Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2015-2016

C o n f e r e n c e 21

13 April 2016

Jey Koehler, DVM, PhD, DACVP
Director, Histopathology Core Laboratory
Department of Pathobiology
Auburn University College of Veterinary Medicine

CASE I: 1888-15 (JPC 4070542).

Signalment: 6-year-old castrated male Yorkshire terrier dog (*Canis familiaris)*

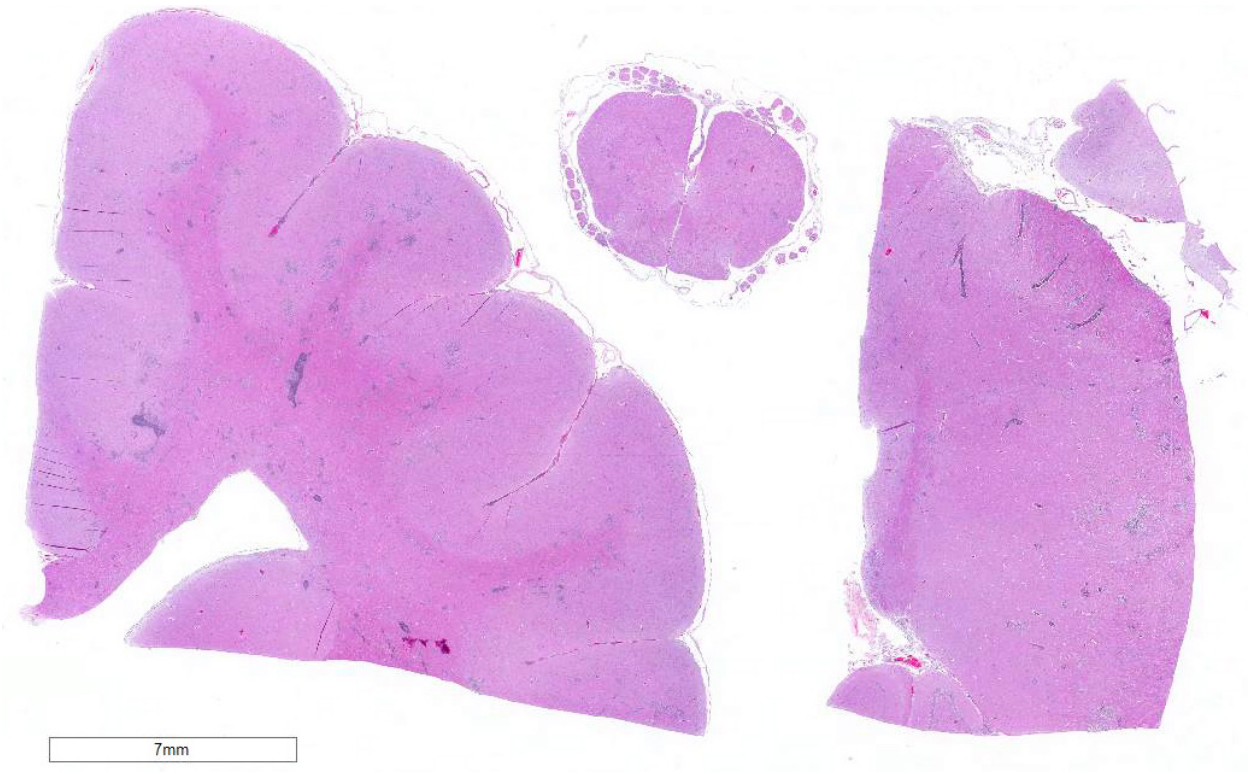
History: Per history provided, Milo is a 6-year-old castrated male Yorkshire terrier dog that was diagnosed with tracheobronchitis for the past month and was placed on antibiotic therapy. The dog developed sudden onset of hindlimb weakness and was unable to walk on both hind limbs. He collapsed acutely, failed to revive when administered cardiopulmonary resuscitation, and was submitted for necropsy.

Gross Pathology: There were no significant gross findings noted.

Laboratory Results: N/A

Histopathologic Description: Brain: Multifocally, expanding the meninges and Virchow-Robins space primarily within the white matter and less severely affecting the grey matter of the cerebrum, cerebellum and brainstem, are numerous intense inflammatory foci that often coalesce. Inflammatory cells are comprised of large numbers of macrophages, lymphocytes, with fewer plasma cells and neutrophils. The macrophages often appear epithelioid cells and form granulomatous foci that efface and replace neuropil. Within neuropil adjacent to granulomatous foci are large numbers of glial cells (gliosis) and reactive gemistocytic astrocytes. Both lymphocytes and macrophages are arranged in dense and often whirling, perivascular cuffs. Mitoses in the macrophage population are present at 1-3 per high power field (400x) in areas of perivascular cuffs. Endothelium associated with the perivascular cuffing is reactive, and lined by plump and hypertrophied endothelial cells, and vessel walls are infiltrated by the mononuclear cells described above.

Spinal cord: Diffusely expanding meninges and multifocally replacing white and grey matter of the spinal cord are similar



Cerebrum, dog. In cross sections from the cerebrum and cervical spinal cord, the vessels (especially at the interface of the grey and white matter) are accentuated by a perivascular inflammatory infiltrate. (HE, 5X)

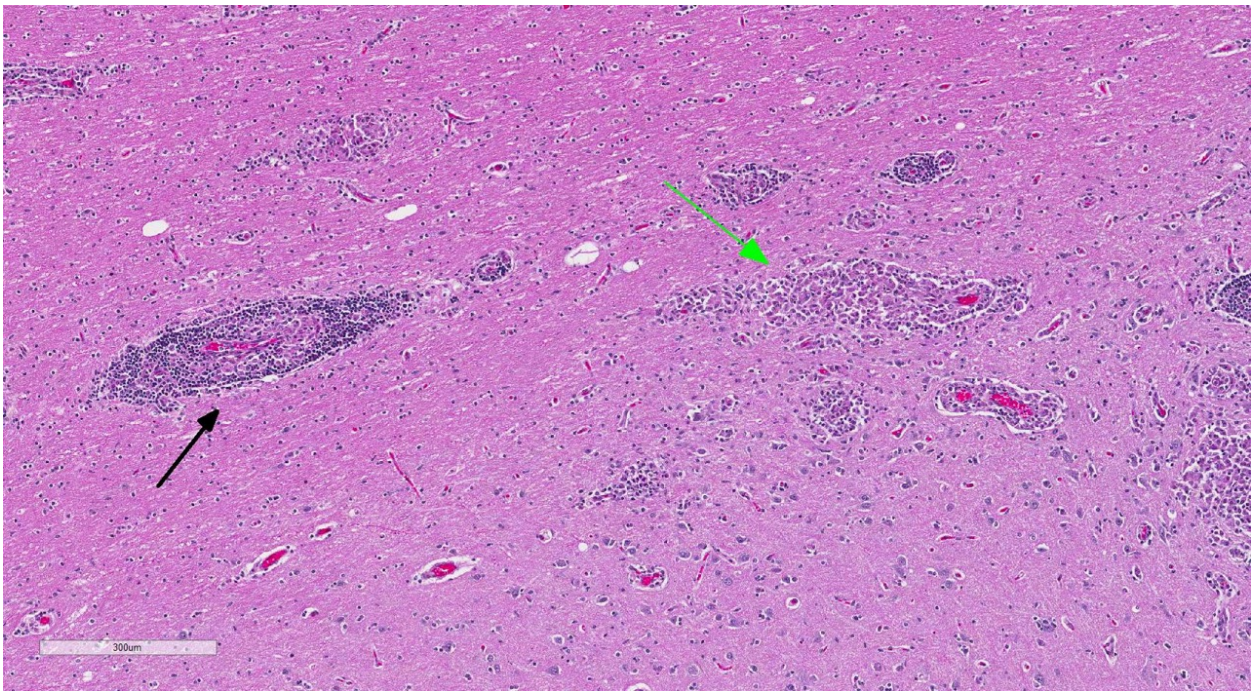
inflammatory cells as those described above for the brain sections. Perivascular granulomatous, lymphocytic cuffs are present in neuropil and meninges.

Contributor's Morphologic Diagnosis:
Brain: 1. Severe, multifocal to coalescing lymphohistiocytic encephalitis
2. Severe, diffuse lymphohistiocytic meningitis
Spinal cord: 1. Severe, multifocal lymphohistiocytic myelitis
2. Severe, diffuse lymphohistiocytic meningitis

Contributor's Comment: This dog had disseminated granulomatous meningoencephalomyelitis (GME) that affected the brain and spinal cord (and optic nerve, not included on slide). GME is an idiopathic disease that affects the central nervous system of predominantly young to middle-aged, small toy breed dogs, although age and breed of affected dogs can vary vastly.[3,4] The disease was first reported in 1978, and represents 5-25% of central nervous system disease of dogs.[3] A higher incidence of this disease may be seen in females, and affected dogs are typically mature.[2] The disease is almost always progressive and is associated clinical signs include ataxia, non-ambulation, paresis, paralysis, as well as a wide range of other neurological signs.[2] Dogs with GME may develop neutrophilic leukocytosis, with increased protein and cellularity in CSF fluid.[2] While the disease is normally progressive and prolonged, some affected dogs can die spontaneously.[2] Grossly, the lesions of GME can be hard to identify, and affected white matter may appear as small foci of malacia.[4] The disease tends to affect white matter of cerebrum and cerebellum, and occasionally occurs in the

optic nerve and spinal cord. Histological findings of prominent perivascular cuffing of mononuclear cells and discrete granulomatous aggregates are distinctive features of the disease.[4]

GME can be classified as focal, disseminated (multifocal) and ocular.[3] The focal type is characterized by coalescing granulomas that form a space-occupying lesion and associated clinical signs, and are more common in the cerebrum and brainstem.[3] The disseminated form affects more than one of the following sites: cerebrum, brainstem, spinal cord, cerebellum, meninges and optic nerves.[3] The ocular form is associated with optic neuritis and occasionally uveitis, retinal hemorrhage or retinal detachment.[3] The exact etiology of GME is unknown, although infectious causes and immune-mediated disease have been proposed. Antemortem diagnosis of GME is typically based on CSF analysis, characterized by increased leukocyte count, mononuclear pleocytosis and increased protein content.[3] However, changes can be variable and may be steroid dependent.[3] Definitive diagnosis of the disease is based on brain biopsy.[3] Treatment for GME is largely based on immunosuppression with corticosteroid therapy, and azathioprine, cytosine arabinoside, procarbazine, cyclosporine and radiation therapy have more recently been attempted in treatment regime.[3] In general, prognosis of dogs with GME is poor. Considering the clinical history of tracheobronchitis in this dog and the nature of inflammatory cells, the differential diagnosis includes canine distemper (*Morbillivirus*) infection. There were no intranuclear or intracytoplasmic viral inclusions present in any of the sections examined to further support a viral etiology in this case. Ancillary testing using immunohistochemistry to label viral antigen in the brain sections would be useful to definitively rule out canine distemper in this case. Fungal or protozoa infection are lower on the differential list as organisms were not observed in any of the examined sections.



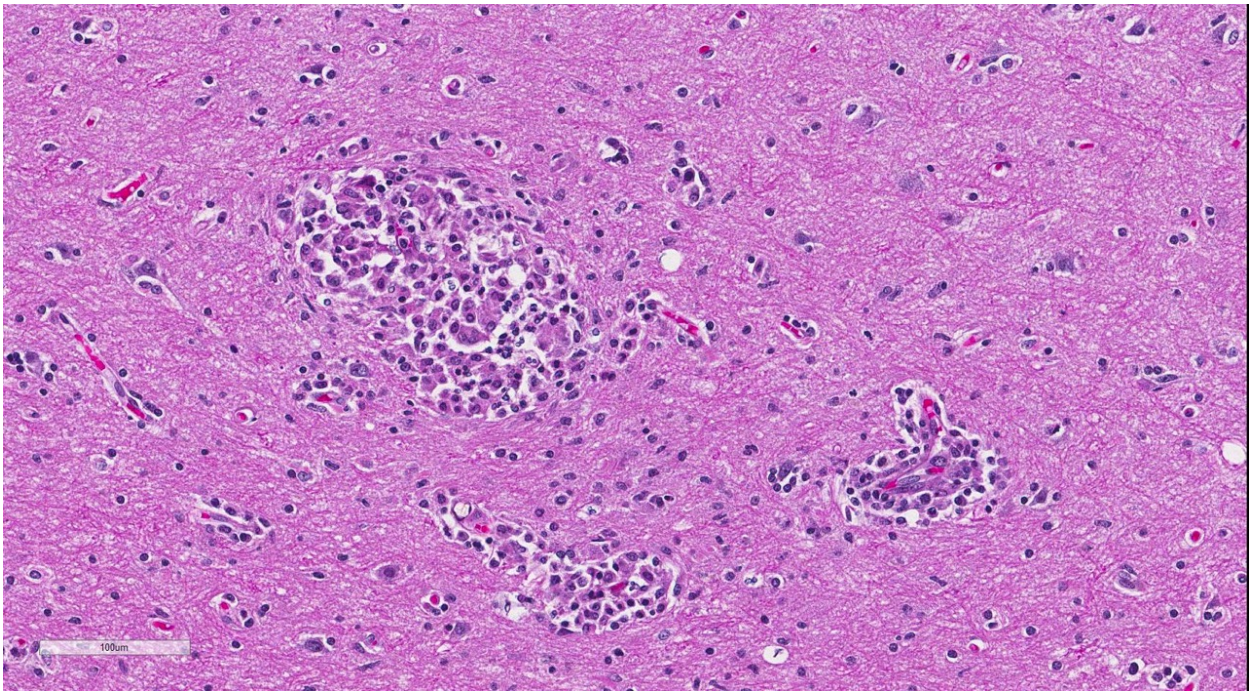
Cerebrum, dog. Virchow-Robins spaces are filled by various combinations of histiocytes and lymphocytes (black arrow). Some vessels have a predominantly histiocytic infiltrate (green arrow). (HE, 81X)

JPC Diagnosis: Brain, cerebrum, brainstem; spinal cord: Meningoencephalomyelitis, lymphohistiocytic, chronic, multifocal, marked.

Conference Comment: In addition to granulomatous meningoencephalitis (GME), other idiopathic inflammatory conditions that affect the nervous system include necrotizing meningoencephalitis (NME) (see case 2 of this conference) and necrotizing leukoencephalitis (NLE). Each of these entities has unique features with reference to breed predilection, histologic lesions, anatomic location and clinical presentation. While NME and NLE are associated with specific dog breeds, GME is not. CD3-positive T-cells appear to play an important role in development of lesions, particularly in GME. CD163-positive macrophages also play an important role in GME where they exhibit a unique, predominantly perivascular distribution.[5] Histologic findings in GME include granulomatous foci composed of primarily macrophages and lymphocytes, with fewer plasma cells and neutrophils, located in the cerebral white matter, subcortical region, cerebellum, and midbrain. In GME, interleukin 17 (IL-17) levels are often elevated within inflammatory cells (primarily macrophages / microglia) and may contribute to the development of the associated lesions. IL-17 is a component of the Th17 immune response and is produced in some autoimmune conditions, such as rheumatoid arthritis and Crohn's disease, among others. It has been postulated that GME may be represent a delayed type hypersensitivity reaction and that mast cells may play a role in development of early lesions.[6]

Aside from (or in conjunction with) an autoimmune disorder, infectious agents have long been suspected to be involved in the pathogenesis of GME (and NME), and the phenomenon of molecular mimicry has also been implicated. Infectious agents have not been identified via light microscopy or culture and molecular techniques have failed to identify a viral agent; however, molecular techniques have identified *Mycoplasma canis* (an agent not typically associated with CNS disease) in cases of both GME and NME. To date, the precise role of *M. canis* in the pathogenesis of GME and NME remains unclear.[1] Aside from explanations involving an infectious agent and immune dysregulation, it has also been postulated that GME may represent a lymphoproliferative disorder.[7] Overall, the common view is that this condition is multifactorial with a complex pathogenesis involving genetic factors, immune dysregulation and environmental factors (i.e. pathogens).[1,7]

The conference histologic description mirrored the contributor's description above. Additional features described include mild rarefaction and vacuolation of the neuropil adjacent to inflammatory aggregates, and microgliosis (in addition to the astrocytosis noted above). Although many of the astrocytes appear hypertrophied/reactive, the majority have not yet reached the characteristic gemistocytic stage. Low numbers of necrotic neurons are also present, although this is not a prominent feature. Perivascular cuffs consist primarily of lymphocytes, while most of the histiocytes are localized to the neuropil adjacent to perivascular cuffs. The moderator noted that the distinct asymmetric nature of the lesions, particularly within the perivascular cuffs, is a characteristic feature of GME. She also pointed out the vasculocentric nature of the meningeal inflammation and how it is not evenly distributed, which is another common feature of this entity. Conference



Cerebrum, dog. Scattered throughout the parenchyma, there are numerous aggregates of histiocytes. Close inspection of these nodules often reveals one or more vessels contained within it, suggesting that these nodules are likely perivascular cuffs as well. (HE, 120X)

participants briefly discussed differential diagnosis for GME, including viral encephalitis, pointing out that viruses generally result in primarily lymphocytic inflammation. The histiocytic component in GME is an important diagnostic clue, as well as the fact it affects both grey and white matter. The moderate commented that, in general, GME is easier to diagnose in more chronic, severe cases and that it is important to trim in the eyes in suspected cases of GME, as inflammation is often seen surrounding the optic nerve.

Contributing Institution:

Advanced Molecular Pathology Laboratory
Institute of Molecular and Cell Biology
61 Biopolis Drive, Proteos
Singapore 138673

References:

1. Barber RM, Porter BF, Li Q, May M, *et al*. Broadly reactive polymerase chain reaction for pathogen detection in canine granulomatous meningoencephalomyelitis and necrotizing meningoencephalitis. *J Vet Intern Med*. 2012; 26:962-968.

2. Cordy DR. Canine granulomatous eningoencephalomyelitis. *Vet Pathol*. 1979; 16(3):325-33.

3. Emma JO, Merrett D, and Jones B. Granulomatous meningoencephalomyelitis in dogs: A review. *Ir Vet J*. 2005 Feb 1; 58(2):86-92.

4. Maxie MG, Youssef S. Nervous system. In: Maxie, MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol 1. 5th ed. Philadelphia, PA: Elsevier; 2007: 442-443.

5. Park ES, Uchida K, Nakayama H. Comprehensive immunohistochemical studies on canine necrotizing meningoencephalitis (NME), necrotizing leukoencephalitis (NLE) and granulomatous meningoencephalomyelitis (GME). *Vet Pathol*. 2012; 49(4):682-692.

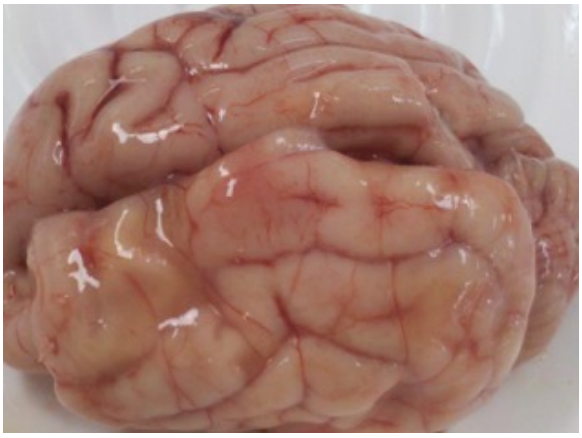
6. Park ES, Uchida K, Nakayama H. Th1-, Th2-, and Th17-related cytokine and chemokine receptor mRNA and protein

expression in brain tissues, T cells, and macrophages of dogs with necrotizing and granulomatous meningoencephalitis. *Vet Pathol*. 2013; 50(6):1127-1134.
7. Schatzberg SJ. Idiopathic gran-ulomatous and necrotizing inflam-matory disorders of the canine central nervous system. *Vet Clin North Am Small Anim Pract*. 2010; 40(1):101-20.

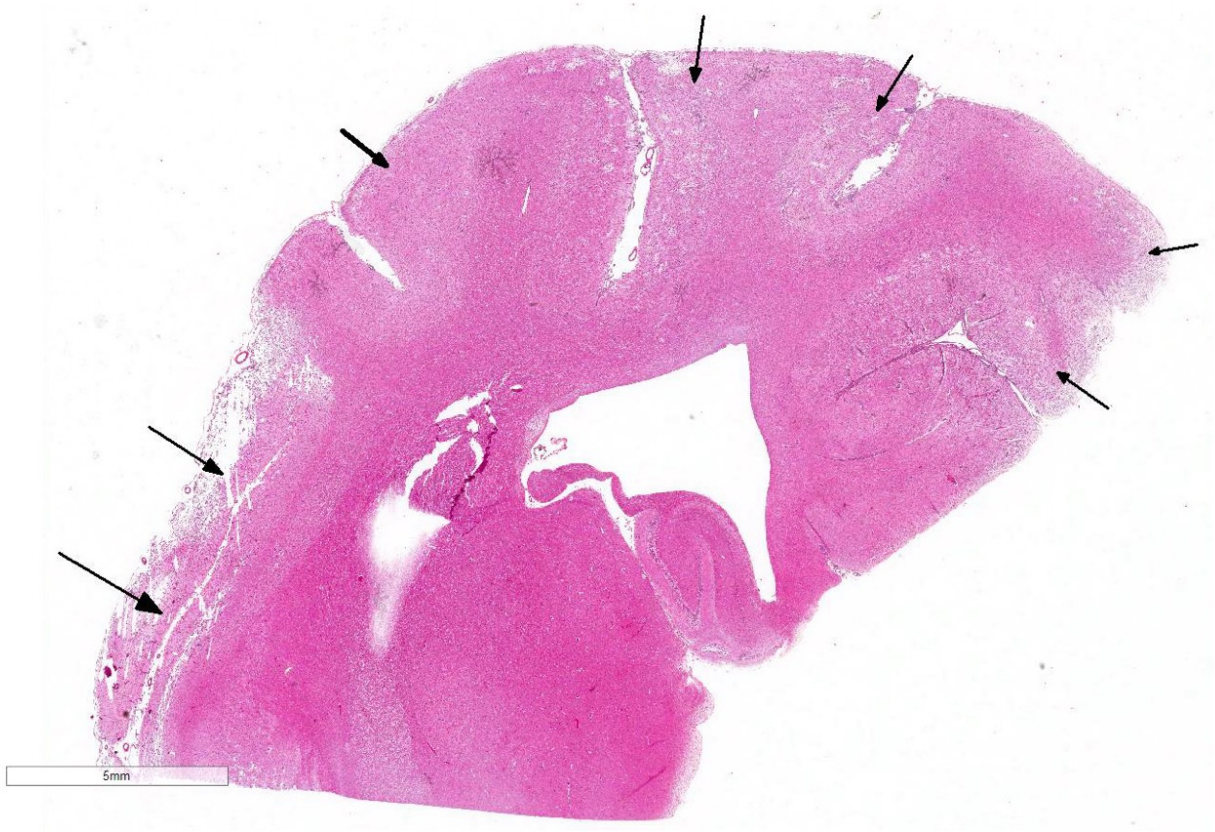
CASE II: UFMG 1 (JPC 4035763).

Signalment: A 2-year-old, intact female Maltese dog (*Canis familiaris).*

History: A 2-year-old, intact female Maltese dog was presented to the veterinarian with a history of acute neurological signs characterized by seizures, constant howling, and circling to the right side. The owner informed that the immunization schedule had been followed. On neurological examination, the dog presented deficit of mental status (apathy and depression), head turn, compulsive circling to the right side and falls to the left side. During ambulation the animal turned away from obstacles, indicating no visual deficit. Abnormal movements and proprioception deficit to the left side were detected. There was also left deficit to menace response and cervical sensibility. A multifocal intracranial lesion involving the telencephalon was suspected. The complete blood count and serum chemistry profiling were unremarkable. Magnetic resonance imaging (MRI) and CSF analysis could not be performed because of the client's refusal. According with breed, age, history and neuroanatomic localization of the lesions, an inflammatory neurological disease of unknown cause was suspected. On the basis of the suspicion, phenobarbital (6mg/kg bid), doxycycline (10mg/kg bid), prednisolone (2mg/kg bid), and A and B vitamins were prescribed. A re-check five days after the onset of therapy was performed and no changes were detected when thorough clinical examination was performed. On neurological examination, reduction of circling and seizures was observed but there was no improvement in posture, proprioception and menace response. Because clinical signs improved gradually, the initial prescription was maintained. Fifty three days after the first presentation the dog was checked again. The owner reported that the initial dosage of prednisolone was maintained but, arbitrarily the client did not maintained the medicine after three weeks, and the seizures episodes started again after discontinuing the corticoid therapy. The veterinarian detected that all clinical signs mentioned above worsened. A new treatment protocol adding cyclosporine (6 mg/kg bid) to prednisolone was prescribed. However, ten days later, the clinical signs worsened dramatically. The dog presented severe changes of the mental status (disorientation, aggression and apathy), increased postural deficit, and bilateral deficit to menace response and cluster seizures. Due to the poor prognosis, the owner elected euthanasia.



Cerebrum, dog. At necropsy, there were multiple yellowish areas of malacia throughout the all lobes. (Photo courtesy of: Universidade Federal de Minas Gerais, Escola de Veterinária, Departamento de Clínica e Cirurgia Veterinárias, Av. Antônio Carlos, 6627; 31270-901. Belo Horizonte, MG, Brazil www.vet.ufmg.br)



Cerebrum, dog. There is marked diffuse malacia of the submeningeal grey matter (arrows) (HE, 5X)

Gross Pathology: At necropsy, there was mild asymmetry between the cerebral hemispheres. In the right hemisphere there were multifocal to coalescing depressed, markedly friable and yellowish areas measuring approximately 0.5 to 1.0 cm of diameter. In the frontal lobe there was a locally extensive malacic area measuring around 2.0 cm of diameter. The left contralateral frontal lobe was edematous, slightly yellowish and friable. On the corresponding cut surface of the right frontal lobe, there was partial loss of cortical parenchyma and, demarcation between grey and white matter was not evident. The frontal lobes of both hemispheres were markedly affected followed by parietal and occipital lobes. No gross lesions were observed in the temporal lobe, hippocampus, cerebellum and brain stem. Extraneural gross lesions included moderate diffuse pulmonary congestion and edema.

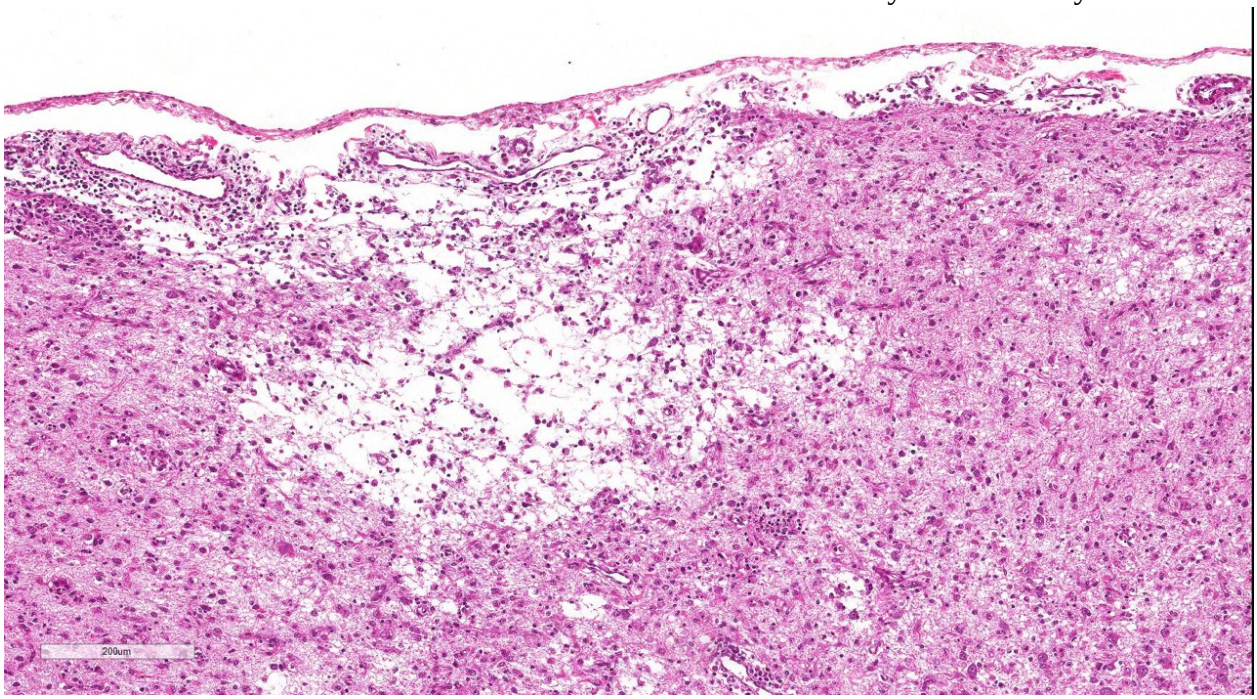
Laboratory Results: N/A

Histopathologic Description:
The cerebral cortex showed areas with markedly increased cellularity interspersed with multifocal cavitation, partially filled by numerous Gitter cells, characterizing malacia. Moderate to marked infiltration of cells, such as plasma cells and lymphocytes were observed around vessels and diffuse in the leptomeninges. In addition, the non-cavitation areas were characterized for neuropil vacuolization, neuronal necrosis, neuronophagia, astroglyosis with various gemistocytes, endothelial hyperplasia and hypertrophy. Single or binucleate gem-istocytes were more commonly seen adjacent to necrotic areas. These areas were

more intense in the frontal and parietal lobes of right side. In the white matter subjacent to the necrotic right frontal cortex there were plasma cells and lymphocytes perivascular cuffs associated to mild vacuolization and axonal degeneration. No lesions were found in the hippocampus, diencephalon (thalamus and hypothalamus), mesencephalon, cerebellum and medulla oblongata. Paraffin blocks of the cerebrum were selected and immunohistochemistry for CD3 (lymphocytes T marker) and CD 79a (lymphocytes B marker) was performed. The immunohistochemical analysis showed that positive CD3 cells were predominant in the perivascular cuffs, leptomeninges and also in the neuroparenchyma. Less numbers of positive CD79a cells were observed in the perivascular cuffs and leptomeninges but they were rarely observed in the neuroparenchyma.

Contributor's Morphologic Diagnosis: Cerebrum: marked multifocal to coalescing, necrotizing non-suppurative meningoencephalitis.

Contributor's Comment: Gross and clinical findings were consistent with multifocal to coalescing malacia involving the cerebral cortex. The histopathology allowed the definitive diagnosis of necrotizing meningoencephalitis (NME) characterized by multifocal to coalescing necrotizing non-suppurative meningoencephalitis, affecting the grey matter of frontal, parietal and occipital lobes, predominating in the right cerebral hemisphere. NME is a central nervous system (CNS) nonsuppurative inflammatory disorder of dogs, whose etiopathogenesis is poorly understood. Necrotizing leukoencephalitis (NLE) and granulomatous meningoencephalomyelitis (GME) are also CNS idiopathic inflammatory conditions. Nevertheless, each disease has unique histopathological features.[13] The NLE is characterized by inflammatory and necrotic



Cerebrum, dog. Higher magnification of necrotic lesions within the submeningeal grey matter. The neuropil surrounding this focus of cavitation is hypercellular with numerous astrocytes, Gitter cells, and extremely prominent vessels. (HE, 130X)

lesions similar to NME, however, the lesions are predominately observed in the white matter. The GME is another idiopathic canine disorder affecting mainly the cerebellum and brainstem. The disease is characterized by nodular granulomatous lesions containing macrophages and epithelioid cells, especially in subcortical regions. In addition, there were perivascular cuffs constituted of lymphocytes, plasma cells, macrophages, and some neutrophils.[9,13]

NME has been reported in various toy breeds including Pug dogs, Yorkshire terrier, Maltese, Chihuahua, Shih Tzu, West highland white terrier, Boston terrier, Spitz Japanese and Pinscher, Pekingese and French bulldog.[9,13] NME seems to be more common in females[5,8] and, has been diagnosed in dogs with six months to seven years of age.[13] However, the most common age range is from two[13] to four years.[5]

The clinical signs associated with NME are rapidly progressive and associate to the neuroanatomical localization.[13] The most common signs include seizures, depression, circling, visual deficit,[5,6] postural reaction deficits[4] and vestibule-cerebellar signs.[6,12] Definitive antemortem diagnosis is challenging because histopathology is mandatory. For most cases, the clinical diagnosis is presumptive, associating clinical signs and neuroanatomic localization, CSF analysis and advanced imaging tests to exclude other causes.[5,12,13] The prognosis of NME cases is poor due the progressive course of the disease, with lower survival rate in animals with seizures.[5] The dog of the present study showed clinical signs rapidly progressive and associated with the neuroanatomic localization of the lesions as observed in other studies.[13] The lesions and clinical signs of most cases of NME are related exclusively to the cerebrum, being an important characteristic for this condition.[12] Detailed neurological examination allows to determining the sites of the lesions, which are extremely important for presumptive diagnosis and treatment. In addition to neurological examination for supporting de clinical suspicion, is fundamental the epidemiological data, CSF analysis, cross-sectional imaging via computed tomography (CT) scan or magnetic resonance imaging (MRI) of the CNS and infectious diseases testing. Ct-guided brain biopsy and histopathological evaluation of brain tissue may be considered in cases of suspected NME.[5,13]

An autoimmune pathogenesis has been suggested for NME based on the presence of anti-astrocytic and anti-glial fibrillary acid protein (GFAP) autoantibodies in the cerebrospinal fluid (CSF) of affected dogs.[14] However, similar antibody levels occur in the CSF of dogs with GME, brain tumors and even in some clinically normal dogs.[14] A genomic study in Pugs with NME showed a single strong association with dog leukocyte antigen (DLA) class II, and supports the role of the immune system in the disorder.[6] Genetic predisposition also has been confirmed in Pug dogs with NME but it is believed there are additional influences contributing to the phenotypic expression of the disease.[2,6]

The immunohistochemical study showed predominance of T lymphocytes in the leptomeninges, around vessels and in the neuroparenchyma similar to the observed in other studies in dogs with NME.[7,12] A study using double-labeling immunofluorescence antibody demonstrated predominance of CD3+ T lymphocytes in close proximity or attached to astrocytes, and the cytoplasm and astrocytic processes were positive for IgG in the NME and NLE lesions. The involvement of the autoantibody to astrocytes in the NME cases supports the immune mediated pathogenesis hypothesis however, does not confirm if anti-astrocytic and anti-GFAP antibodies are a primary

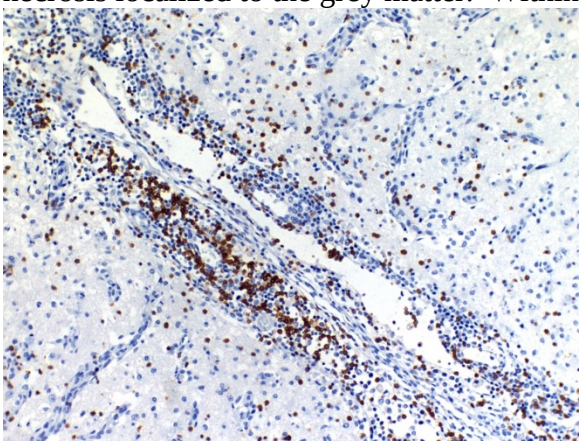
cause or a secondary consequence of NME.[12] A recent study showed that viral pathogens are not common in the brain of dogs with NME.[1]

JPC Diagnosis: Cerebrum: Meningo-encephalitis, necrotizing, lymp-hohistiocytic, multifocal to coalescing severe.

Conference Comment: As mentioned above, a genetic predisposition has been identified in Pug dogs with NME and a genetic susceptibility test is currently available for this breed. Additionally, a potential breed predisposition has also been identified in Maltese dogs, with gene abnormalities similar to what was seen in Pugs, although the degree of gene variation and sus-ceptibility may vary between breeds.[10] The specific genes involved, including those involved with the major histocompatibility complex class II (MHC II), are suspected to play a role in immune system regulation and have been implicated in central nervous system inflammatory conditions in people. The precise role by which MHC II abnormalities contribute to development of this disease is not completely understood, but MHC II molecules play an important role in antigen presentation and determining the reactivity of T cells. Other genes of interest include one that encodes for part of the interleukin 7 receptor, which is important in proliferation and survival of T and B lymphocytes. NME has been postulated to share some pathogenic mechanisms with multiple sclerosis in people and the above described genetic research may provide support for this.[10]

Although most commonly associated with small/toy breed dogs, NME has also been reported in a Staffordshire bull terrier.[4] Other small breeds not mentioned above that have been documented with NME include Coton de Tulear, Papillon, and Brussels Griffon although it is very uncommon in these breeds and their development of this condition is considered atypical.[3] The pathogenesis involved in development of this condition in atypical breeds is unclear as much remains elusive regarding the development, progression and pathogenesis of NME, as well as NLE and GME. In this case, the lesions were fairly characteristic for NME, particularly given the signalment. However, in some early or mild cases in less commonly affected breeds, there may be clinical and/or pathologic overlap with infectious diseases such as *Neospora caninum*, *Toxoplasma gondii* and viruses (e.g., canine distemper virus). Therefore, when the diagnosis is less straightforward but NME is still considered a top differential diagnosis, it is important for the diagnostic pathologist to consider and rule out infectious causes.

The subgross view of this section is striking and the lesions are characteristic for this entity. Within up to 50% of the section, there is loss of differential staining with areas of pallor, drop-out and liquefactive necrosis localized to the grey matter. Within



Cerebrum, dog. CD-3 positive cells predominate within perivascular cuffs throughout the neuropil. (Photo courtesy of: Universidade Federal de Minas Gerais, Escola de Veterinária, Departamento de Clínica e Cirurgia Veterinárias, Av. Antônio Carlos, *31270-901. Belo Horizonte, MG, Brazil* *www.vet.ufmg.br)*

necrotic areas, there is loss of neuropil and increased numbers of microglial cells, lymphocytes, and plasma cells; moderate numbers of gitter cells and hypertrophied astrocytes are also present. There is multifocal perivascular cuffing in the grey matter with affected vessels have reactive endothelium. Spongiosis is present multifocally and necrotic neurons are seen in low numbers. The meninges are multifocally expanded with edema and infiltrated by macrophages, lymphocytes and plasma cells. Conference participants discussed and contrasted the histologic lesion described here to what was seen in the GME case. Ventricular dilation and hydrocephalus ex-vacuo were also described; however, the moderator cautioned against over-interpreting this change in brachycephalic breeds which may normally have some degree of ventricular dilation.

Contributing Institution:
Universidade Federal de Minas Gerais, Escola de Veterinária, Departamento de Clínica e Cirurgia Veterinárias, Av. Antônio Carlos, 6627; 31270-901. Belo Horizonte, MG, Brazil www.vet.ufmg.br

References:

1. Barber RM, Porter BF, Li Q, et al. Broadly reactive polymerase chain reaction for pathogen detection in canine granulomatous meningoencephalomyelitis and necrotizing meningoencephalitis. *J Vet Intern Med.* 2012; 26(4):962-968.

2. Barber RM, Schatzberg SJ, Corneveaux JJ, et al. Identification of risk loci for necrotizing meningoencephalitis in pug dogs. *J. Hered.* 2011;102: 540-546.

3. Cooper JJ, Schatzberg SJ, Vernau KM, Summers BA, et al. Necrotizing Meningoencephalitis in atypical dog breeds: A case series and literature review. *J Vet Intern Med*. 2014; 28:198-203.

4. Estey CM, Scott SJ, Cerda-Gonzalez S. Necrotizing Meningoencephalitis in a large mixed-breed dog. *J Am Vet Med Assoc*. 2014; 245(11):1274-8.

5. Granger N, Smith PM, Jeffery ND. Clinical findings and treatment of non-infectious meningoencephalomyelitis in dogs: A systematic review of 457 published cases from 1962 to 2008. *Vet J***.** 2010; 184: 290-297.

6. Greer KA, Schatzberg SJ, Porter BF, et al. Heritability and transmission analysis of necrotizing meningoencephalitis in the Pug. *Res. Vet. Sci.*, 2009; 86: 438–442.

7. Higgins RJ, Dickinson PJ, Kube SA, et al. Necrotizing meningoencephalitis in five chihuahua dogs. *Vet Pathol.* 2008; 45: 336–346.

8. Levine JM, Fosgate GT, Porter B, et al. Epidemiology of Necrotizing Meningoencephalitis in Pug Dogs. *J Vet Intern Med.* 2008; 22: 961–968.

9. Park ES, Uchida K, Nakayama H. Comprehensive immunohistochemical studies on canine necrotizing meningoencephalitis (NME), necrotizing leukoencephalitis (NLE), and granulomatous meningoencephalomyelitis (GME). *Vet Pathol.* 2012, 49 (4):682-92.

10. Schrauwen I, Barber RM, Schatzberg SJ, Siniard AL, et al. Identification Identification of Novel Genetic Risk Loci in Maltese Dogs with Necrotizing Meningoencephalitis and Evidence of a Shared Genetic Risk across Toy Dog Breeds. *PLoS One*. 2014;9(11):e112755.

11. Shibuya MN, Fujiwara K, Imajoh-Ohmi S, et al. Autoantibodies against glial

fibrillary acidic protein (GFAP) in cerebrospinal fluids from Pug dogs with necrotizing meningoencephalitis. *J Vet Med Sci*. 2007; 69(3): 241-245.

12. Spitzbarth I, Schenk HC, Tipold A, Beineke A. Immunohistochemical Characterization of Inflammatory and Glial Responses in a Case of Necrotizing Leucoencephalitis in a French Bulldog. *J Comp Path***.** 2010; 142: 235-241.

13. Talarico LR., Schatzberg SJ. Idiopathic granulomatous and necrotizing inflammatory disorders of the canine central nervous system: a review and future perspectives. *J Sm Anim Pract.* 2010; 51: 138-149.

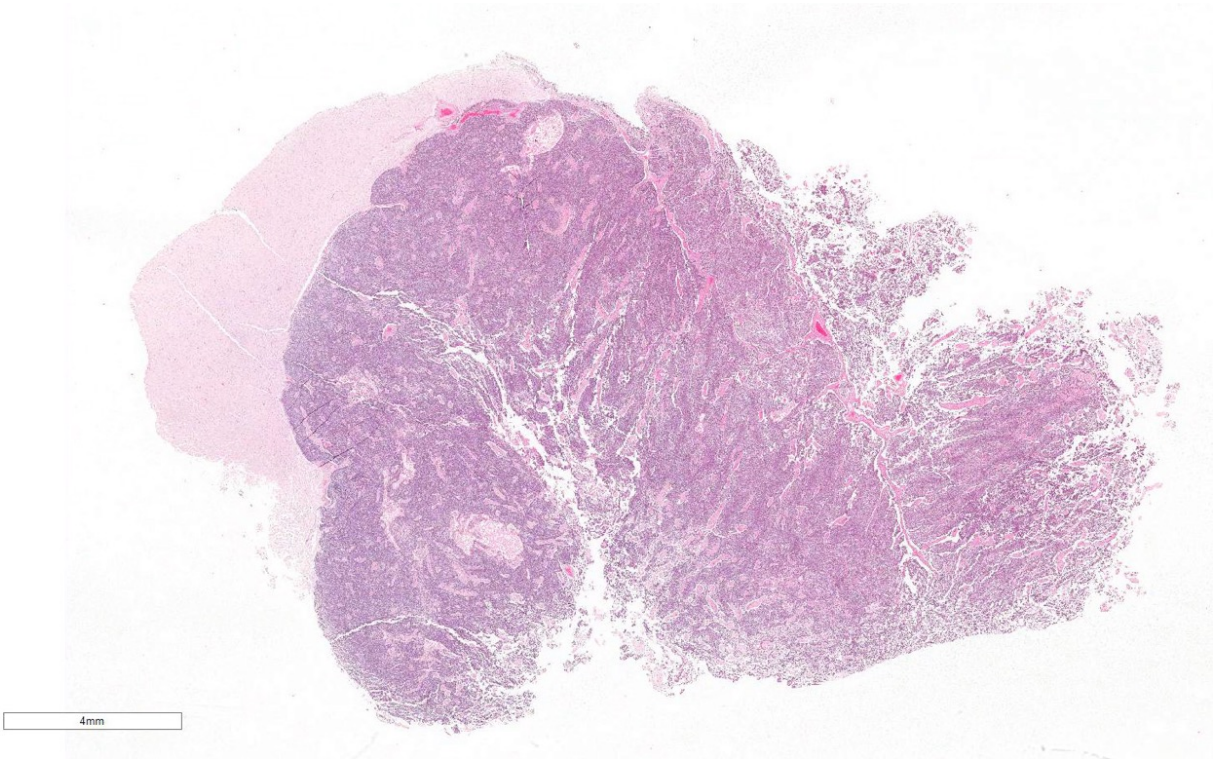
14. Toda Y, Matsuki N, Shibuya M, et al. Glial fibrillary acidic protein (GFAP) and anti-GFAP autoantibody in canine necrotising meningoencephalitis. *Vet Rec.* 2007; 161(8): 261-264.

CASE III: 11-123787 (JPC 4018117).

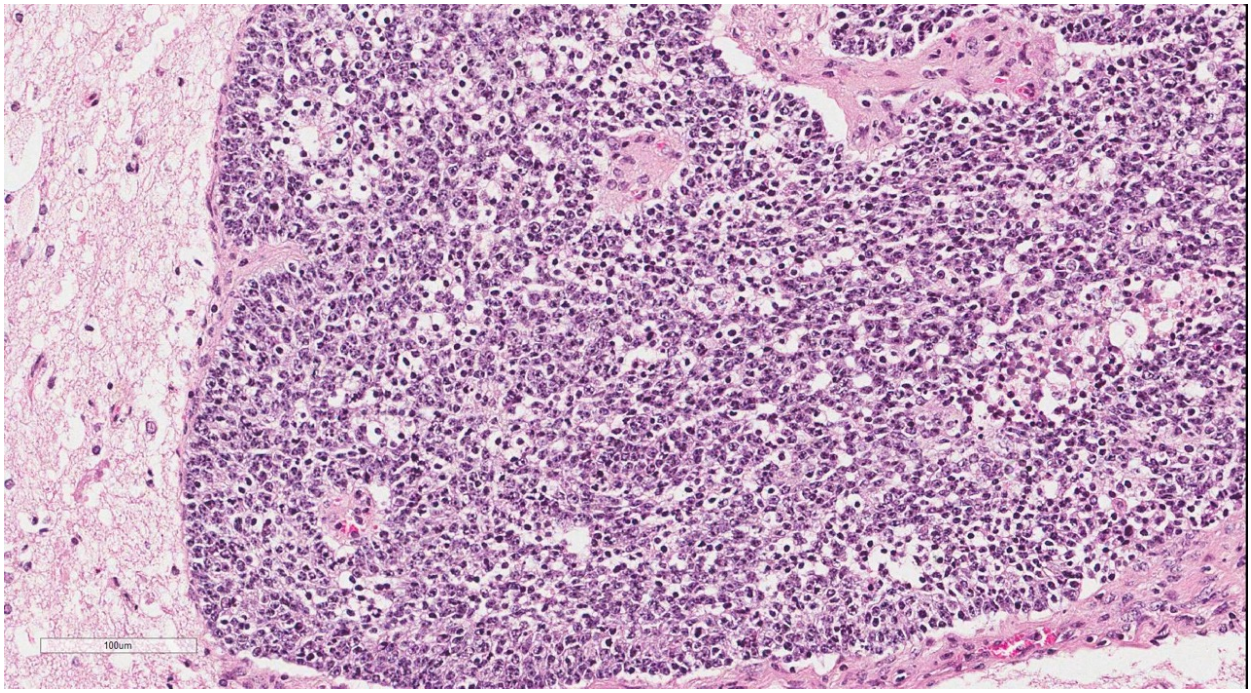
Signalment: Adult female white-tailed deer (*Odocoileus virginianus).*

History: The deer had no fear of humans or vehicles. He had human contact including petting and hand feeding. The animal was euthanized by gunshot wounds to the chest. The head was removed and send intact.

Gross Pathology: The head from the deer was submitted as part of routine Rabies surveillance by New York State Department of Health. A soft, white gelatinous mass effaced the right caudal nasal passage, cribriform plate and extended to involve the



Cerebrum, deer. The olfactory lobe of the cerebrum is compressed and largely effaced by a large, densely cellular neoplasm. (HE, 5X).



Cerebrum, deer. The neoplasm is composed of polygonal cells arranged in nests and cords which often palisade along vessels and the advancing edge of the neoplasm. (HE, 216X)

olfactory bulb of the brain.

Laboratory Results: Brain:

1. Fluorescent antibody test was negative for rabies
2. Immunohistochemistry for chronic wasting disease was negative
3. The brain mass was sent for aerobic bacterial culture and many *Aeromonas* sp. were isolated.

Histopathologic Description:

Brain: Compressing and effacing the neural parenchyma, invading into neuropil in locally extensive areas and extending to cut borders is a fairly well demarcated, large, densely cellular, expansile, variably encapsulated mass composed of sheets of neoplastic cells within an eosinophilic fibrillar background occasionally separated by few large blood vessels. The neoplastic cells are round to polygonal and occasionally palisade around central cores of fibrillary eosinophilic tangles (Homer-Wright rosettes) (Fig. 1), central empty lumens (Flexner-Wintersteiner rosettes) (Fig. 2) and blood vessels (pseudorosettes) (Fig. 3). Neoplastic cells are approximately 12-15µm in diameter with scant eosinophilic cytoplasm and indistinct cell margins. Nuclei are round with coarsely stippled chromatin and indistinct nucleoli. There is mild anisocytosis and anisokaryosis. Up to 10 mitotic figures are counted in 10 high power (40X) fields. Admixed with the neoplastic cell population are few round 8-10µm diameter cells with hyperchromatic nuclei, variably distinct cell borders and pale vacuolated or scant fibrillar eosinophilic cytoplasm. There are scattered locally extensive areas of necrosis and small foci of hemorrhage. Within the periphery of the mass are few scattered hemosiderin laden macrophages.

Immunohistochemistry was performed on a section of the brain mass. Diffusely, neoplastic cells exhibited moderate to strong cytoplasmic immunoreactivity for neuron specific enolase (NSE) (Fig. 4) and a

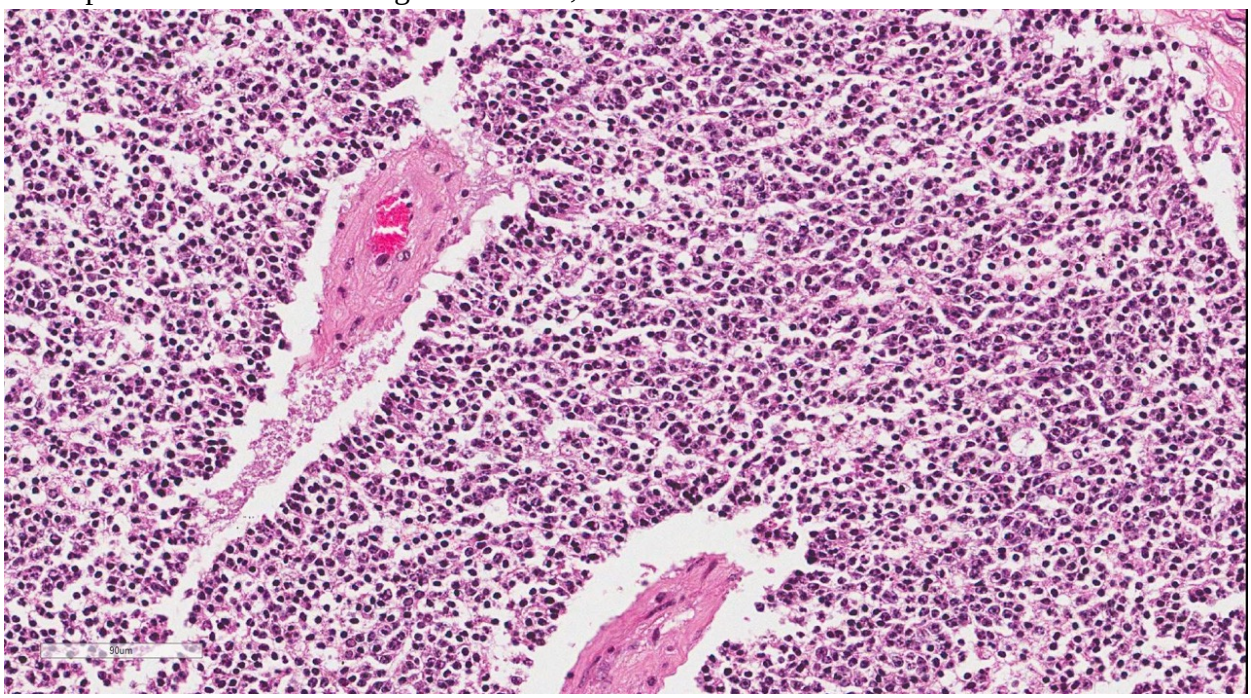
subpopulation of the cells showed strong cytokeratin immunoreactivity (Fig. 5).

Contributor's Morphologic Diagnosis:
Brain, olfactory lobe: Neuroendocrine carcinoma

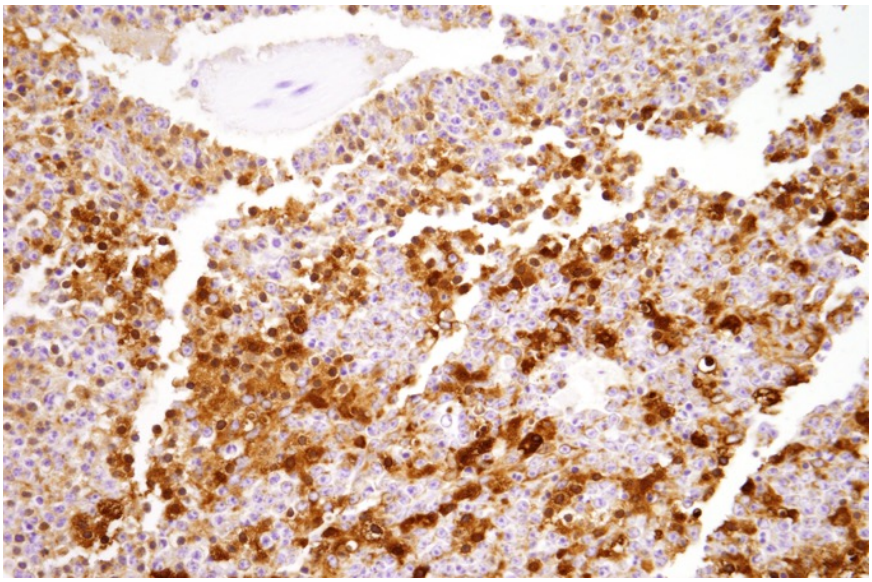
Contributor's Comment: The two primary differential diagnoses for this tumor are neuroendocrine carcinoma and olfactory neuroblastoma. These two tumor types have similar histologic features and diff-erentiation often requires ultrastructural analysis and/or immunohistochemistry.

Neuroendocrine tumors are a diverse group of neoplasms derived from cells which (A) produce a neurotransmitter, neuromodulator or neuropeptide hormone, (B) contain dense core granules on electron microscopy and (C) do not have axons or form synapses.[10] Neuroendocrine tumors have been described in the almost every organ and are characterized by a typical pattern of small to medium sized cells with granular eosinophilic chromatin arranged in nests, cords and packets separated by fine fibrovascular stroma. Rosette formation and peripheral palisading is variably present. Ultrastructurally, tumor cells contain round, membrane bound dense core granules and have distinct intercellular junctions. Cells are argyrophilic (Grimelius positive) and show positive immunoreactivity for neuron specific enolase, chromogranin, cytokeratin and neuropeptides. Nasal neuroendocrine carcinomas have been reported in dogs[14] and horses.[16] In dogs, nasal neuroendocrine tumors have been reported to invade the underlying bone and adjacent sinuses, but not the brain.[13]

Olfactory neuroblastomas (ONB) or esthesioneuroblastomas are rare tumors, believed to be derived from the olfactory neuroepithelium. These tumors arise primarily in the upper nasal cavity and adjacent paranasal sinuses and frequently invade the cribriform plate and the intracranial cavity. Histological features of well-differentiated ONBs include growth in circumscribed lobules separated by richly



Cerebrum, deer. Neoplastic cells form pseudorosettes around blood vessels (and their edematous borders) due to their propensity to form palisades. (HE, 240X)



Cerebrum, deer. Neoplastic cells exhibit strong cytoplasmic positivity for neuron-specific enolase. (anti-NSE, 400X). (Photo courtesy of: Department of Biomedical Sciences, College of Veterinary Medicine, T4 018 Veterinary Research Tower, Cornell University, Ithaca, NY 14853, http://www.vet.cornell.edu/biosci/pathology/services.cfm)

vascularized fibrous stroma, or less commonly, a diffuse growth pattern. Pseudorosettes and true rosettes can be found. The neoplastic cells are surrounded by neurofibrillary matrix and have sparse cytoplasm and round to oval nuclei with inconspicuous nucleoli. Nuclear pleomorphism, high mitotic activity and necrosis are frequently observed. Ultrastructural characteristics include dense core neurosecretory granules and neurite-like cell processes with neurofilaments and neurotubules. Like neuroendocrine carcinomas, these tumors are argyrophilic and often show positive immunoreactivity for neuron specific enolase. Synaptophysin, neurofilament protein, class III beta-tubulin and microtubule-associated protein-2 are less frequently detected. Cells are typically not cytokeratin immunoreactive. Olfactory neuroblastomas have been described previously in dogs, cats,[2, 4, 13] horses[5] and a cow.[1]

Other than cutaneous fibromas, neoplasms are uncommonly reported in white tailed deer. Central nervous tumors described in white tailed deer include astrocytomas,[7] ependymomas[10] and a mixed primitive neuroectodermal and rhabdomyoblastic tumor.[6] Nasal tumors have not been previously reported in white tailed deer, but nasal adenocarcinomas have been described in Persian fallow deer and Eld's deer.[3,9]

A tentative diagnosis of neuroendocrine carcinoma was made in this case based on cytokeratin immunopositivity, which is a consistent feature of neuroendocrine carcinomas and uncommon in olfactory neuroblastomas; however, invasion through the cribriform plate and the fibril background of this tumor are more consistent with a diagnosis of olfactory neuroblastoma. A definitive diagnosis would require electron microscopy.

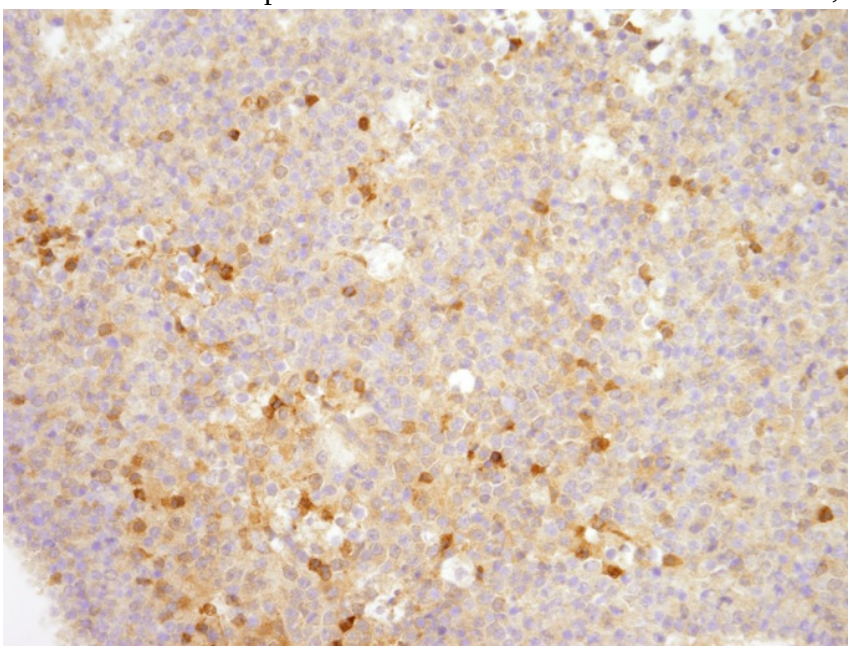
JPC Diagnosis: Cerebrum: Neuroendocrine carcinoma.

Conference Comment: The conference description was closely aligned with the contributor's description above; however, there was considerable discussion regarding the presence of and types of rosettes, with agreement that the poor fixation in this case gave a false appearance of Homer-Wright and Flexner-Wintersteiner rosettes. The moderator commented on the marked degree of vascular hyalinization and expansion of the perivascular space by dense, hyalinized, bright eosinophilic material. The differential diagnosis list for this lesion included oligodendroglioma in addition to olfactory neuroblastoma discussed above.

Immunohistochemical stains that can be useful in diagnosing oligodendroglioma include cyclic nucleotide phosphatase (CNPase) and oligodendrocyte transcription factor 2 (olig2); the absence of glial fibrillary acidic protein (GFAP) may also aid in the diagnosis.[8] Other differentials discussed included primitive neuroectodermal tumor and ependymoma. In general, neoplasms associated with the cribiform plate often extend from outside the CNS into the olfactory bulb / frontal lobe as opposed to extending from the brain outward through the cribiform plate.

Primitive neuroectodermal tumors include neuroblastoma, medulloblastoma and neuresthesioblastoma and are composed of 'neuroblasts' and generally arranged in sheets with the presence of Homer-Wright rosettes. Immunohistochemical markers used in the diagnosis of this type of tumor in dogs and cats include neuron-specific enolase (NSE), synaptophysin, a neuron-specific nuclear protein known as neuronal nuclei (NeuN) and neurofilament protein (NFP).[8] These tumors may have a heterogenous or irregular staining pattern due to the presence of both neural and glial differentiation, which may aid in the diagnosis. Other stains which are reported as positive in some subsets of PNETs include vimentin and S100 among others. Stem cell markers which may be useful include nestin, beta III tubulin and doublecortin. Ependymomas are most often associated with the ventricle in some fashion and classically have a papillary pattern with ependymal rosette formation, although these features are not present in all cases. These tumors are generally GFAP and vimentin positive.[8]

Neuroendocrine carcinomas are uncommon neoplasms which derive from neuroendocrine cells distributed throughout the body. Other locations for neuroendocrine neoplasms include the gastrointestinal tract, lungs and integument. The most germane differential diagnosis in this case is olfactory neuroblastoma as discussed above, due in large part to overlapping histologic features with neuroendocrine carcinoma. Features which can aid in ultrastructural differentiation include the lack of microtubule containing neural processes in neuroendocrine carcinoma,[9] as well as the IHC profiles discussed above. A pancytokeratin immunohistochemical (IHC) stain performed at the JPC showed diffuse,



Cerebrum, deer. Some neoplastic cells exhibit strong cytoplasmic positivity for cytokeratin. (anti-NSE, 400X). (Photo courtesy of: Department of Biomedical Sciences, College of Veterinary Medicine, T4 018 Veterinary Research Tower, Cornell University, Ithaca, NY 14853, http://www.vet.cornell.edu/biosci/pathology/services.cfm)

strong, cytoplasmic immunoreactivity and a neurofilament IHC was negative, supporting the contributor's diagnosis of neuroendocrine carcinoma.

Contributing Institution:

Department of Biomedical Sciences
College of Veterinary Medicine
T4 018 Veterinary Research Tower
Cornell University
Ithaca, NY 14853
http://www.vet.cornell.edu/biosci/pathology/services.cfm

References:

1. Anderson BC, Cordy DR. Olfactory neuroblastoma in a heifer. *Vet Pathol.* 1981; 18: 536.

2. Brosinski K, Janik D, Polkinghorne A, Von Bomhard W, Schmahl W. Olfactory neuroblastoma in dogs and cats--a histological and immunohistochemical analysis. *J Comp Pathol*. 2012; 146: 152-9.

3. Brownstein DG, Montali RJ, Bush M, James AE. Nasal carcinoma in a captive Eld's deer. *J Am Vet Med Assoc*. 1975; 167: 569-71.

4. Cox NR, Powers RD. Olfactory neuroblastomas in two cats. *Vet Pathol.* 1989; 26: 341-3.

5. Do¨pke C, Gro¨ne A, Borstel Mv, et al. Metastatic esthesioneuroblastoma in a horse. *J Comp Path*. 2005; 132: 218–222.

6. Holscher MA, Page DL, Powell HS, Kord CE. Mixed primitive neuroectodermal and rhabdomyoblastic tumor in the brain of a wild deer. *J Wildl Dis*. 1974; 10: 201.

7. Howard DR, Drehbiel JD, Fay LD, Whitenack DL. Visual defects in white-tailed deer from Michigan: six case reports. *J Wildl Dis*. 1976; 12: 143-7.

8. Johnson GC, Coates JR, Wininger F. Diagnostic immunohistochemistry of canine and feline intracalvarial tumors in the age of brain biopsies. *Vet Pathol*. 2014; 51(1):146-160.

9. Kubo M, Matsuo Y, Okano T, Sakai H, et al. Nasal neuroendocrine carcinoma in a free-living Japanese Raccoon dog (*Nyctereutes procyonoides viverrinus*). *J. Comp Path*. 2009;140:67-71.

10. Langley K. The neuroendocrine concept today. *Annals New York Academy Sci.* 1994; 733: 1–17.

11. Movassaghi AR, Davazdah Emami MR. Ethmoturbinate adenocarcinoma in a Persian fallow deer (Cervus dama mesopotamica). *Vet Rec*. 2001; 149: 493-4.

12. Nettles VF, Vandevelde M. Thalamic ependymoma in a white-tailed deer. *Vet Pathol.* 1978; 15:133-5.

13. Parker VJ, Morrison JA, Yaeger MJ. Olfactory neuroblastoma in a cat. *J Feline Med Surg.* 2010;12: 867-71.

14. Patnaik AK, Ludwig LL, Erlandson RA. Neuroendocrine carcinoma of the nasopharynx in a dog. *Vet Pathol*. 2002; 39: 496.

15. Sako T, Shimoyama Y, Akihara Y, Ohmachi T, Yamashita K, Kadosawa T, Nakade T, Uchida E, Okamoto M, Hirayama K, Taniyama H. Neuroendocrine Carcinoma in the Nasal Cavity of Ten Dogs. *J Comp Pathol*. 2005; 133: 155-163.

16. van Maanen C, Klein WR, Dik KJ, van den Ingh TS. Three cases of carcinoid in the equine nasal cavity and maxillary sinuses: histologic and immunohistochemical features. *Vet Pathol.* 1996; 33(1): 92-5.

CASE IV: WHL 14317601 (JPC 4067276).

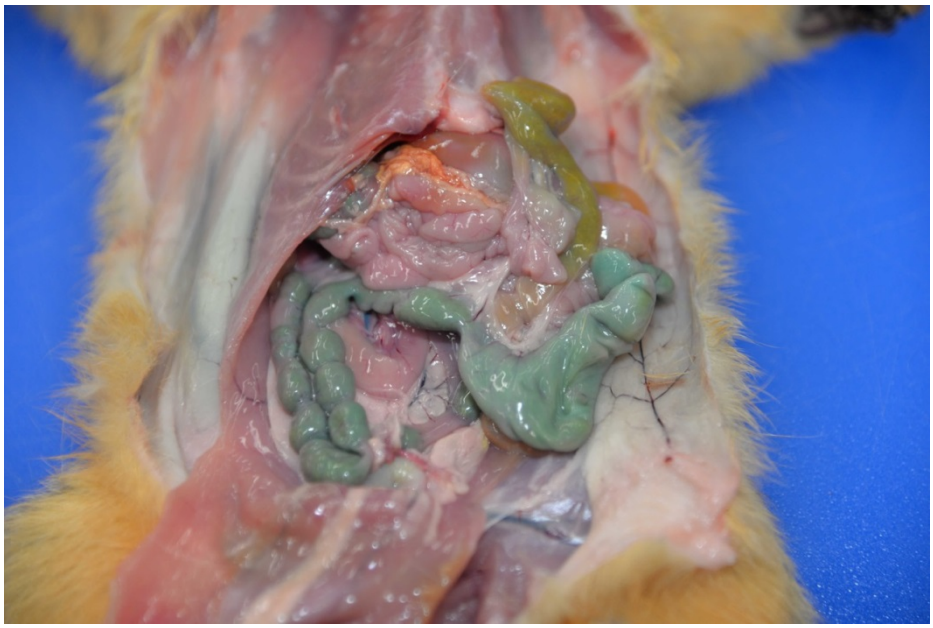
Signalment: Adult male fox squirrel (*Sciurus niger*)
Adult female fox squirrel (*Sciurus niger*)

History: Over the course of several days, five squirrels were found dead under a tree at a residence in Colorado. Prior to death, all of the squirrels had similar clinical signs which included hindquarter paralysis, lethargy, and heavy breathing.

Gross Pathology: Two fox squirrels were presented for postmortem examination in good body condition with minimal autolysis. No evidence of trauma was identified. The adult male squirrel had no significant gross lesions and stomach contents were within normal limits, including grainy yellow-brown ingesta. The adult female had turquoise-green granular material on the fur of the upper lip and similar material within the stomach. The colonic contents were stained a distinct turquoise-green.

Laboratory Results: Adipose tissue contained desmethylbromethalin.

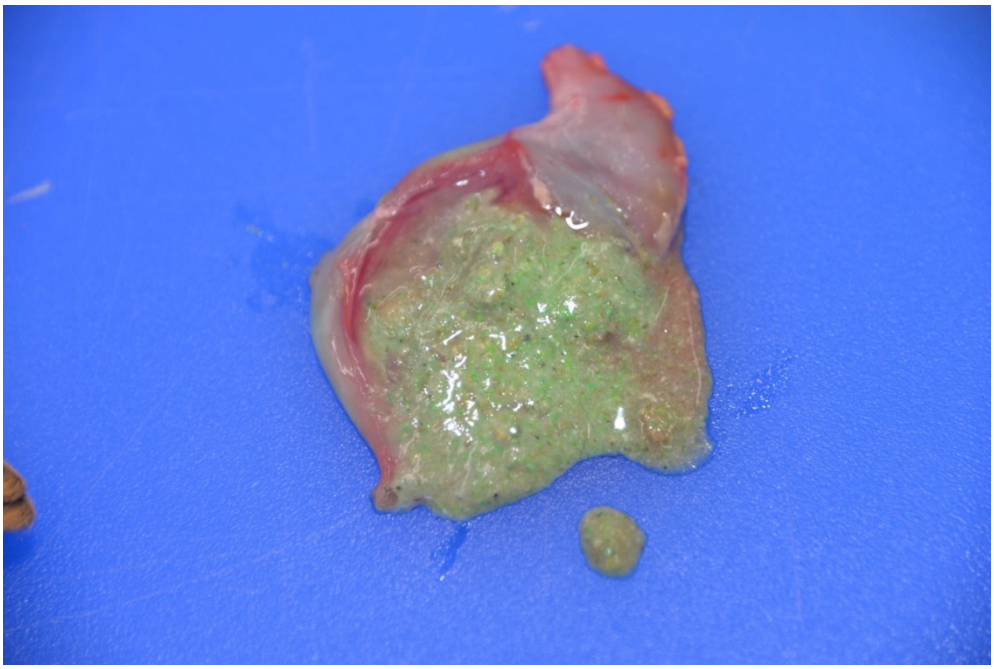
Histopathologic Description:
Brain, 2 sections (cerebellum and cerebral cortex including hippocampus): Diffusely throughout both sections, the white matter is characterized by moderate to severe extracellular vacuolization. Vacuoles are formed by variably swollen myelin sheaths which occasionally coalesce into large extracellular clear spaces. Dilated myelin sheaths contain normal to minimally swollen axons. Scattered throughout the grey matter of the cerebral cortex and rarely within the hippocampus are low numbers of neurons with degenerative changes, including central chromatolysis and occasional pyknosis. Rare neurons are shrunken, angular, and hypereosinophilic with karyolysis (necrosis). Clefts within the perikaryon of multiple neuronal cell bodies are consistent with fixation artifact.



Colon, squirrel. At necropsy, the colon of one squirrel had bright turquoise green contents. (Photo courtesy of: Colorado State University, Department of Microbiology, Immunology and Pathology,College of Veterinary Medicine and Biomedical Sciences, http://csu-cvmbs.colostate.edu/academics/mip/)

Contributor's Morphologic Diagnosis: Cerebellum and cerebral cortex: Vacuolar myelinopathy, severe, diffuse, with mild, multifocal neuronal degeneration and necrosis.

Contributor's Comment: Bromethalin toxicosis was strongly suspected based on the history and collective gross and histologic findings. This suspicion was



Stomach, squirrel. The stomach of one squirrel had a small amount of turquoise green grainy ingesta. (Photo courtesy of: Colorado State University, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences , http://csu-cvmbs.colostate.edu/academics/mip/)

confirmed by the presence of desmethylbromethalin in the adipose tissue. Desmethylbromethalin is a toxic metabolite of bromethalin, a potent neurotoxin and the active ingredient in a variety of rodenticides. The mechanism of action involves un-coupling of oxidative phosphorylation, resulting in decreased ATP production and diminished Na+/K+ pump activity.[9,10] In the CNS, the net result is severe, acute fluid retention and a dramatic elevation in cerebrospinal fluid pressure. Bromethalin is metabolized to desmethylbromethalin th-rough N-demethylation by hepatic mixed-function oxygenases and is excreted predominantly in the bile. The oral LD_{50} is 2.38-5.6 mg/kg in the dog and 0.4-0.71 mg/kg in the cat.[3,9] A relative resistance to toxicity has been demonstrated in species unable to metabolize bromethalin to desmethylbromethalin (e.g. guinea pigs with an LD_{50} of 1000 mg/kg).[10] Short of chemical confirmation, diagnosis of bromethalin toxicosis is based on likelihood of exposure and development of corresponding clinical signs, including muscle tremors, seizures, dypsnea, hyperexcitability, hind limb ataxia, and paresis to paralysis. Severity and onset (2-14 hours post-ingestion) are dose-dependent.[3]

Bromethalin is indistinguishable from anticoagulant rodenticides in appearance and color, and gross lesions are uncommon. Diffuse white matter vacuolization is the characteristic histologic lesion, and ultrastructural studies have demonstrated intramyelinic vacuoles with separation and splitting of myelin lamellae.[4,5] Luxol fast blue-periodic acid Schiff stain has demonstrated myelin displacement due to edema with no apparent net myelin loss.[5] Hypertrophied astrocytes and oli-godendrocytes have also been reported.[5] Vacuolization of the optic nerve occurs in most cases.[4,5] Similar white matter vac-uolization is seen with triethyltin and hexachlorophene neurotoxicosis.[7,8]

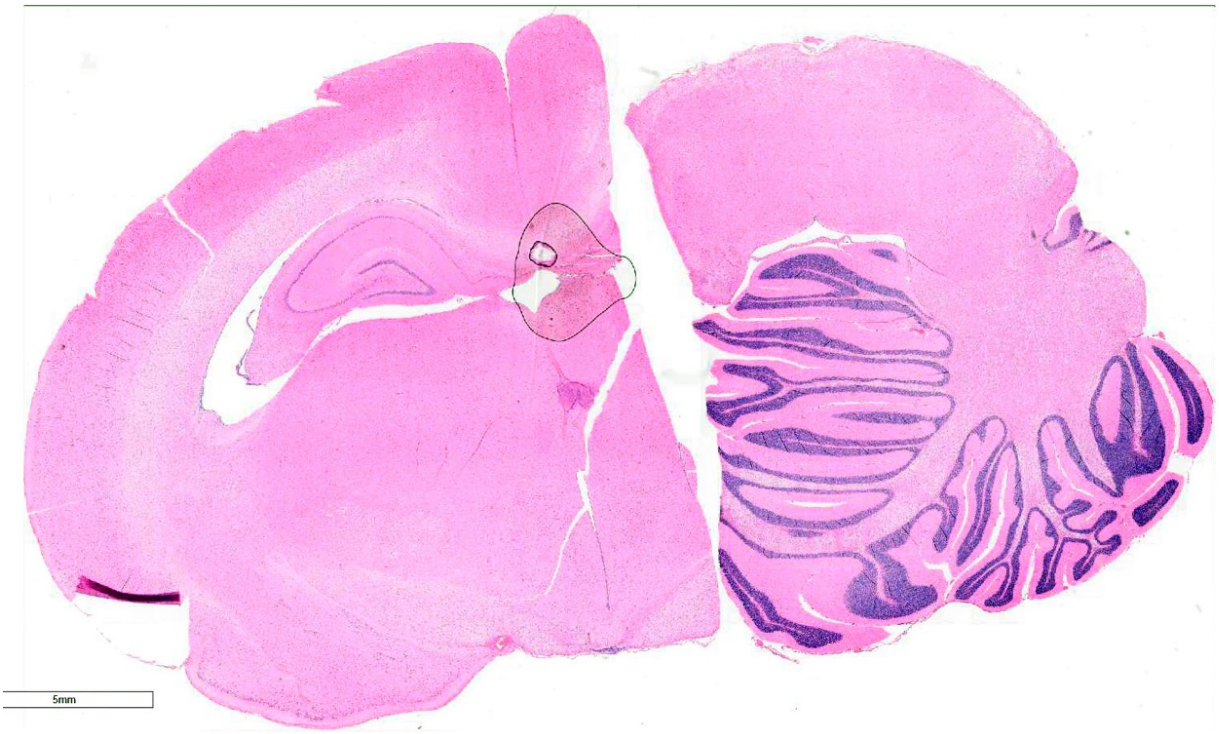
Bromethalin use has increased in recent years in association with new regulations prohibiting residential use of second generation anticoagulant rodenticides. While bromethalin remains readily available for over-the-counter sales, many anticoagulant rodenticides with similar names (e.g. brodifacoum, bromadiolone) have been removed. Thus, bromethalin

toxicity is gaining in importance due to increased popularity of neurotoxic rodenticides.

JPC Diagnosis: Cerebrum and cerebellum, white matter: Vacuolar myelinopathy, diffuse, severe.

Conference Comment: Conference participants discussed this lesion as being very 'quiet' histologically, with minimal if any response to the swelling of myelin sheaths. The moderator discussed how this lesion contrasts with a demyelinating lesion, which manifests histologically as a patchy, less diffuse distribution and with at least some degree of glial response. In this example of bromethalin toxicity, there is a distinct absence of swollen axons and spheroids, and the oligodendrocytes appear quiescent.

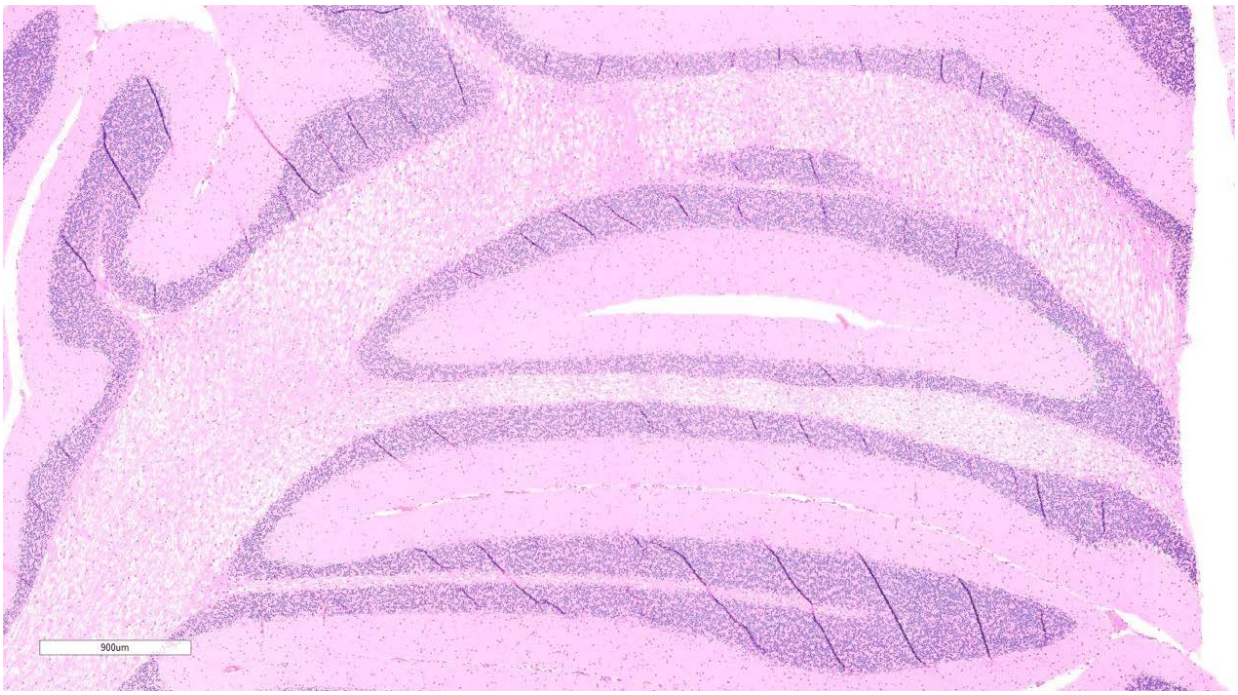
The differential diagnosis discussed by participants for a similar histologic lesion in other species included other toxicants, such as hexachlorophene and ammonia, as well as plant toxins seen in various parts of the world, such as *Stypandra* spp. in Australia and *Helichrysum* spp. in Africa. The lesions of bromethalin in the central nervous system of these squirrels also bear resemblance to those of avian vacuolar myelinopathy, which is seen in North America secondary to a cyanobacterial toxin that grows on non-native aquatic vegetation. The condition is frequently lethal and affects various avian species, such as bald eagles and American coots in the southeastern United States.[6] Another cause of similar white matter specific vacuolar change includes branched-chain alpha-ketoacid decarboxylase deficiency (maple syrup urine disease) in cattle.



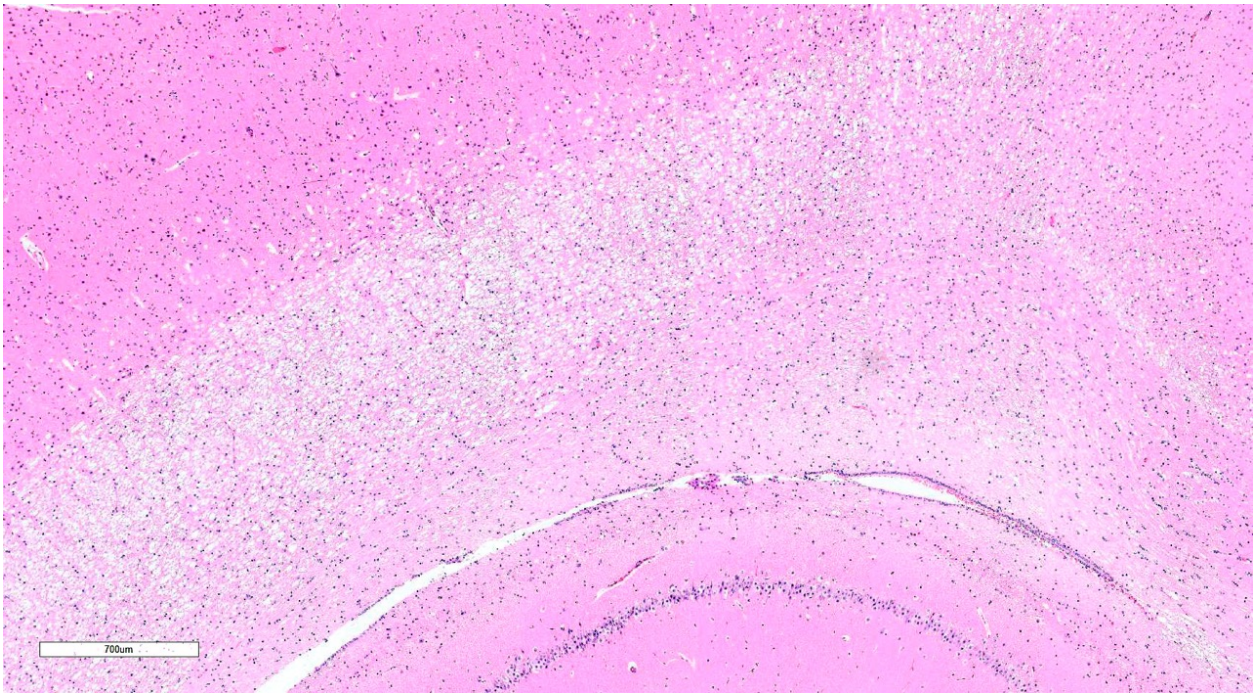
Cerebrum and cerebellum. squirrel: The white matter of the corona radiate, corpus callosum, and spinocerebellar tracts, as well as within the cerebellar folia exhibits diffuse pallor. (HE, 5X)

Despite the apparent frequency with which bromethalin intoxication occurs in domestic animals and wildlife species, there are surprisingly few reports in the recent professional literature. In addition to the more acute syndrome discussed above, a paralytic syndrome is also described when concentrations below the LD_{50} are ingested; it includes ataxia, CNS depression, and paralysis which may develop over a period of days and worsen over a period of weeks.[2] The acute syndrome has also been reported to occur when smaller doses (below the LD_{50}) are ingested in dogs and may relate to treatment with activated charcoal, which can result in idiosyncratic hypernatremia in rare cases. Dramatic changes in sodium levels can result in CNS associated clinical signs and lesions, including cortical laminar necrosis in cases of salt intoxication; conversely, osmotic demyelination can occur in cases where there is a sudden increase in sodium in a hyponatremic animal. Histologic lesions in osmotic demyelination and the changes seen in bromethalin toxicity can have similarities, although in osmotic demyelination there is myelin and oligodendrocyte loss which does not occur with bromethalin intoxication.[1]

The green-tinged or turquoise coloring seen in the gross image is characteristic of dyes used in certain rodenticides, as well as in some fertilizers and pesticides,[2] which can make confirmation of bromethalin intoxication challenging.[1] The highest concentration of desmethylbromethalin is usually found in adipose tissue, which is the most important tissue sample for diagnostic toxicology testing in suspected cases of bromethalin intoxication.[2] Bromethalin is not only lipid-soluble, but also readily crosses the blood brain barrier.[1] Additionally, in cases of mild white matter vacuolation, both light microscopy and ultrastructural examination may not be able to precisely differentiate the changes of bromethalin intoxication from those of autolysis, the latter of which are especially common in wildlife species.[2] Neuron-specific nuclear protein (NeuN) and glial fibrillary acidic protein (GFAP) may be



Cerebellum, squirrel. The cerebellar white matter contains numerous well-defined vacuoles which impart a diffuse pallor to it. (HE, 80X)



Cerebrum, squirrel. The vacuolated white matter of the internal capsule does not have a cellular infiltrate, as the lesion is primarily intramyelinic edema without axonal destruction. (HE, 80X).

useful in distinguishing subtle changes from autolytic artifact in questionable cases. Decreased NeuN immunoreactivity can indicate neuronal loss and/or metabolic stress and increased GFAP immunoreactivity is indicative of reactive astrocytosis.[1]

Contributing Institution:
Colorado State University
Department of Microbiology, Immunology and Pathology
College of Veterinary Medicine and Biomedical Sciences
http://csu-cvmbs.colostate.edu/academics/mip/

References

1. Bates MC, Roady P, Lehner AF, Buchweitz JP, et al. Atypical bromethalin intoxication in a dog: pathologic features and identification of an isomeric breakdown product. *BMC Vet Res*. 2015; 11(244):1-9.

2. Bautista AC, Woods LW, Filigenzi MS, Puschner B. Bromethalin poisoning in a raccoon (*Procyon lotor*): diagnostic considerations and relevance to nontarget wildlife. *J Vet Diagn Invest*. 2014; 26(1):154-157.

3. Dorman, DC, Parker, AJ, Buck, WB. Bromethalin Toxicosis in the dogs. Part I: Clinical Effects. *J Am Anim Hosp Assoc*. 1990; 26(6): 589-594.

4. Dorman, DC, Simon, J, Harlin, KA, Buck, WB. Diagnosis of bromethalin toxicosis in the dog. *J Vet Diagn Invest*. 1990; 2:123-128.

5. Dorman, DC, Zachary, JF, Buck, WB. Neuropathologic findings of bromethalin toxicosis in the cat. *Vet Pathol*. 1992; 29:139-144.

6. Haynie RS, Bowerman WW, Williams SK, Morrison SK. Triploid grass carp susceptibility and potential for disease transfer when used to control aquatic

vegetation in reservoirs with avian vacuolar myelinopathy. *J Aquat Anim Health*. 2013; 25(4):252-259.

7. Nakaue, HS, Dost, FN, Buhler, DR. Studies on the toxicity of hexachlorophene in the rat. *Tox and Appl Pharm.* 1973; 24: 239-249.

8. O'Shaughnessy, DJ, Losos, GJ. Peripheral and central nervous system lesions caused by triethyl- and trimethyltin salts in rats. *Tox Path*. 1986; 14(2).

9. Peterson, ME. Bromethalin Topical Review. *Topics in Compan An Med*. 2013; 28:21-23.

10. van Lier, RB, Cherry, LD. The toxicity and mechanism of action of bromethalin: a new single-feeding rodenticide. *Fundam Appl Toxicol*. 1988 Nov; 11(4):664-72.