Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2017-2018

C o n f e r e n c e 22

18 April 2018

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CASE I: 15L-2067C (JPC 4066542).

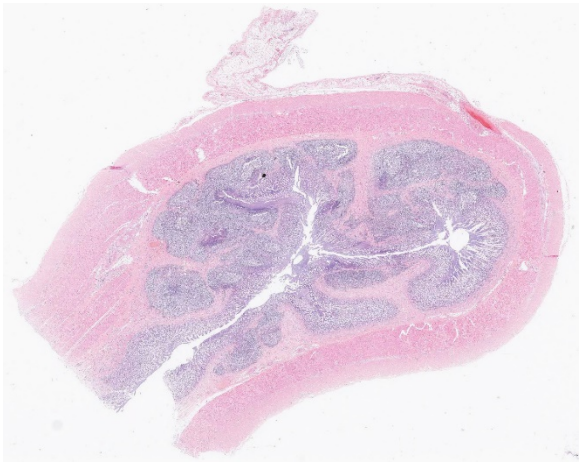
Signalment: 3-year-old, male, Warmblood (*Equus caballus*), equine.

History: Acute signs of colic, poor general condition

Gross Pathology: Diffuse thickening and white discoloration of the caudal 2/3 of the small intestine, evident from the serosal surface.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.):

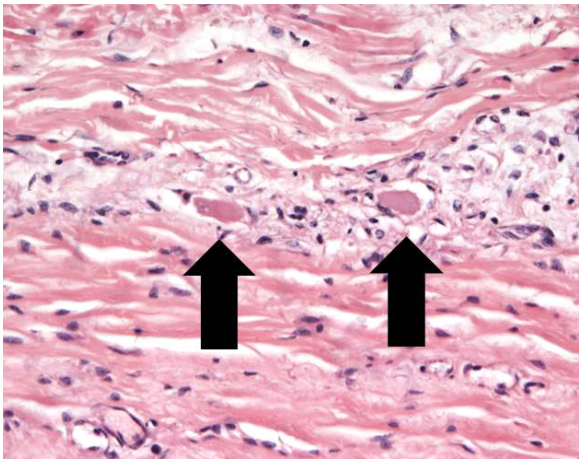
- CD3: Diffusely well-differentiated small lymphocytes show moderate to strong immunopositivity for CD3. Large blastic cells show diffuse moderate membranous positive signal for CD3. CD3 positive cells represent >95% of the lymphoid cells recognized.
- CD79a: Scattered very rare small well-differentiated lymphocytes are positive for CD79a antibody. Large blastic cells are negative. CD79a positive cells represent <5% of the lymphoid cells recognized.
- CD20: Large blastic cells are diffusely negative for CD20 antibody. CD20 positive cells represent <5% of the lymphoid cells recognized.



Ileum, horse. A neoplastic infiltrate extends from the Peyer's patches into the surrounding mucosa and submucosa. (HE, 4X)

- Synaptophysin: An intense strong cytoplasmic positive stain is detected in the scattered remaining neurons of the Auerbach's and Meissner's plexuses

Microscopic Description:
Ileum – The intestinal epithelium is diffusely effaced by amorphous eosinophilc material and nuclear debris (post mortem autolysis – artifact). Multifocally, some crypts are expanded by eosinophilic and basophilic amorphous material (cellular and nuclear debris/crypt abscesses) with occasional areas characterized by granular basophilic material (mineralization – dystrophic). The autonomic ganglia of the submucosa (Meissner's) and tunica muscularis (Auerbach's) exhibited a marked reduction in numbers of neuronal bodies which occasionally appear hyper-eosinophilic with rounded cell margins and peripheralized hyperchromatic nuclei (chromatolysis). Within the plexuses an increased number of satellite cells is also recognized. Expanding from the submucosal gut associated lymphoid tissue (GALT), and diffusely infiltrating and effacing the mucosa, submucosa and occasionally reaching the superficial tunica muscularis of the ileum there is a densely cellular, poorly demarcated, unencapsulated and infiltrative proliferation of round cells arranged in sheets within a fine fibrous stroma. Cells are 20-25 μm in diameter, round with distinct cell borders. They exhibit scant to moderate lightly basophilic cytoplasm and central round nuclei with finely stippled to vesicular chromatin and one to three nucleoli. The follicular structure of GALT is diffusely effaced or lost. Mitotic index is 1-4 mitosis per high power field and occasional bizarre mitoses are recognized. Large numbers of small well-differentiated lymphocytes are infiltrating in between the atypical blastic cells. Large numbers of atypical lymphoid cells are found infiltrating lymphatic vessels and/or veins of the lamina propria,



Ileum, horse. There is a significant reduction of numbers of neurons within the Auerbach's and myenteric plexi. Remaining neurons exhibit chromatolysis. (HE, 400X) (Photo courtesy of: of Liverpool, Leahurst Campus, Chester High Road, Neston, Wirral, UK, CH64 7TE.)

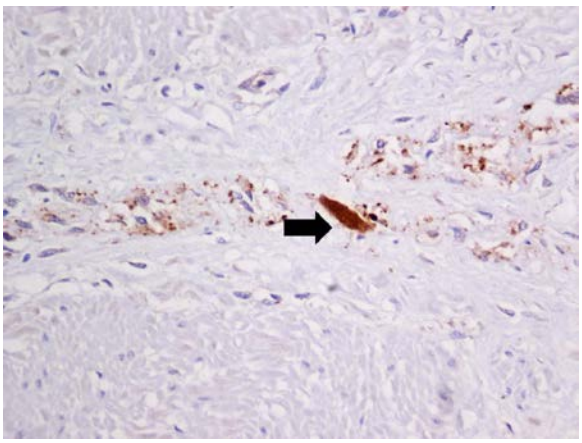
submucosa, muscularis and serosa. Scattered basophilic, star-shaped bodies (mineralization – asteroid bodies) are recognized within the intima of medium sized to large submucosal arteries (incidental finding).

Contributor's Morphologic Diagnosis:
Ileum, diffuse sub-acute severe Meissner's and Auerbach's plexuses chromatolysis and neuron loss.
Ileum, atypical lymphoproliferative disorder consistent with intestinal lymphoma.
Name of the condition: Equine dysautonomia
Name of the disease: Equine grass sickness

Contributor's Comment: In the present case, the submucosal (Meissner's) and myenteric (Auerbach's) plexuses showed severe neuronal chromatolysis (de-generation) consistent with equine grass sickness (equine dysautonomia). Immuno-histochemical staining with an anti-synaptophysin antibody revealed a marked reduction in numbers of neurons within the neuronal ganglia of the Meissner's and Auerbach's plexuses and a highly increased

immune-signal within the soma of remnant neurons indicative of neuronal degeneration.[3,20]

Equine grass sickness is a polyneuronopathy of the central and peripheral nervous system that only affects grazing horses, ponies and donkeys. It is a seasonal disease with peak of incidence between April-July in the northern hemisphere. This pathological condition was first described in Scotland in 1909, since then equine grass sickness has been described in numerous Northern European countries, Cyprus, Falkland Islands and Australia.[17] Although the etiology is still unknown, toxins produced by *Clostridium* spp. are suspected to play a role in the disease as low serum antibody levels for this bacterium were found to be a risk factor.[10] The most common gross pathological manifestations are fluid distention of the proximal gastrointestinal tract (stomach and small intestine) and large intestine impaction with dry and corrugated digesta often coated by copious mucus.[1] Histologically the main findings consist in neuronal chromatolysis of the peripheral nervous system and central nervous system, with changes particularly severe in the peripheral autonomic ganglia and enteric neurons. Whilst less significant, different studies also have identified involvement of the central nervous system including degeneration of cranial nerves (III, V, VI, VIII, XII), dorsal motor nucleus of the X, accessory cuneate nucleus, red nucleus and reticular formation.[17] The histological appearance of the chromatolytic neurons consists in swelling of cell body (soma), loss and/or peripheralization of Nissl substance, central eosinophilic spheroid bodies, foamy cytoplasm and peripheral margination and flattening of the nucleus.[9] The best gastrointestinal location to identify these changes is the ileum (submucosal and myenteric plexuses), especially in acute cases.[13] Nonetheless, chromatolysis has been also reported in stomach, duodenum, jejunum, caecum, large colon, small colon and rectum,[17] and the duodenum has been identified as the best location to identify degenerated neurons in chronic cases.[13] Neuronal lesions have been documented in the following ganglia of the autonomic system: ciliary ganglion, cranial cervical ganglion, caudal mesenteric ganglion, stellate ganglion, thoracic and abdominal sympathetic trunk, celiaco-mesenteric, caudal mesenteric ganglion and parasympathetic terminal cardiac ganglion.[17] Additionally, loss of interstitial cells of Cajal have also been reported in cases of acute grass sickness, suggesting the loss of these pacemarkers may also contribute to development of the dysmotility.[6] The only way to diagnose equine grass sickness ante mortem is by mean of an ileal biopsy which allows the identification of neuronal loss and / or neuronal chromatolysis with 100% sensitivity and specificity.[12] Anti-synaptophysin immunostaining has been proposed as a good diagnostic tool to identify degenerating neurons which show an increase of synaptophysin signal within the cytoplasm.[3,20]

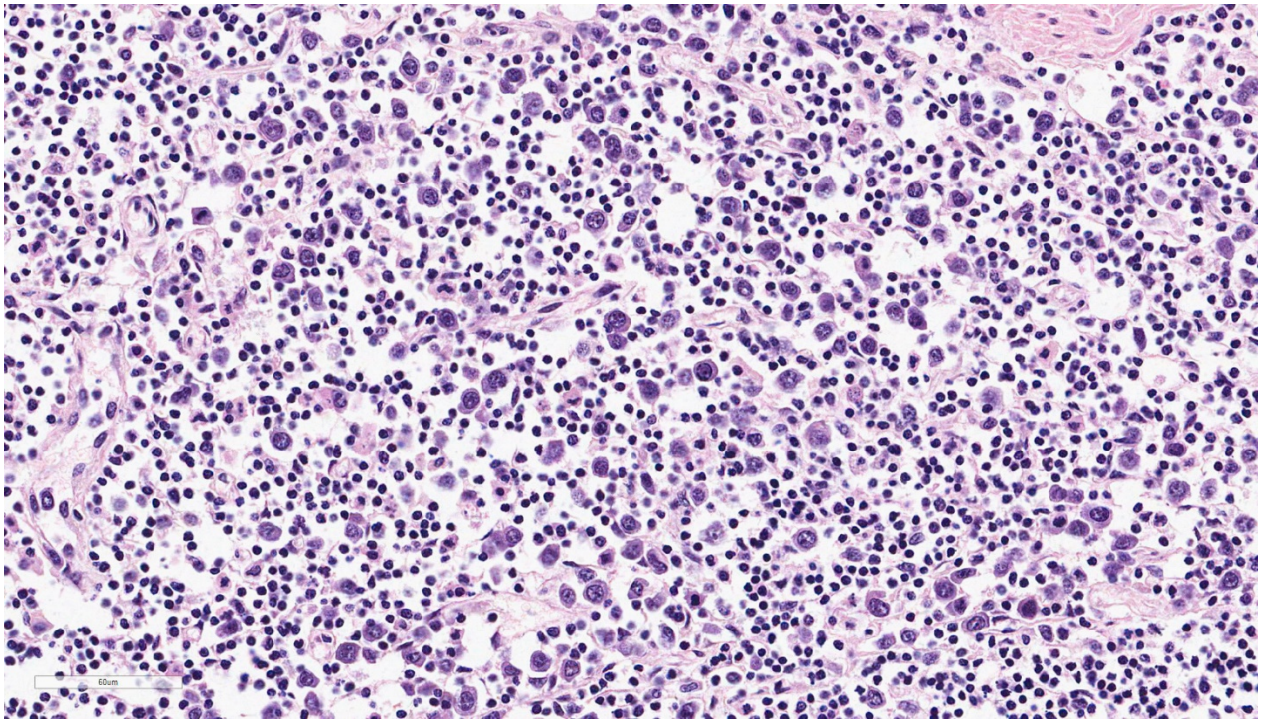


Ileum, horse. Enhanced synaptophysin immunopositivity is consistent with neuronal degeneration. (HE, 400X) (Photo courtesy of: University of Liverpool, Leahurst Campus, Chester High Road, Neston, Wirral, UK, CH64 7TE.)

Associated with the typical features of equine dysautonomia, an atypical proliferation of the lymphoid tissue arising from the GALT was also recognized in this case, that can explain the grossly observed focally extensive thickening and white discoloration of the intestine, which is not a usual feature of equine grass sickness per se. In our opinion there are several features that support the diagnosis of a lymphoma against a lymphoid hyperplasia: grossly the intestinal wall was diffusely severely thickened and white in color. Histologically the blastic lymphoid cells exhibited features of atypia (large cellular size, with vesicular to finely stippled chromatin, occasional nuclear membrane indentations and scattered bizarre mitotic figures) and the infiltrative behavior (invasion of the lamina propria and vessels) with loss of follicular structure of numerous GALT areas. On the basis of the morphological appearance provided by the H&E the tumor was provisionally classified as a T-cell rich B-cell lymphoma.

Immunohistochemichal results however identified a constant CD3 positivity in both small and blastic lymphoid cells, with lack of positivity for B markers (CD79a / CD20). CD3 positive cells were also detected within vessels. On this basis a T cell lympho-proliferative disorder consistent with a T cell lymphoma was the final diagnosis in this case. Unfortunately no other organ was available for examination to further investigate the possible leukemic phase of the neoplasm as suggested by the vascular invasion, in distant organs. A clonality test was not available to test the clonality of the proliferation.

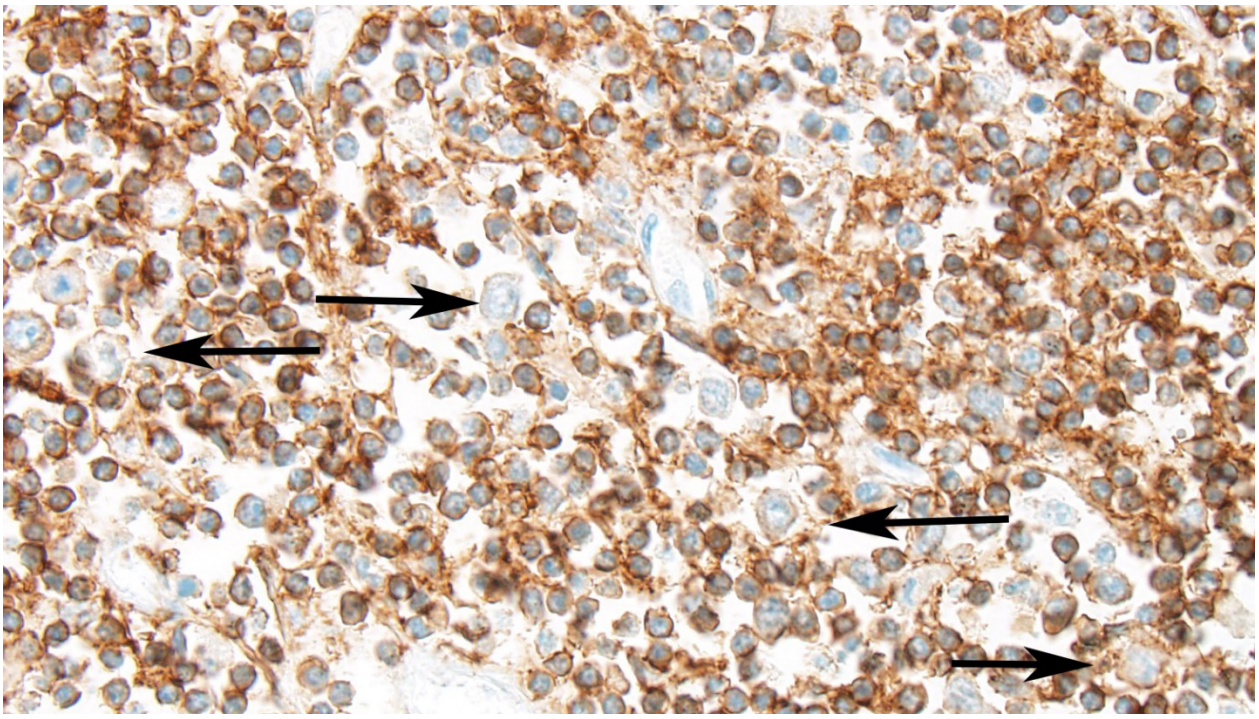
Alimentary lymphoma is a commonly reported tumor of the gastrointestinal system in horse.[1] The mean age of presentation of



Ileum, horse. The presence of small numbers of large lymphocytes in a sea of smaller mature T-cells is consistent with T-cell rich B-cell lymphoma. (HE, 400X)

intestinal lymphoma is 16 years. This information, gained from a recent publication[19], differs from older papers of equine lymphoma in which the mean age was 7.5-10 years.[5,14,15] This difference may suggest alimentary lymphoma is more represented in older animals compared to other lymphomas or perhaps life expectancy of horses has increased during recent years.[19] The major clinical signs are weight loss, ventral edema and ascites probably due malabsorption and protein loss, lethargy, occasionally diarrhea, pyrexia, and abdominal pain with sings of colic.[1,5] In one study lymphoma was found as the most common diagnosis in a group of horses showing frequent recurrent episodes of colic with high mortality, lymphoma was the fourth most common etiology in horses showing chronic colic signs.[4,8] The most affected anatomical region is the small intestine closely followed by the large intestine, although segmental distribution is also described usually associated with younger animals.[19] The gross appearance of this tumor consists of diffuse thickening of the intestinal wall, enlargement of the regional lymph nodes and sometimes nodular bulges in the serosal surface.[1,5,18] Histologically it is commonly characterized by diffuse infiltration of the mucosa and submucosa and sometimes the tunica muscularis.[1] The cytological features of the tumor may vary depending on phenotype of the primary neoplastic lymphocytes.[7,11]

Alimentary lymphoma has been traditionally correlated with B-cell neoplastic proliferation since it is thought to arise from GALT, therefore large centroblastic cells are the predominant cell population.[1,18] Other subtypes of lymphoma have been later documented such as epitheliotrophic T-cell lymphoma[16] and T-cell rich B-cell lymphoma (TCRBCL), been this latter the most common lymphoma subtype according to a recent publication.[2] Histologically TCRBCL is composed of two cell populations, the



Ileum, horse. T-cells are strongly positive for CD3; larger B-cells are not. (HE, 400X)

predominant one is characterized by well-differentiated small T-lymphocytes, inter-mingled within these T-cells there are large neoplastic cells ~2-3 times the size of the aforementioned cells which are derived from B-lymphocytes.[2,7] Most of T-cell lymphomas are characterized by small to medium T-lymphocyte cell population infiltrating lamina propria and mucosa occasionally showing epitheliotropsim.[16] In all lymphomas there is a marked shortening and fusion of the villi.

JPC Diagnosis: 1. Small intestine (ileum per contributor): Lymphoma (consistent with TCRBCL), Warm blood (*Equus caballus*), equine.
2. Small intestine (ileum per contributor), neurons: Neuronal degeneration and loss, multifocal, moderate.

Conference Comment: Gastrointestinal lymphomas are discussed by the contributor and were reviewed by conference attendees including: enteropathy-associated T-cell lymphomas, type I and II (EATL), diffuse large B cell lymphomas, and large granular lymphocyte (LGL) lymphoma. EATL is most common in the jejunum of dogs and cats. EATL type I is composed of intermediate to large T-cells, whereas EATL type II is composed of small T-cells (often with histologic overlap with inflammatory bowel disease early in disease process).LGL lymphoma is composed of large cells with brightly eosinophilic granules that contain granzymes. The granules are much easier to see cytologically, so an impression smear done by the surgeon prior to formalin fixation is often high yield.
In this case, there are large cells (that are often mitotic) admixed with a reactive population of small lymphocytes. In the horse, this mixed cell population is most consistent with T cell rich large B cell lymphoma (TCRBCL), which is the most common subtype of lymphoma in this species. For this reason, we re-ran special stains to take a second look at the two cell populations. CD3, a marker for T-cells, was positive (with strong intracytoplasmic immunoreactivity) for the majority of the cells, particularly the cells that expand the lamina propria; CD20 and Pax-5, markers for B-cells were positive (with strong intracytoplasmic immunoreactivity) for the smaller population of large neoplastic cells admixed among the T-cell aggregates or forming germinal centers (presumed to be residual GALT) or was non-contributory, respectively. It is ideal to use more than one B-cell marker in suspected lymphoma cases. CD79a was not run in this case as it has not been proven effective in the horse. Two other B-cell markers (CD20 and Pax-5) This immunohistochemical pattern is compatible of TCRBCL. PARR was discussed as an option for diagnosis, but the paucity of neoplastic cells and the abundance of small reactive T cells may confound this test.

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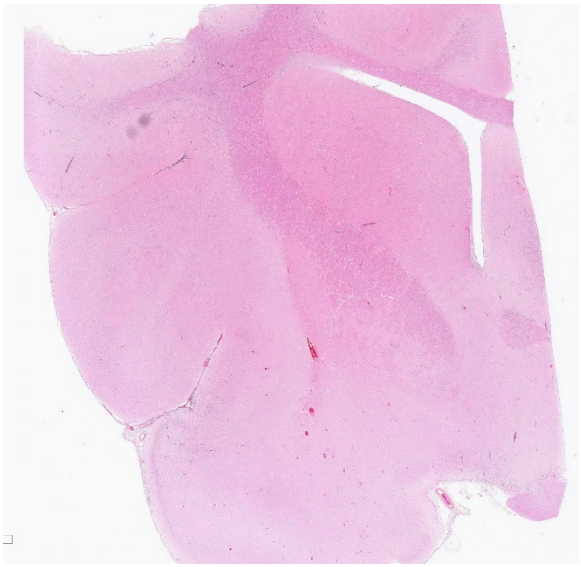
CASE II: P636-14 (JPC 4066540).

Signalment: 8-week-old, intact, female, Braque francais (French pointer) (*Canis familiaris*), canine.

History: This puppy was presented to a veterinary clinic for a sudden onset of drooling (sialorrhea), vomiting, tremors and seizures. Due to the severity and rapid evolution of the clinical signs, the owner



Cerebrum, puppy: A section of cranial diencephalon was submitted for examination. (HE, 4X)

elected for euthanasia without any further investigation. The dog was submitted to our diagnostic laboratory and a complete necropsy was performed.

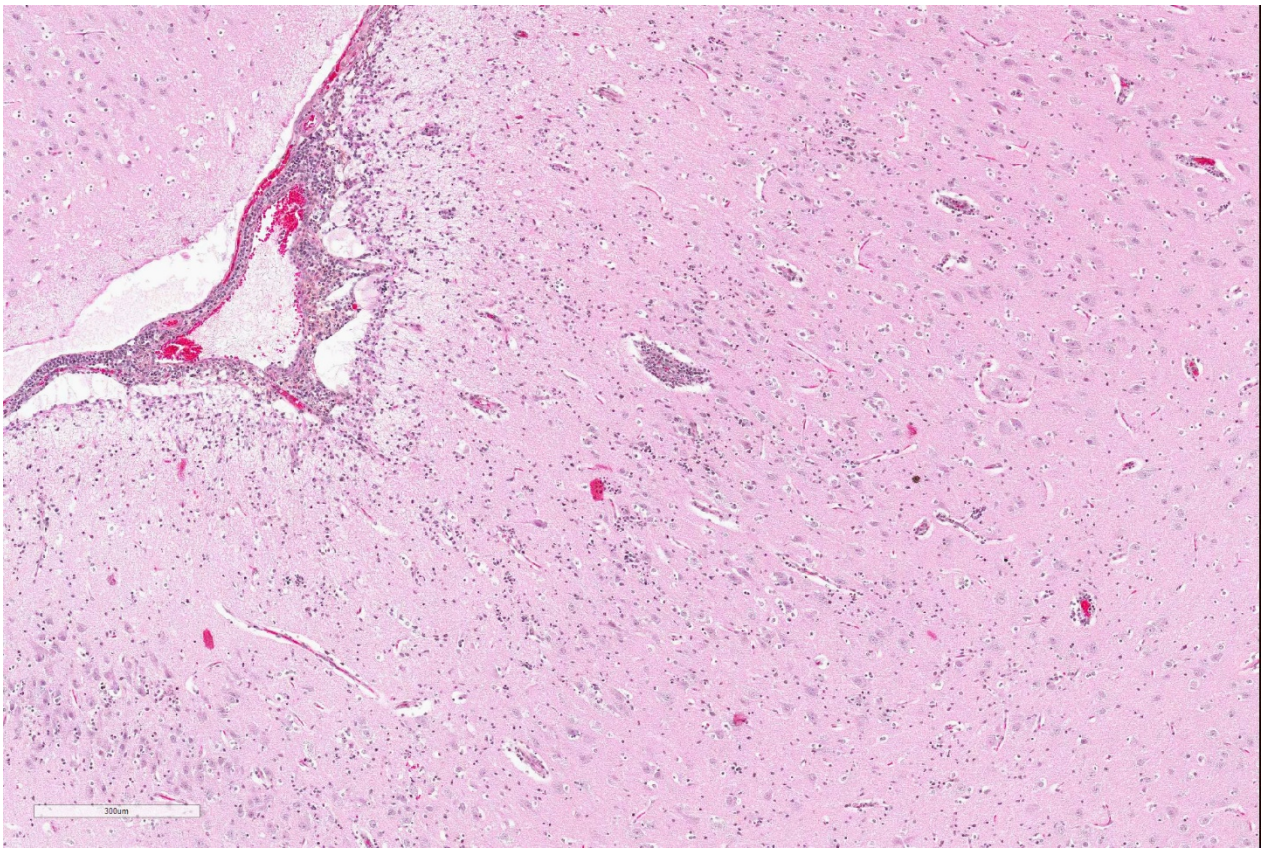
Gross Pathology: The dog was in good body condition and there were no remarkable findings on gross examination.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.): The dog's brain tested negative for rabies (immuno-peroxidase) and herpesvirus (PCR → panherpesvirus/DNA polymerase on FFPE tissue).

Microscopic Description: Similar lesions were observed throughout the brain and the cranial portion of the cervical spinal cord, with relatively minor variations in intensity. The submitted sections are from the cerebrum. Multifocally in the neuro-parenchyma and, to a lesser degree, the leptomeninges (subarachnoid space), there is a population of relatively monomorphic lymphoid cells that have a mainly perivascular and, in the neuroparenchyma, vascular distribution (Fig.1). In the neuroparenchyma, these cells are located in the Virchow-Robin spaces (up to 8 cells thick) and/or the vascular walls in both white and grey matter; in the latter case, there is sometimes associated edema, fibrin and/or erythrocytes (Figs 1 and 2). Multifocally, especially around affected blood vessels, the neuropil has an increased cellularity due to the presence of apparently similar lymphoid cells and possibly glial cells (some of which are reactive) (Fig.1). The lymphoid cells are small (nuclei ≤ 1.5 RBC), and are characterized by scant eosinophilic cytoplasm, a round to oval nucleus with finely stippled chromatin. There are several to numerous cells, interpreted as lymphoid cells, that are karyorrhectic or sometimes pyknotic (apoptosis). Anisocytosis and anisokaryosis are minimal, and no mitoses were detected. Immunohistochemistry (IHC) for CD3 and CD79a was performed on sections of the brain. The overwhelming majority (> 99%) of lymphoid cells, including in the hypercellular neuropil, were strongly positive for CD3 (Figs.3 and 4), with very rare cells (< 1%) positive for CD79a. There were no significant changes in other organs examined, including the eyes.

Contributor's Morphologic Diagnosis:
Primary CNS T-cell lymphoma (PCNSL)

Contributor's Comment: Although a diagnosis of viral mononuclear/lymphocytic meningoencephalomyelitis was initially considered and investigated (CDV and herpesvirus), primary CNS T-cell lymphoma was the final diagnosis based on the morphologically monomorphic nature of the lymphocytic infiltrate (including the absence of plasma cells) that was confirmed to be almost purely of T cell nature, and its angiocentric nature; although not assessed by IHC, macrophages did not seem to be present, at least in significant numbers, and glial nodules were not observed. Assessment of T-cell clonality by either T-cell antigen receptor gene rearrangement analysis (PCR)



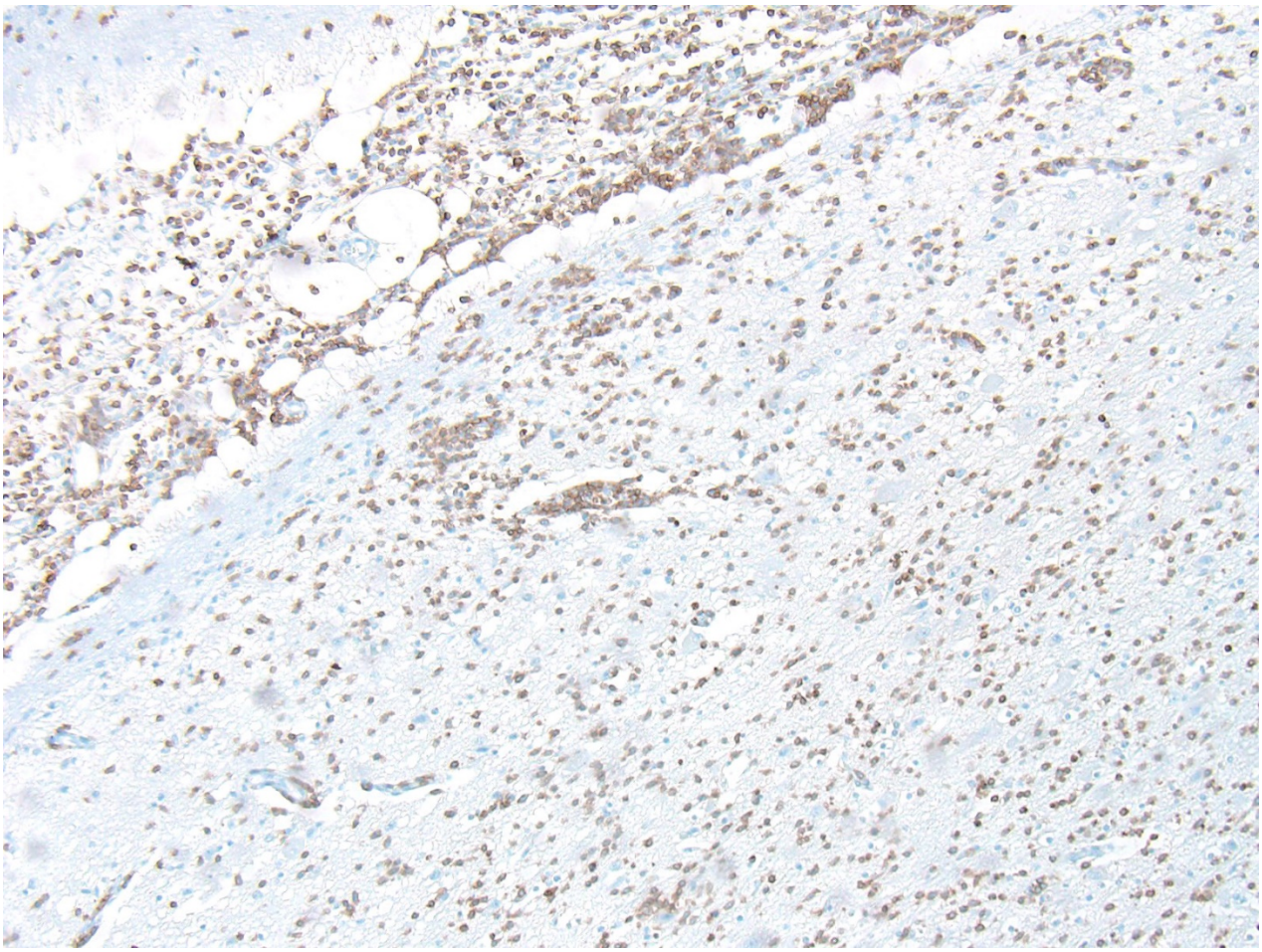
Cerebrum, puppy: Multifocally, the meninges (upper left) as well as Virchow-Robins spaces are expanded by numerous lymphocytes. Lymphocytes also infiltrate the gray matter as well. (HE, 100X)

or X-chromosome inactivation pattern (XCIP) analysis was not performed. Demonstration of clonality would have further supported our diagnosis, although it is not totally confirmative; the XCIP technique has more limitations, especially with regards to gender (females) and hematopoietic neoplasms.[9]

Primary CNS lymphomas (PCNSL) are uncommon to rare neoplasms defined as non-Hodgkin lymphomas that are confined, at least initially, to the CNS and/or the eye.[1,3,12,13] In the CNS, they can involve the neuroparenchyma and/or the meninges. Primary ocular lymphomas included in the PCNSLs in humans are vitreoretinal lymphomas (PVRL); 65-90% of patients with PVRLs develop lymphoma in the CNS.[2] In contrast to PCNSLs, secondary CNS lymphomas represent a metastatic process from a lymphoma outside the CNS, and are more common.[1,3,4,13] In humans and cats, 5% of patients with systemic lymphomas have CNS involvement, predominantly in the leptomeninges.[8,13] PCNSLs are neoplasms mainly seen in humans, dogs and, to a lesser degree cats, but they have also been described in ruminants, a dolphin and a harbor seal;[1,3-5,7,8,12,13] there is a probable case reported in a horse.[11] In humans and dogs, the reported incidence is about 3% of intracranial neoplasms; in humans they account for 1-2% of all non-Hodgkin lymphomas. In cats, most PCNSLs involve the spinal cord.[4] In animals, the majority of PCNSLs are of T-cell lineage, in contrast with humans in which 80-95% are large B-cell lymphomas which differ from their systemic counterparts with regards to behavior and treatment.[1,3,7,12] The etiology is

unknown in both animals and humans, except in cats in which FeLV is often involved and in immunocompromised humans in which the Epstein-Barr virus (EBV) plays a major role (EBV has not been associated with PCNSL in immunocompetent human patients).[1,12] Most PCNSL cases have gross lesions and the diagnosis of lymphoma is straightforward on microscopic examination, but in a few reported cases in cats and dogs,[4,5,7,8] there were no conspicuous masses and histopathology could not readily differentiate between lymphoma and mononuclear/ lymphocytic inflammation. In human PCNSLs, neoplastic lymphocytes in the neuroparenchyma characteristically invade walls of small blood vessels, accumulate in perivascular spaces and spread into the neuropil.[1,3]

In the present case, the age of the animal, absence of gross lesions and, histologically, absence of "masses" of lymphoid cells, atypia and mitosis initially led to a diagnosis of mononuclear (non-suppurative) meningo-encephalomyelitis of probable viral origin. Other infectious diseases (e.g. neosporosis), granulomatous meningoencephalitis (GME) and necrotizing meningoencephalitis (NME) were included in the initial differential diagnosis, but the histopathologic findings were not consistent with these conditions. Based on aforementioned findings (homogenous infiltrate, angiocentrism and

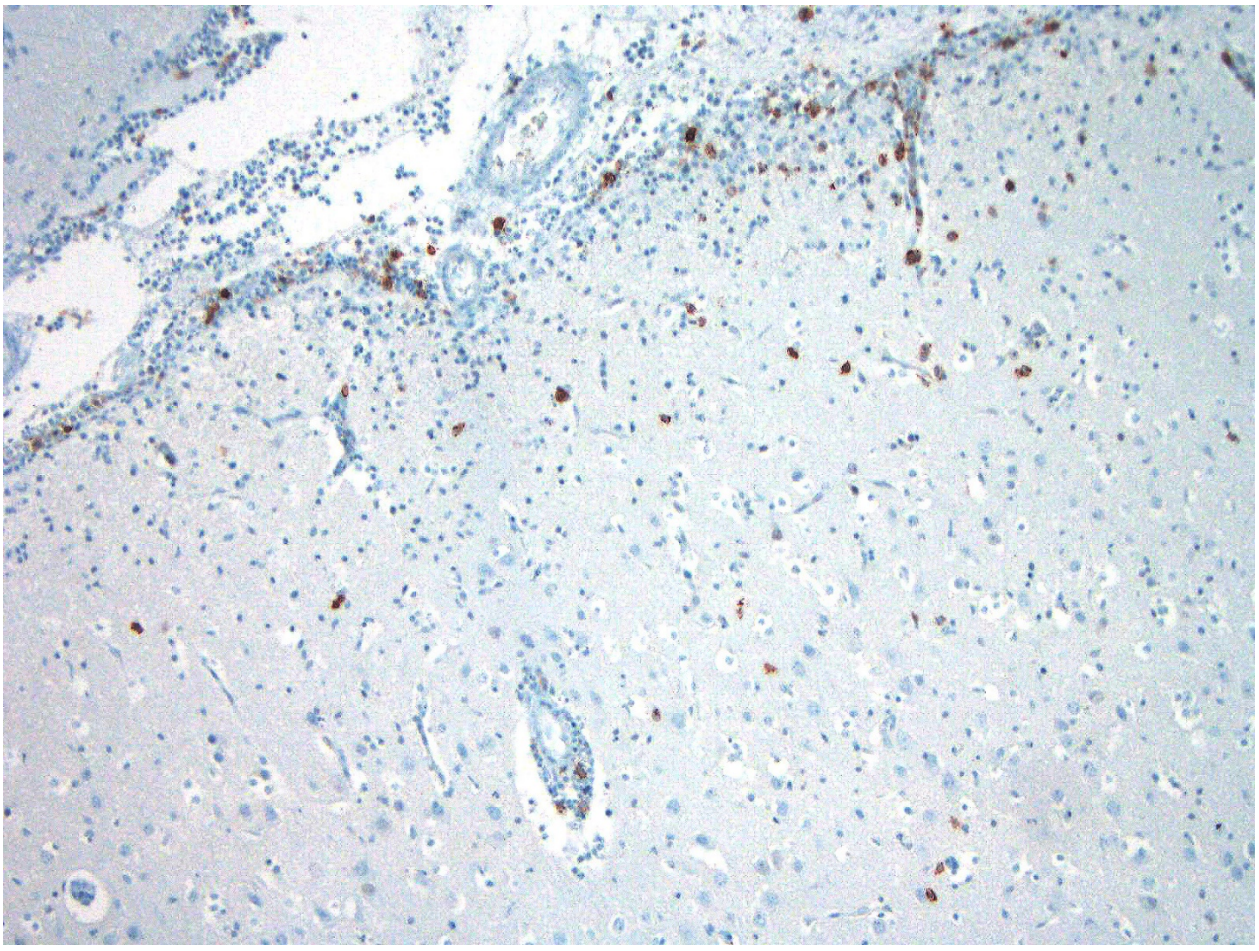


Cerebrum, puppy: The majority of the infiltrating lymphocytes stain strongly positive for CD-3. (anti-CD3, 100X)

IHC), some published veterinary cases and a consultation with a veterinary neuropathologist, we finally concluded to a PCNSL. An equine case with similarities to our case was termed "lymphoproliferative disease with features of lymphoma in the CNS".[11] In human pathology, small-cell lymphomas have been distinguished from mononuclear encephalitides by two morphologic criteria: 1) encephalitis generally has a more polymorphous infiltrate with at least some plasma cells, and 2) vascular invasion by lymphocytes, characteristic in lymphomas, is not a feature of encephalitis.[3] The age of the animal is also not typical of canine lymphomas which are mostly seen in middle-aged dogs, even though they have been seen in puppies as young as 4 months. In the bovine species, lymphomas have been reported in aborted fetuses and neonatal calves.[6]

JPC Diagnosis: Cerebrum: Meningoencephalitis, lymphocytic, multifocal to coalescing, moderate, Braque francais (French pointer) (*Canis familiaris*), canine.

Conference Comment: As described by the contributor above, primary central nervous system (CNS) lymphoma is rare in humans and animals. In humans the majority are large B-cell variants, whereas, in animals most of the reported cases have been T-cell variants. Microscopically, the diagnosis of lymphoma



Cerebrum, puppy: CD20-positive B cells are present within the infiltrate around vessels as well as in the parenchyma. (anti-CD20), 100X

is challenging because the perivascular and periventricular distribution of neoplastic cells may be confused with an inflammatory response (e.g. viral encephalitis). However, inflammatory responses often have several other cell types depending on chronicity, exhibiting neutrophilic infiltrates initially, followed by plasma cells, lymphocytes, and potentially histiocytes as the infection progresses. In humans, an atypical variant of CNS lymphoma has been described termed "lymphomatosis cerebri" which is characterized by diffuse infiltration of deep cerebral white matter by individualized lymphoma cells with no mass formation. Another differential which has been reported in humans and domestic animals is lymphomatoid granulomatosis, a rare primary pulmonary lymphoproliferative disease which has had CNS involvement described in late stages. This is characterized microscopically by angiocentric and angiodestructive atypical lymphoid cells with rare binucleate and multinucleate cells.[10]

The conference attendees discussed the alternative diagnoses, given the clinical presentation (young puppy). Conference attendees reviewed common viral encephalitides (distemper, alphaviruses, West Nile virus, rabies, canine herpesvirus-1) with their microscopic characteristics. It was noted that immunohistochemistry for B cells or tests for T-cell clonality were not performed as part of the workup. Several immunohistochemical stains were run by the JPC in this case to further classify the cell type in these specimens. While the majority of cells exhibited strong cytoplasmic positivity for T-cells, a number of lymphocytes both in perivascular and parenchymal locations stained positively for CD20, a B-lymphocyte marker. PARR testing was discussed as an important next step to determine if this represents a clonal expansion of T cells. Based on the morphologic appearance of the lesion, the relatively frequency of both subclinical viral infections in puppies versus the frequency of primary T-cell lymphomas (the age of this puppy notwithstanding), the presence of B-cells within the lesion, and the absence of evidence of clonality, the moderator and attendees favored a diagnosis of inflammatory disease in this case rather than T-cell lymphoma.

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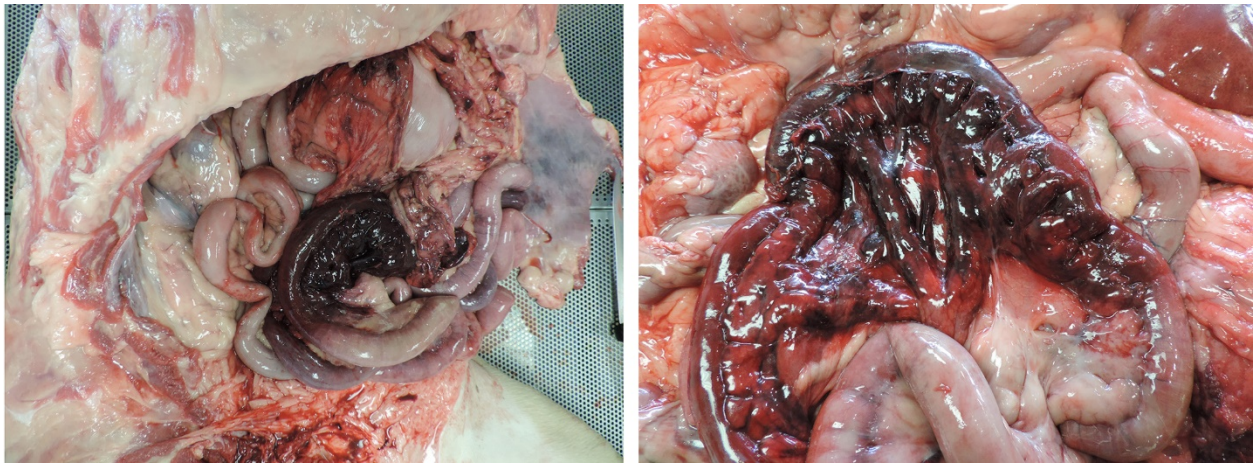
CASE III: 15-23253 (JPC 4066861).

Signalment: 7-year-old, spayed, female, Bullmastiff (*Canis familiaris*), canine.

History: A 7 year old spayed female Mastiff was referred to the Cornell University Hospital for Animals Emergency Service for evaluation of hemoabdomen and a two day history of weakness, lethargy, and in appetence. On presentation, the patient was febrile with tachycardia and tachypnea. Abdominal ultrasound and abdominocentesis confirmed a hemorrhagic effusion. Platelet numbers were moderately decreased, and coagulation parameters were within normal limits. The patient also had a one month history of an ulcerated mass on the right



Intestine, dog: There is extensive infarction and hemorrhage of the small intestine (Photo courtesy of: Department of Anatomic Pathology, Cornell University College of Veterinary Medicine http://www.vet.cornell.edu/biosci/pathology/)

lateral thigh that had been unresponsive to treatment with antimicrobials.

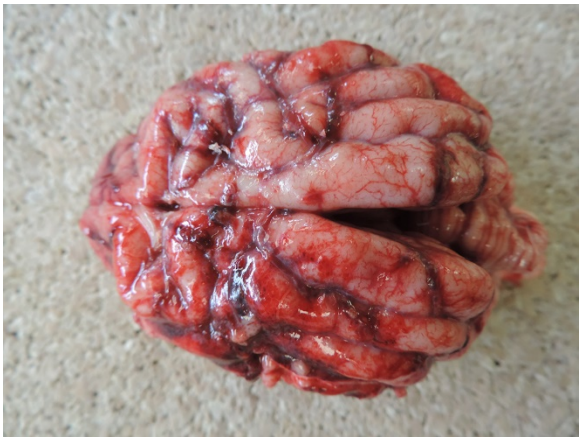
While hospitalized the patient developed neurologic signs, including a vestibular episode with nystagmus and ataxia, circling, head-pressing, and knuckling. Her neurologic signs progressed to tetraplegia, and she became obtunded. The patient's condition further declined, and she died in the hospital.

Gross Pathology: Gross examination confirmed hemoabdomen and focal cutaneous ulceration at the lateral right thigh. Examination further revealed multiple sites of acute infarction and hemorrhage in the meninges and cerebrum, small intestine, lung, and a mesenteric lymph node. More chronic sites of infarction were found in the kidney and myocardium.

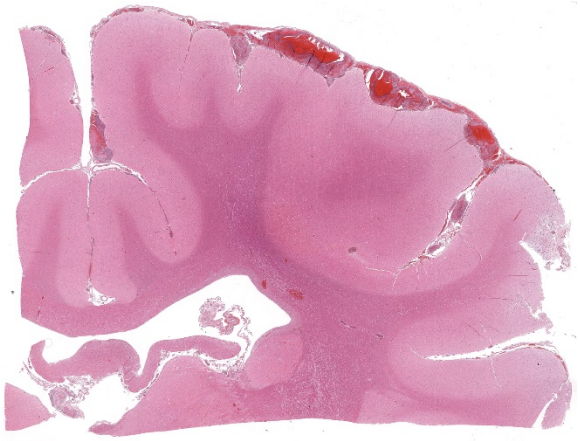
Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.): None provided.

Microscopic Description:

The submitted slide includes a section of liver. Distending portal and central veins and expanding sinusoids are sheets and clusters of intravascular neoplastic cells. Neoplastic cells are round with a high nuclear to cytoplasmic ratio. Nuclei are up to twice the diameter of a neutrophil and have distinct cell margins, scant amphophilic to pale basophilic cytoplasm, and an often eccentric, round or indented nucleus with coarsely clumped chromatin and 1-3 magenta nucleoli. There is marked anisocytosis and anisokaryosis and up to 4 mitotic figures per high magnification (400x) field. There are scattered apoptotic neoplastic cells. Sinusoids also contain increased numbers of circulating neutrophils and lymphocytes. Hepatic cords are thin with mildly dilated sinusoidal spaces (atrophy). Portal and central vein lymphatics are occasionally dilated.



Cerebrum, dog: There is multifocal to coalescing hemorrhage within the cerebral meninges. (Photo courtesy of: Department of Anatomic Pathology, Cornell University College of Veterinary Medicine, http://www.vet.cornell.edu/biosci/pathology/)

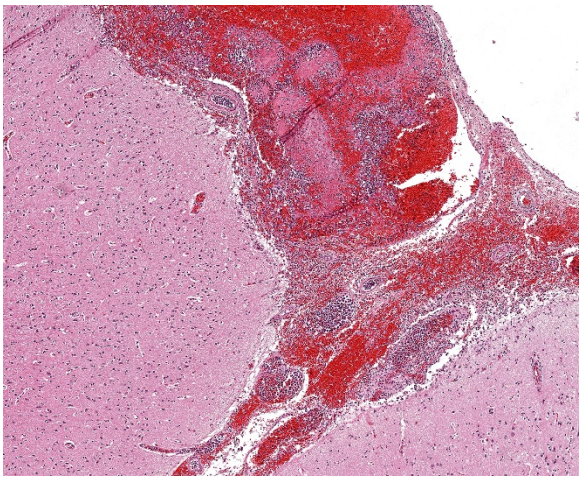


Cerebrum, dog: At subgross magnification, there is focally extensive meningeal hemorrhage as well as multiple hemorrhages in the periventricular white matter. (HE, 6X) (Photo courtesy of: Department of Anatomic Pathology, Cornell University College of Veterinary Medicine http://www.vet.cornell.edu/biosci/pathology/)

Immunohistochemistry revealed positive reactivity for CD3, consistent with a T cell origin. Similar intravascular neoplastic populations were observed in the lung, heart, kidney, jejunal mesentery, pancreas, mesenteric lymph node, skin, and brain, often associated with thrombosis and infarction.

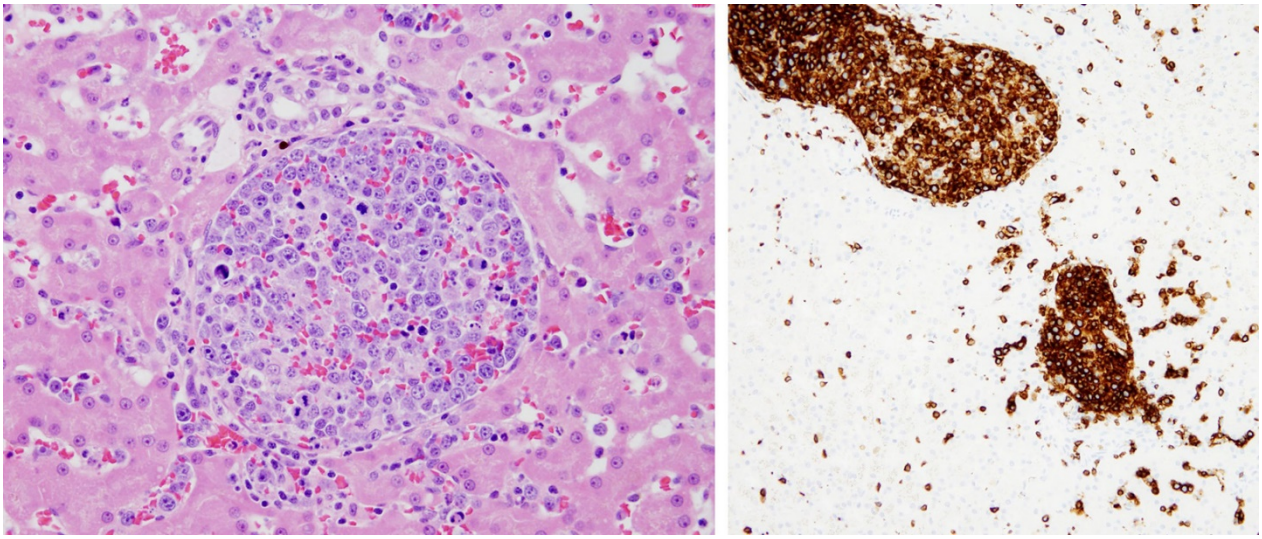
Contributor's Morphologic Diagnosis:
Liver: Intravascular lymphoma, large T cell

Contributor's Comment: Intravascular lymphoma (IVL) is a rare large-cell lymphoma, defined by its confinement within blood vessel lumina in the absence of leukemia or a primary extravascular mass. Typical clinical and pathologic features are related to vascular occlusion, injury, and fibrin thrombosis, often with central nervous system signs. In a review of 17 cases of IVL in dogs, predominant clinical signs included spinal cord ataxia, seizures, vestibular disease, lethargy, diarrhea, and fever.[4] Skin involvement was apparent in one of the cases.[4] IVL accounted for the spectrum of clinical findings in this dog, including the neurologic signs (due to cerebral thrombosis and infarction), hemoabdomen (small intestinal infarction), and ulcerative dermatitis. No extravascular lymphomatous masses were found at necropsy, and ante-mortem blood work was not consistent with leukemia.



Cerebrum, dog: Within the meninges, there is extensive hemorrhage, vessels are dilated and by cellular fibrinocellular thrombi. (HE, 40X) (Photo courtesy of: Department of Anatomic Pathology, Cornell University College of Veterinary Medicine, http://www.vet.cornell.edu/biosci/pathology/)

Intravascular lymphoma was first described in the dog as angioendotheliomatosis, under the assumption that the neoplastic cells were derived from the endothelium, but subsequent immunohistochemical data determined the lymphocyte-derivation.[4,6,7] Refinement of understanding of intravascular lymphoma in dogs was supported by work on immunohistochemical and ultrastructural data on similar human neoplasms.[6] While the



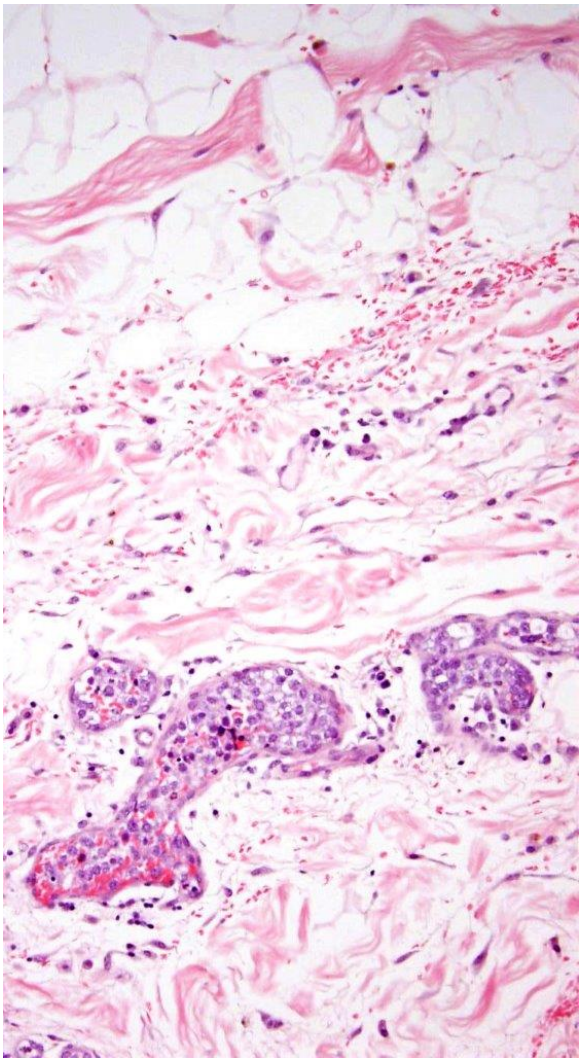
Liver, dog: Portal veins are expanded by accumulations of numerous neoplastic lymphocytes, which stain strongly positive for CD-3, indicated T-cell origin. (Left: HE, 400X, right: anti-CD-3, 400X) (Photo courtesy of: Department of Anatomic Pathology, Cornell University College of Veterinary Medicine, http://www.vet.cornell.edu/biosci/pathology/)

majority of human cases are derived from B cells, most reported intravascular lymphomas in dogs are derived from T cells or non-B, non-T lymphocytes.[2,4,8] Human and canine cases show a similar predilection for nervous system involvement, but cutaneous presentation appears to be more common in humans.[4]

Neoplastic lymphocytes are generally most abundant in capillaries and small to medium veins, but also appear in smaller numbers within arteries.[4] The mechanism for the selective intravascular growth of IVL is unknown, but defective cell-to-cell adhesion between the neoplastic lymphocytes and endothelial cells has been proposed.[1,5]

JPC Diagnosis: Liver, veins, arteries, and sinusoids: Intravascular lymphoma, multifocal, Bull mastiff (*Canis familiaris*), canine.

Conference Comment: Angiotropic intravascular lymphoma, previously also referred to as malignant angioendo-theliomatosis, is a rare tumor in humans and dogs, and has been reported a cat. Microscopically, it is characterized by a proliferation of neoplastic lymphocytes within the lumen and wall of blood vessels with no primary extravascular mass. In most human cases, these cells have been identified as B-cells, but in canine and feline cases, the majority are of T-cell origin.[3,4] Clinically, most of these cases present with neurologic signs and microscopically neoplastic cells are seen occluding cerebral arteries and veins. The vessels of the lungs are also commonly affected. In the affected cat,[3] there was severe involvement of the kidneys and cerebrum with absence of neoplastic cells in other visceral organs. Diagnosis usually follows the onset of neurologic signs and is typically postmortem. There are no neoplastic cells identified in routine blood samples. In humans, even with chemotherapy, prognosis is poor. There is no treatment data in domestic animals.[3]



Skin, dog: Vessels in the skin are also occluded by numerous T-cells. (HE, 200X) (Photo courtesy of: Department of Anatomic Pathology, Cornell University College of Veterinary Medicine, http://www.vet.cornell.edu/biosci/pathology/)

The moderator shared a case that she had also of a female Bullmastiff that had intravascular lymphoma who presented with neurologic signs, acute renal failure, antibiotic-resistant urinary tract infection, and autoimmune hemolytic anemia. She then graciously reviewed images of the case and features of the entity.

Contributing Institution:
Anatomic Pathology
Cornell University College of Veterinary Medicine
http://www.vet.cornell.edu/biosci/pathology/

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CASE IV: #1 (JPC 4101495).

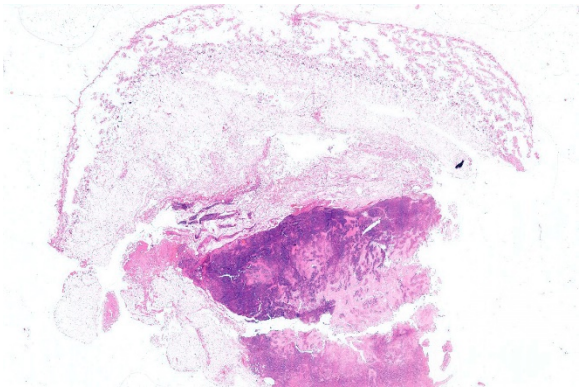
Signalment: 12-year-old, neutered male, European domestic shorthair (*Felis catus*), feline.

History: Mass developing rapidly in the right mid-thigh. Previous history of multiple antibiotic and vaccine injection on the site were reported by the referring veterinarian.

Gross Pathology: Subcutaneous soft mass of large diameter, white tan, blending in the muscle

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.): CBC, Serum biochemistry, urine analysis unremarkable. Clinical staging negative for internal disease.

Microscopic Description:
Haired skin (not always present): The deep dermis, the panniculus and the skeletal muscles are characterized by a variably cellular neoplastic infiltration associated with areas of necrosis. The tumor is not encapsulated, infiltrative, and extending to the borders of the biopsy.



Hared skin and subcutis, cat: The subcutis and underlying skeletal muscle contains a nodular and half-necrotic round cell neoplasm. (HE, 4X)

The neoplasm is composed by round neoplastic organized in variably dense sheets or that tightly encircle and invade blood vessels walls (angiocentric and angioinvasive pattern) in association with locally extensive to coalescing areas of necrosis.

Neoplastic cells range from 20 to 35 microns in diameter, are round, with variably distinct cell borders, high N/C ratio, complete rim of variably eosinophilic homogeneous cyto-plasm. Nuclei are round, oval indented, paracentral, 15-30 micron in diameter, with finely granular chromatin and 1 to 4 round, prominent, basophilic nucleoli. Anisocytosis and anisokaryosis are severe. Mitoses are common and range from 2-6 per HPF and are often atypical. Tingible body macrophages range from 1 to 4 per HPF.

Contributor's Morphologic Diagnosis:
Angiocentric angiodestructive large cell subcutaneous lymphoma (injection site type) with necrosis

Contributor's Comment: Lymphomas represent more than 50% of all tumors in cats, with a prevalence of approximately 1.6% of the general feline population and 4.7% of hospitalized sick cats.[11,23] Primary cutaneous lymphomas account for 0.2 to 3%[21] of all feline lymphomas. Cutaneous, non-epitheliotropic lymphomas seem more frequent in cats[10] than dogs and include indolent T cell lymphoma, also referred to as cutaneous lymphocytosis,[6,7] diffuse T cell lymphoma, T cell rich large B cell lymphoma, and lymphoplasmacytic lymphoma.

In this case, microscopic features parallel descriptions and history of injection site lymphoma in cats.[15,17] Primary cutaneous lymphomas developing following injection have been reported in cats.[15,17] These lymphomas exhibit several peculiarities, including clinical presentation as a solitary dermal to subcutaneous nodule, development at confirmed previous injection sites (lateral thorax, interscapular region, thighs), microscopical presence of necrosis leading to central cavitation, peripheral inflammation, angiocentric, angioinvasive and angiodestructive growth patterns,[15,17] and presence of peripheral inflammation characterized by perivascular nodular lymphoid cell aggregates.[17] An additional unusual feature of feline injection site primary cutaneous lymphomas as a group is the prevalence of large B cell lymphomas with centroblastic, immunoblastic and anaplastic morphology[17] that are considered rare to exceptional tumors in cats.[7,10,21,23]

Primary cutaneous diffuse large B cell lymphomas (DLBCL) in man manifest as a solitary nodule or as multiple tumors restricted to one anatomic area (regional disease) and have a relatively poor prognosis compared with other primary cutaneous lymphomas, with a 5-year survival rate of 20-55%.[19] The most common morphological variants of human DLBCL are centroblastic, immunoblastic and anaplastic.[19] All these features are shared by a large prevalence of feline injection site skin lymphomas.[17]

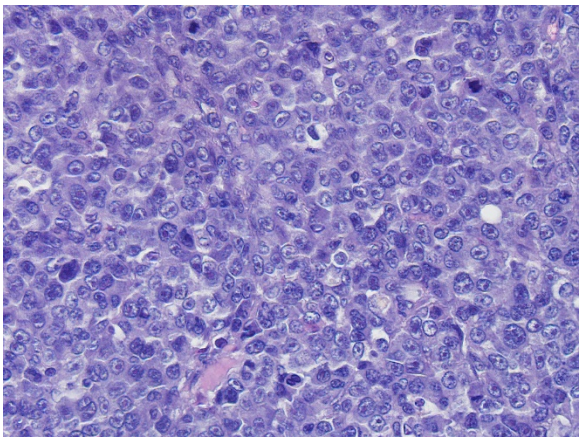
The microscopic angiocentric, angioinvasive and angiodestructive pattern described for cutaneous feline injection site lymphomas resembles descriptions of human angiocentric lymphomas (ALs).[18] In man, primary cutaneous ALs represent a localized disease with propensity to relapse developing primarily in male patients.[18] Distinctively, human ALs are also characterized by extensive tissue necrosis and/or severe inflammation that often obscures the tissue and the neoplastic process itself.[16]

Pathogenesis of feline cutaneous injection lymphomas may also resemble pathogenesis of human lymphomas emerging in the

context of chronic inflammation with transformation of lymphoid cells.[4,5,8,9] Chronic inflammation has long been linked to emergence of a wide range of human malignancies and is now generally accepted as a risk factor for development of a variety of cancers including hematopoietic malignancies such as cutaneous T and B cell lymphomas in man.[5,8,9] In cats, the development of sarcomas at injections sites (e.g. rabies vaccine, long acting antibiotics or steroids) or at sites of implanted foreign material (non-absorbable suture material, microchip implants, retained surgical sponges or trauma) have been well documented and their pathogenesis has been attributed to the chronic inflammation elicited.[12] Progression of chronic inflammation to feline cutaneous lymphoma has also been hypothesized.[17] Previously, cutaneous lymphomas have been reported to arise in areas of feline injection site sarcomas following chemotherapy or radiation therapy of the primary tumor, and lymphomagenesis in these cases was hypothesized to derive from the mutagenic action of chemotherapy or radiation treatment.[14]

Many primary human cutaneous lymphomas including ALs and DLBCL have been consistently associated with inflammation and EBV infection.[2,5] DLBCL associated with chronic inflammation (DLBCL-ACI) is a B cell lymphoma included in the WHO classification as a specific entity[2] This tumor develops in the context of long standing inflammation associated most frequently with EBV infection.[2,4] Most cases of DLBLC-ACI have been described in patients with pyothorax resulting from artificial pneumothorax prescribed for pleural tuberculosis.[2] DLBLC-ACI is also angiocentric and, similarly to feline injection site lymphoma, is more frequent in middle aged to old male patients and develops after a long latency period of over 10 years from terminally differentiated B cells.[2] In these instances, inflammation has been implicated in the reactivation and proliferation of EBV transformed B cells and seems to be the most accredited pathogenesis for DLBCL-ACI.[2] Chronic inflammation enables virally transformed B cells to escape from host immune surveillance trough production of IL-10 and providing autocrine and paracrine cell growth stimuli via IL-6 production. Noteworthy, also for other DLBCL occurring in settings of long standing inflammation such as osteomyelitis, metallic implants, and chronic skin ulceration, EBV positivity of neoplastic cells has been demonstrated. All the above observations parallel the finding of inflammation and concurrent FeLV positivity documented in several cases of CFIL.[17] While Feline injection site sarcoma seems not related with a specific viral etiology[10,12] the role of FeLV in lymphoma development has been well established.[11,13] Overall, approximately 70 percent of cats with lymphoma have FeLV antigenemia. Rate of FeLV serological positivity has been correlated with the anatomical form of lymphoma with percentages of positive cats maximal for mediastinal lymphoma (90%) and multicentric lymphoma (80%) and decreasing to less than 10% for cutaneous lymphoma.[11,13]

In feline injection site lymphomas, expression of FeLV p27 capsidic and gp70 envelope proteins has been detected in neoplastic cells.[13] Expression of p27 indicates that viral infection has occurred but does not confirm viral assembly (nonproductive infection) thus, p27 detection



Hared skin and subcutis, cat: High magnification of neoplastic cells reveals a diffuse infiltrate numerous large neoplastic lymphocytes with a high mitotic rate. Unfortunately, unstained slides were not available for immunophenotyping, and immunophenotyping results were not included by the contributor. (HE, 4X)

does not imply that FeLV infection is in progress. Gp70 expression denotes viral particle assembly confirming viral integration, viral replication and productive infection.[20] In FeLV latent infection, cats are seronegative but FeLV provirus has been demonstrated in peripheral blood and bone marrow cells by PCR. Thus, old seronegative cats may still bear the virus in their genome, but the virus may be inserted and not transcribed until reactivation and/or neoplastic transformation of infected cells occurs. Like what is described for DLBCL-ACI in man, chronic inflammation elicited by the injection may have contributed to FeLV reactivation and transcription with neoplastic transformation of lymphoid cells. The most likely hypothesis linking persistent antigenic stimulation with chronic inflammation and lymphoma development derives from the nature of the lymphoid proliferation. During chronic antigenic stimulation, lymphoid cell proliferation and gene rearrangements of TCR and BCR increase with increasing production of normal cells or cells with genetic mutations or translocations.

JPC Diagnosis: Haired skin (not present on all sections) and subcutis: Lymphoma with angioinvasion, angiodestruction, and coagulative necrosis, European domestic shorthair (*Felis catus*), feline.

Conference Comment: Throughout the 20th century, lymphomas in domestic animals were classified based on the non-Hodgkin lymphoma classification system in humans. The Rappaport classification, designed in 1966, was one of the earliest systems used in veterinary medicine, especially the dog. This system was based solely on morphologic characteristics (which fallaciously classified many large cell lymphomas as histiocytic). It wasn't until the advancement of immunohistochemical practices that classification systems were again updated. The Lukes-Collins (North America) and Kiel (Europe) classification systems were published based on immunologic more than morphologic concepts, but often yielded different diagnoses. To unify lymphoma classification, in 1982, the National Cancer Institute initiated a broad study oriented on clinical outcome rather than morphologic features and published the Working Formulation. This classification system was even more unreliable because survival times were based on human clinical trials. Finally, an updated Kiel classification was produced which until recently was the most useful prognostic tool for canine malignant lymphomas.[22]

The current system used is based on the WHO classification system for hema-topoietic neoplasms which has been applied to lymphomas in multiple veterinary species. These classification schemes characterized each type of lymphoma as a specific disease entity. The WHO classification for lymphoma diagnosis in domestic animals entails grouping subtypes based on pattern (diffuse or nodular), cell size, grade,

postulated normal cell counterpart, and defining histopathologic features.[1,22] Cell size is determined based on the size of a red blood cell (RBC) with large lymphocytes being greater than twice the size of an RBC, intermediate lymphocytes being 1.5 times the size of an RBC, and small lymphocytes being 1 to 1.25 times the size of an RBC. Grade is determined by the mitotic count per 400x field with indolent being 0-1/HPF, low grade being 2-5/HPF, medium 6-10/HPF, and high greater than 10 mitotic figures per 400x HPF. Cell size can be difficult to appreciate, secondarily, chromatin pattern can be used to distinguish between different forms of lymphoma. For example, small cell lymphomas have very dense chromatin and intermediate in a few subtypes have prominent nucleoli (Burkitt-like subtype) whereas others have hazy chromatin with indistinct nucleoli (lymphoblastic lymphomas). Immunohistochemically, there are several available B-cell markers (e.g CD20, CD79a or b, Pax5), and it is optimal to use more than one in order to capture the distinct maturation phases of B-cells. CD3 is an excellent pan-T-cell marker. It is important to note that PARR is a genotyping test for clonality, and must always be run in concurrence with immunophenotyping protocols (e.g. IHC, ICC, flow cytometry).

The moderator briefly discussed cancer arising in an inflammatory background. Inflammation is causally related to cancer via: genotoxicity, aberrant tissue repair, proliferative responses, invasion, and metastasis. For example, STAT3 and NF-kB pathways are involved in diffuse large B-cell lymphoma oncogenesis. Additionally, tumor cells secrete soluble growth factors, and render inflammatory cells suppressive against host immune responses. Finally, some microbial organisms are causative agents of cancer inducing inflammation, and commensal microbiota, if altered, can predispose to neoplastic transformation.[3]

Unfortunately, the unstained slides submitted contained a different tissue than what was submitted on H&E with the tumor. We were therefore unable to fully subtype the lymphoma in this case.

Contributing Institution:
DIMEVET, Faculty of Veterinary Medicine of Milan, Italy
http://www.dimevet.unimi.it/ecm/home

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