of 105 dogs in Sweden. *J Small Anim Pract*. 2000; 41:248-253.
7. Mayhew IG, Hill KE, Ahn Y, Jones BR. Can drug-induced aseptic meningitis account for some cases of eosinophilic meningitis/meningoencephalitis in dogs? *N Z Vet J*. 2022;70(3):184-185.
8. Olivier AK, Parkes JD, Flaherty HA, Kline KL, Haynes JS. Idiopathic eosinophilic meningoencephalomyelitis in a Rottweiler dog. *J Vet Diagn Invest*. 2010; 22(4):646-648.
9. Smith-Maxie LL, Parent JP, Rand J, et al. Cerebrospinal fluid analysis and clinical outcome of eight dogs with eosinophilic meningoencephalomyelitis. *J Vet Intern Med*. 1989;3:167-174.
10. Sykes JE, Weiss DJ, Buoen LC, et al. Idiopathic hypereosinophilic syndrome in 3 Rottweilers. *J Vet Intern Med*. 2001;15: 162-166.
11. Vidana B, Floyd T, Brena C, et al. First case of idiopathic eosinophilic meningoencephalitis in a sheep. *J Comp Pathol*. 2020;174:58-62
12. Williams JH, Koster LS, Naidoo V, et al. Review of idiopathic eosinophilic meningitis in dogs and cats, with a detailed description of two recent cases in dogs. *J S Afr Vet Assoc*. 2008;79(4):194-204.
13. Windsor RC, Sturges BK, Veranu KM, Vernau W. Cerebrospinal fluid eosinophilia in dogs. *J Vet Intern Med*. 2009;23: 275-281.

CASE IV:

Signalment:
5-month-old, intact male Siberian Husky (*Canis lupis familiaris)*

History:
The dog had a history of progressive tetraparesis. Magnetic resonance imaging demonstrated bilaterally symmetric white matter hyperintensities in the corpus callosum, cerebrum, and thalamus. An analysis of the cerebrospinal fluid was normal. Given the breed and signalment, GM1 gangliosidosis was suspected and the dog was euthanized.

Gross Pathology:
There was subtle flattening of the cervical spinal cord.

Laboratory Results:
DNA testing did not identify the gene mutation associated with GM1 gangliosidosis in Huskies.

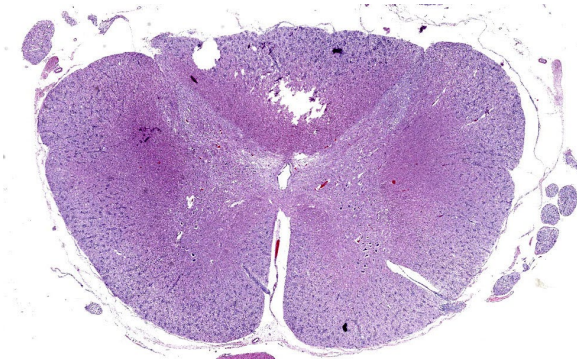

Figure 4-1. Spinal cord, dog. There is diffuse pallor of all funiculi. (HE, 15X)

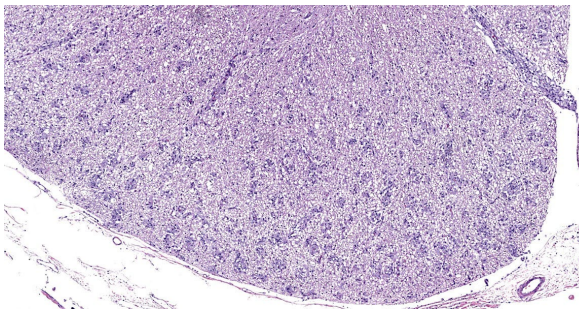

Figure 4-2. Spinal cord, dog. Diffusely, myelin sheaths are dilated. Blood vessels are outlined by a cellular infiltrate. (HE, 16X)

Microscopic Description:
Cervical spinal cord: Within the white matter are aggregates of large round to polygonal cells with finely granular, lightly basophilic cytoplasm and eccentric nuclei (globoid cells). These cells occasionally surround blood vessels. The globoid cells contain faintly magenta material with a periodic acid- Schiff stain. There is marked loss of myelin and axons highlighted by Luxol fast blue and Sevier-Munger stains, respectively. The white matter in other portions of the brain and spinal cord were similarly affected.

Contributor's Morphologic Diagnosis:
Marked widespread axonal degeneration with marked histiocytosis.

Contributor's Comment:
The clinical signs, gross, and microscopic findings are attributed to axonal degeneration in the brain and spinal cord as a result of globoid cell leukodystrophy. Globoid cell leukodystrophy occurs due to deficient activity of the lysosomal enzyme galactocerebrosidase. Globoid cell leukodystrophy is a genetic condition reported in many pure breed dogs (Cairn Terriers, West Highland White Terriers, Miniature Poodles, Bluetick Hounds, Basset Hounds, Beagles, Irish Setters and the Australian Kelpie).[1] It has not been reported in Siberian Huskies. Additionally, sheep, cats and primates may be affected.[3]

The pathophysiology of globoid cell leukodystrophy is complicated and not fully understood. Galactocerebrosidase normally breaks down galactocerebroside and galactosylsphingosine (psychosine).[1] Interestingly, at the time of diagnosis, many individuals have decreased amounts of galactocerebroside; this paradox may reflect the decreased amounts of myelin remaining late in the disease course.[2] There is, however, accumulation of psychosine in the oligodendroglia and Schwann cells. The psychosine is extremely toxic to these cells and results in their death. The loss of oligodendroglial cells and Schwann cells results in decreased myelination of axons and ultimately, axonal degeneration. Macrophages attempt to clear remaining myelin and galactocerebrosides, but without the enzyme they are also unable to break down galactocerebrosides. As a result, the macrophages become swollen with PAS-positive debris and are termed "globoid cells."[1] The globoid cells often accumulate around blood vessels, as in this case. Although a biopsy of peripheral nerves can be useful to make an antemortem diagnosis, in this case, the sciatic nerve was not clearly affected.[1]

Contributing Institution:
University of Tennessee
College of Veterinary Medicine
Department of Biomedical and Diagnostic Sciences
http://www.vet.utk.edu/departments/path/index.php

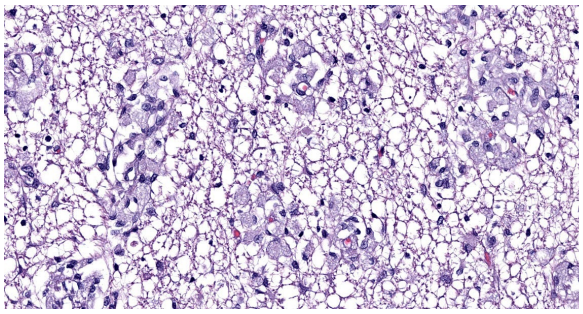

Figure 4-3. Spinal cord, dog. The rarefied white matter is infiltrated by large amphophilic macrophages with abundant vacuolated cytoplasm (Gitter cells or "globoid cells"). (HE, 381X)

JPC Diagnosis:
Spinal cord, white matter: Histiocytosis, perivascular, diffuse, marked, with abundant intracellular myelin.

JPC Comment:
Globoid cell leukodystrophy (GLD), also known as Krabbe disease, is an autosomal recessive, fatal lysosomal storage disease caused by mutations in galactoceramide beta-galactosidase (also known as galactosylceramidase, galactocerebrosidase, or GALC). GLD develops early in life and, as noted by the contributor, is characterized by impaired aberrant myelin metabolism and the subsequent accumulation of galactosylceramide (GalCer) and psychosine, the major substrates of GALC.[3]

Compared with normal cellular membranes, myelin has a very high lipid content and is enriched in GalCer.[2] Myelin is remarkably stable, but is contininously remodeled at a basal rate and at a faster rate in response to injury or disease. The complex myelin degradation process relies on a number of enzymes, including GALC, to break down its consitutent parts, including GalCer.[2] GALC is expressed ubiquitously in the lysosomes of all cell types in the nervous system, and is particularly enriched in oligodendrocytes.[2] In health, once GalCer is processed in the lysosome, its consitutent molecular parts are recycled and become building blocks in the constitutively active myelin remodeling/remyelination pathway.[3]

In GLD, mutations diminish GALC enzyme activity, resulting in impaired degradation of GalCer during myelin remodeling. The resulting lack of sufficient building blocks for remyelination leads to myelin loss.[3] Concurrently, toxic pysochosine, normally produced by oligodendrocytes and also degraded by GALC, begins to accumulate, leading to extensive oligodendrocyte degeneration and death with consequent degeneration of existing myelin and impairment of remyelination.[1]

As the contributor notes, macrophages arrive to clean up the degenerating myelin, but they are also GALC-deficient and cannot metabolize GalCer. The accumulation of the undigested substrate leads to the "globoid cells" for which the condition is named.[1]

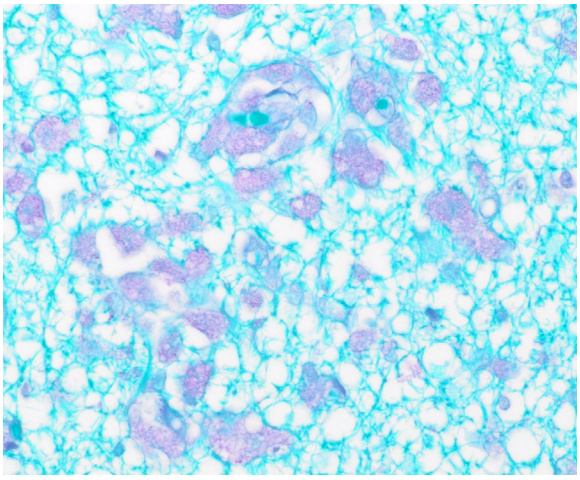

Figure 4-4. Spinal cord, dog. Globoid cells contain PAS-positive material within their cytoplasm. (PAS, 400X)

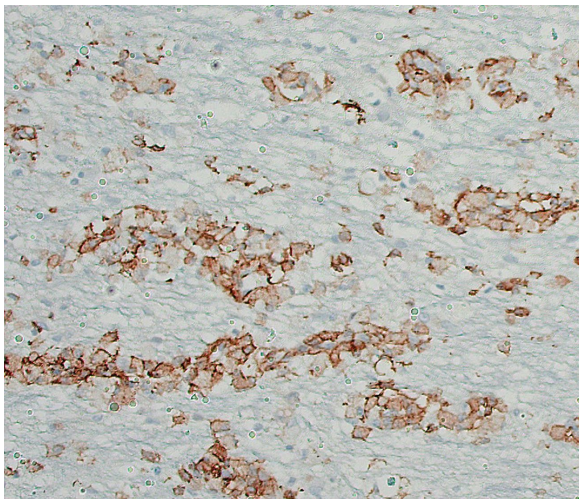

Figure 4-5. Spinal cord, dog. Globoid cells are strongly positive for IBA-1. (HE, 395X)

The globoid cells appear in perivascular cuffs in the white matter of the CNS, in the leptomeninges, and in the endoneurium of peripheral nerves.[1]

This case is an excellent example of the pathognomonic histology of GLD, characterized by marked myelin loss, markedly reduced numbers of oligodendrocytes, and, most dramatically, the infiltration of numerous large, round macrophages filled with PAS-positive storage material, most abundant in areas of active demyelination.[3] The presenting clinical signs in this case–ataxia and limb weakness and tremors that progress to paralysis and muscular atrophy–are also typical.[4] Some animals may also develop blindness. Typical gross lesions include slight gray discolorations of the white matter, particularly in the centrum semiovale of the cerebrum and in the spinal cord.[4]

Conference participants enjoyed the photogenic and straightforward nature of this case. Dr. Miller cautioned residents not to overinterpte the gray matter vacuolation present in some sections of the spinal cord since, as in so many neuroparenchymal lesions, this likely represents autolysis artifact. Dr. Miller discussed leukodystrophies in general, noting that the various diseases have in common a failure of myelinating cells to maintain their myelin sheaths. Such diseases are typically early onset, symmetrical, and typically unaccompanied by significant inflammation.

Dr. Miller briefly reviewed the biology and function of the lysosome as well as sphingolipid metabolism and the diseases that result from associated enzyme deficiencies. Dr. Miller discussed the histochemical staining characteristics of globoid cells and noted that IBA1, an immunohistochemical stain for macrophages; PAS, which stains the material accumulated in globoid cells; and Olig2, an immunohistochemical stain for oligodendrocytes, can be used in concert to demonstrate that the accumulation in GLD is occurring in within histiocytes and not oligodendrocytes.

References:

1. Cantile C and Youssef S. Chapter 4: Nervous System. In: Maxie MG ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 6th ed. vol. 1. Elsevier; 2016.
2. Feltri ML, Weeinstock NI, Favret J, Dhimal N, Wrabetz L, Shin D. Mechanisms of demyelination and neurodegeneration in globoid cell leukodystrophy. *Glia.* 2021. 69:2309-2331.
3. Lee E, Fuller M, Carr M, Manavis J, Finnie J. Globoid cell leukodystrophy (Krabbe's disease) in a Merino sheep. *J Vet Diagn Invest.* 2019;31:118-121.
4. Miller AD, Porter BF. Nervous System. In: Zachary JF, ed. *Pathologic Basis of Veterinary Disease*. 7th ed. Elsevier; 2022:945-946.