Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2015-2016

Conference 4

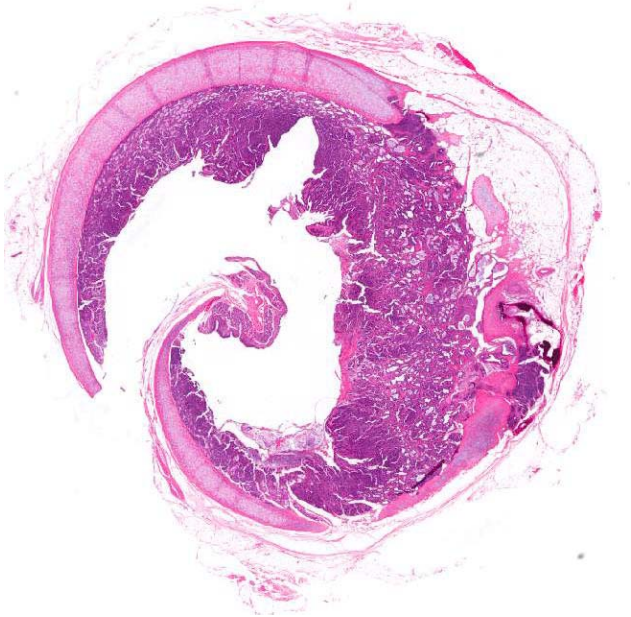
30 September 2015

CASE I: 15H672 (JPC 4066007).

Signalment: 10 year old neutered male domestic short hair cat (*Felis catus*).

History: The cat presented for chronic history of respiratory distress. Mass identified in trachea.

Gross Pathology: Focally extensive thickening/protrusion of the tracheal mucosa that partially occluded the lumen (1.5 cm x 1 cm x 0.25 cm).



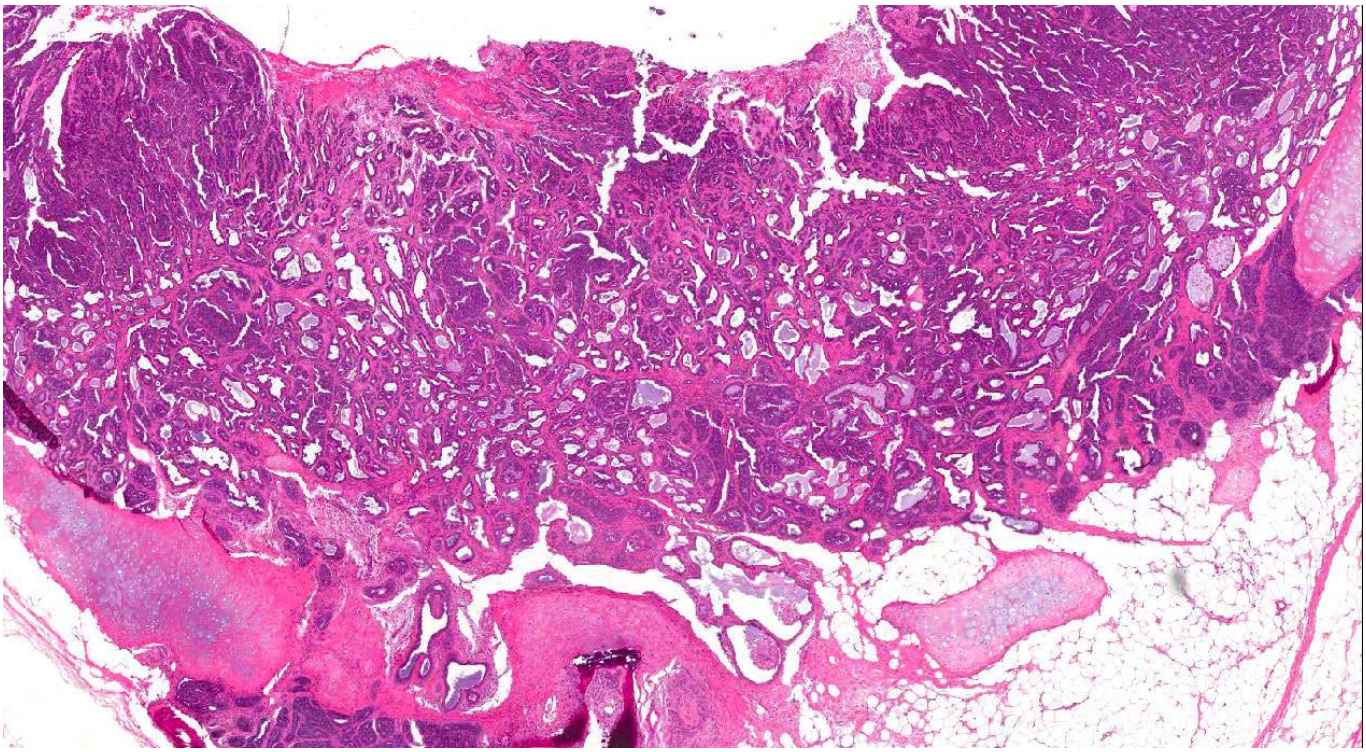
1An infiltrative, well-demarcated neoplasm circumferentially thickens and effaces the tracheal submucoa, multifocally infiltrating into the cartilage of the tracheal rings. (HE 4X)

Laboratory Results: None

Histopathologic Description: Sections of trachea in which there are marked thickening/expansion of the mucosal area by a partly delineated and nonencapsulated mass within the subepithelial/lamina propria area that contains submucosal glands. The mass is composed of cells arranged in numerous variably-sized acini and small cysts with occasional dense clusters in one area supported by a fine fibrovascular stroma. The cysts are lined by one to several layers of cells that are cuboidal with modest amounts of basophilic cytoplasm and a single oval nucleus with finely dispersed chromatin. Mitoses are not seen. Some of the acini and cysts contain small amounts of eosinophilic secretion product. Surrounding many of the cysts are minimal multifocal infiltrates of lymphocytes. A few cells of the mass extend into the subjacent tracheal cartilage in an area.

Contributor's Morphologic Diagnosis: Trachea: Mucosal adenocarcinoma

Contributor's Comment: The mass appears to have arisen from glands of the tracheal mucosa and, as described, protrudes into the tracheal lumen and extends at least in one area into the subjacent tracheal cartilage. Mitotic figures are



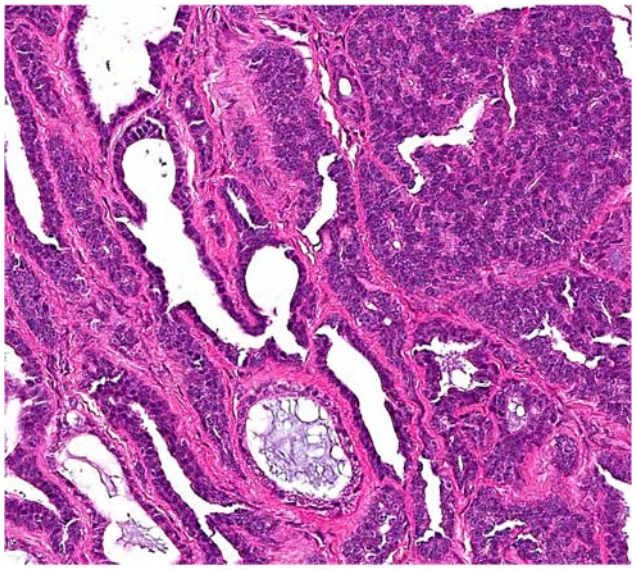
2The infiltrative neoplasm is composed of variably-sized tubules and acini which are often lined with mucin. (HE, 20X)

not common and there is little nuclear atypia; therefore, an adenoma is a consideration. However, the mass appears to be expansile replacing the mucosa in areas and, as indicated, extends to the tracheal cartilage. Tumors of the trachea are very rare in dogs and cats. One publication describes a tracheal tumor in a cat.[3] It is unlikely that the mass is metastatic since: a) Metastasis to the trachea is rare, and b) other neoplastic masses were not identified in the animal. Other types of neoplasms that can occur in the trachea include: Squamous cell carcinoma (especially in humans that smoke), adenoid cystic carcinoma, papilloma, chondroma, hemangioma, and carcinoid. This location of the mass has similarities to adenoid cystic carcinoma seen in humans.[5]

JPC Diagnosis: Trachea: Mucosal adenocarcinoma.

Conference Comment: Characteristics of this unique neoplasm which generated discussion during the conference include the focal area of infiltration into tracheal cartilage (may not be present in all slides) and the fact that it was circumferential in nature, affecting the entirety of tracheal mucosa. Participants noted frequent piling of cells and the focal invasion as two characteristics indicative of a more malignant phenotype. Most agreed the neoplasm was likely primary in the trachea due to the resemblance of the neoplasm to tracheal glands and the fact that another primary tumor was not reported in this animal.

Other primary tracheal neoplasms reported in domestic species, not listed above, include undifferentiated carcinoma, plasmacytoma, leiomyoma, fibrosarcoma, mast cell tumor, rhabdomyosarcoma, osteochondroma, osteosarcoma,[7] and primary intratracheal lymphosarcoma.[2] Epithelial tumors and osteo-chondroma are the most common tumors of the feline and canine trachea, respectively.[4] Laryngeal tumors are also considered rare, but occur more frequently than tracheal tumors. Papillomas and squamous cell carcinomas are the most common laryngeal tumors in dogs, and laryngeal lymphomas are occasionally seen in cats.[6] Neoplasms can be extraluminal or intra-



Higher magnification of the neoplasm showing the marked variation in size of neoplastic acini, as well as the dense fibrous supporting stroma.(HE, 88X)

luminal and cause swelling in the neck and/or tracheal obstruction, respectively, depending on location; dyspnea, cough and dysphagia are common associated clinical signs. Studies have suggested an association between tobacco smoke and oral squamous cell carcinoma in cats,[1] but the precise relationship between tobacco smoke and respiratory tract tumors in cats is unclear, although it can be postulated, based on secondhand smoke being a known human carcinogen.

Contributing Institution:
College of Veterinary Medicine, Iowa State University, Ames, Iowa 50010
Iowastateuniversity.com

References:

1. Bertone ER, Snyder LA, Moore AS. Environmental and lifestyle risk factors for oral squamous cell carcinoma in domestic cats. *J Vet Intern Med*. 2003; 17(4):557-62.
2. Brown MR, Rogers KS, Mansell KJ, Barton C. Primary intratracheal lymphosarcoma in four cats. *J Am Anim Hosp Assoc*. 2003;39(5):468-472.
3. Cain GR, Manley P. Tracheal adenocarcinoma in a cat. *JAVMA*. 1983;182(6):614-616.
4. Carlisle CH, Biery DN, Thrall DE. Tracheal and laryngeal tumors in the dog and cat: Literature review and 13 additional patients. *Vet Radiol*. 1991;32:229-235.
5. Hu Z, Meng Y, Wu H, Shen J, Bi Y, Luo Y, et al. Adenoid cystic carcinoma of the tracheobronchial tree: Cliniocpathologic and immunohistochemical studies of 21 cases. *Int J Clin Exp Pathol*. 2014;7(100):7527-7535.
6. Lopez A. Respiratory system, mediastinum, and pleurae. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby Elsevier; 2012:480.
7. Ramirez GA, Altimira J, Vilafranca M. Cartilaginous tumors of the larynx and trachea in the Dog: Literature review and 10 additional cases (1995-2014). *Vet Pathol*. 2015; Apr 16, Epub: 1-

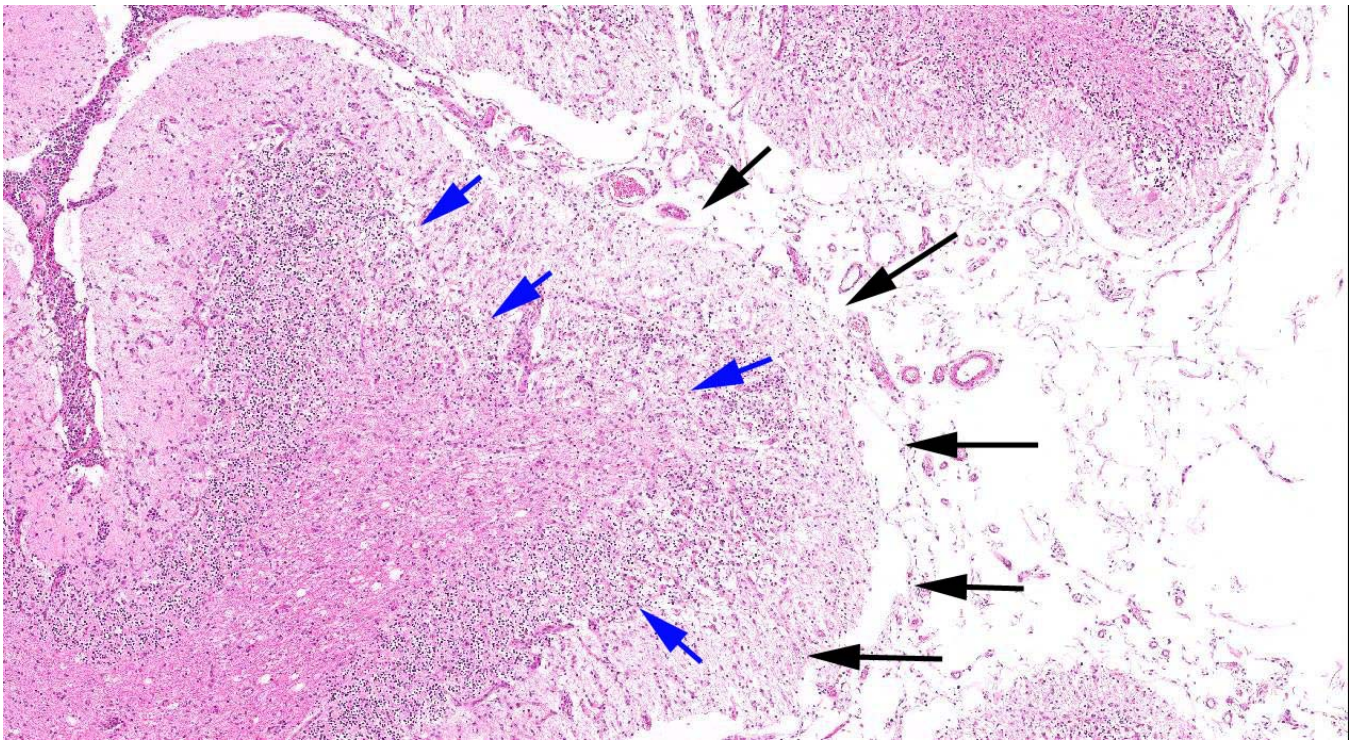
CASE II: 13-338 (JPC 4066222).

Signalment: 5-month-old, female, Labrador retriever, canine (*Canis familiaris).*

History: Approximate 3-month history of progressively abnormal gait and ataxia. Neurological examination revealed mild to moderate tetraparesis and mild proprioceptive ataxia in the pelvic limbs. Evaluation of the spinal reflexes showed reduced flexion in all four limbs and also reduced muscle tone in the pelvic limbs. The patellar reflexes were reduced bilaterally. MRI findings indicated cerebellar atrophy.

Gross Pathology: Examination of the brain reveals mildly increased amounts of clear cerebrospinal fluid surrounding the brain. The cerebellum is diffusely small relative to the cerebrum and medulla with the caudal aspect of the fourth ventricle extending beyond the caudal margin of the vermis. The folia appear diffusely thin with widening of the sulci and moderate congestion of the meninges. The remaining carcass is grossly unremarkable.

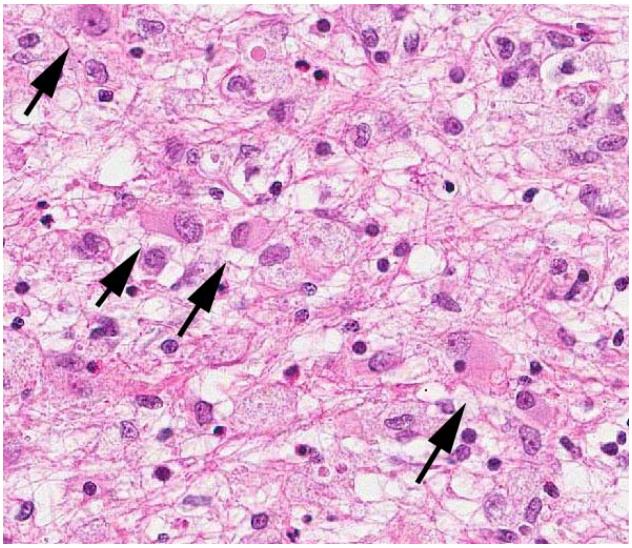
Laboratory Results: Cytological examination of the cerebrospinal fluid shows moderate mixed



There is marked pallor (necrosis) of the submeningeal gray matter of the cerebellar folia (black arrows). The necrosis extends into the underlying molecular layer, which is now hypocellular due to extensive neuronal necrosis (blue arrows). (HE, 37.5X)

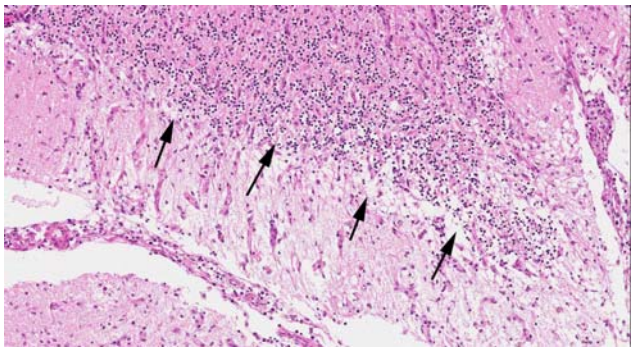
pleocytosis. Samples of brain tissue are PCR positive for *Neospora caninum* and PCR negative of canine herpesvirus, canine parvovirus, *Toxoplasma gondii*, and *Sarcocystis sp*.

Histopathologic Description: Brain (cerebellum and brainstem): The cerebellar folia are partially to extensively disrupted by multifocal to coalescing foci of neuropil rarefaction, loss and collapse with marked Gitter cell infiltration and fibrous astrocyte proliferation, gliosis, and proliferation of small caliber vessels lined by reactive endothelium Low to moderate numbers of plasma cells, lymphocytes, fewer eosinophils and neutrophils are interspersed amongst the collapsed folia, expand the overlying meninges, and distend perivascular spaces within the subjacent cerebellum and brainstem. Smaller, randomly scattered foci of gliosis, nonsuppurative inflammation, and mild rarefaction are scattered randomly within the cerebellar peduncles and brainstem. Within the foci of inflammation, and randomly distributed within the neuropil, are variably frequent protozoal tissue cysts that measure up to 113 µm in diameter. These cysts have a 2 - 3 µm thick cyst wall and contain numerous 8 x 2 µm basophilic bradyzoites (**Figure 4**). Sporadic foci of axonal swelling, digestion chambers containing few debris-filled Gitter cells, and occasional spheroids are noted throughout the white matter.



Necrotic gray matter contains numerous astrocytes with abundant eosinophilic cytoplasm (gemistocytic astrocytes). (HE, 228X)

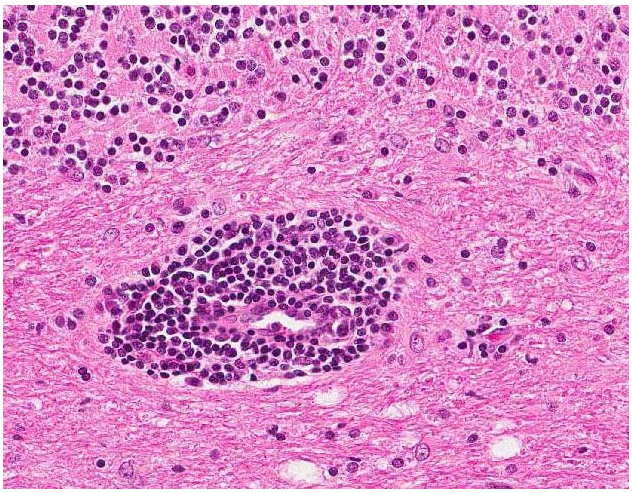
In tissues not included for conference material, also contain similar lesions and protozoal tissue cysts. These lesions were noted in the cerebrum, midbrain, thalamus, and spinal cord with mild infiltration of the spinal nerve roots.



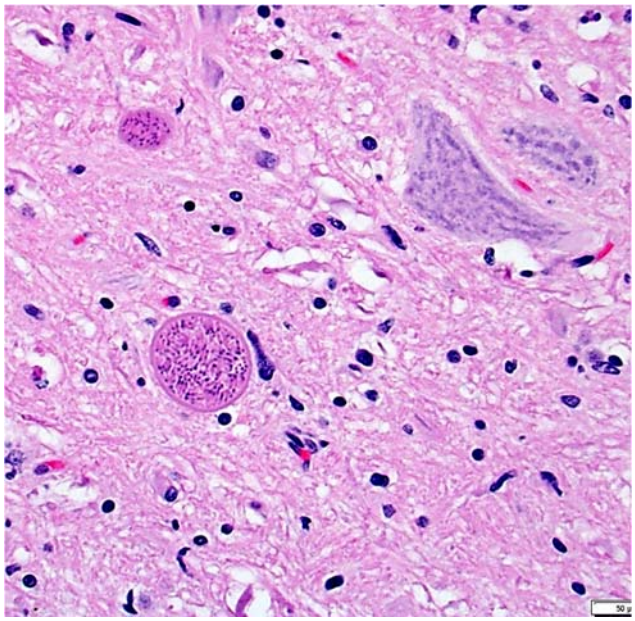
Regionally, there is extensive loss of Purkinje cells (black arrows). (HE, 44X)

Contributor's Morphologic Diagnosis: Brain (cerebellum and brainstem): Meningoencephalitis, lymphoplasmacytic, histiocytic, necrotizing, subacute to chronic, multifocal, moderate to marked with protozoal tissue cysts (consistent with *Neospora caninum*)

Contributor's Comment: Additional findings that were not present in all slides include rare clusters of free 2 x 6 µm, spindle-shaped tachyzoites within the neuropil and fibrinoid degeneration scarcely noted within the walls of arterioles. Overall, the microscopic findings and PCR results confirm the etiologic diagnosis of *Neospora* encephalitis as the underlying cause for the progressive ataxia in this 5-month-old Labrador retriever puppy. *Neospora caninum* is a protozoan parasite of animals and can cause serious neuromuscular and polysystemic disease in dogs,[2,6] as well as significant disease in cattle,[6,9] small ruminants, horses, and wildlife.[5] Infection can be fatal in dogs of any age, but disease is most severe in puppies less than 6 months of age and those that are congenitally infected.[5,7,8] Based on the literature, there is an apparent age-related variation in lesion distribution in the canine. Young dogs and puppies typically develop lesions in the skeletal muscles and spinal nerve roots with resultant ascending paralysis, which tends to be most severe in the hind limbs.[1] Lesions may also be found in multiple organs including the central nervous system, lungs, heart, and liver.[1,8]



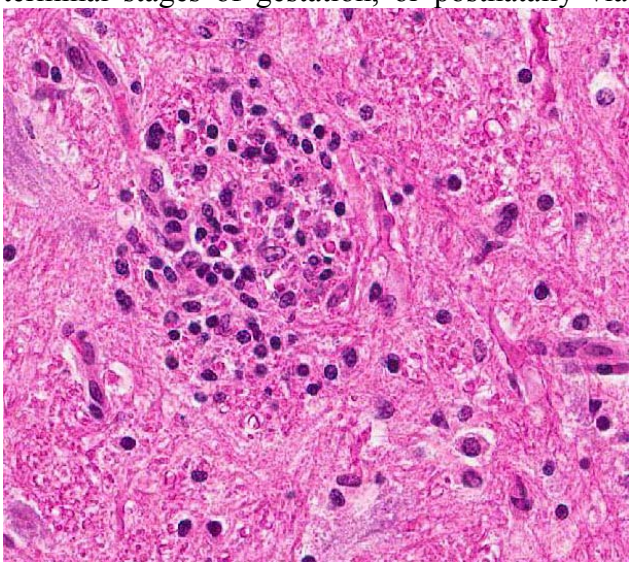
There is multifocal marked lymphohistiocytic perivascular cuffing within the underlying white matter. (HE, 150X)



Randomly distributed throughout both necrotic and unaffected areas are numerous apicomplexan cysts ranging up to 130um. (HE, 400X) (Image courtesy of: University of Calgary Faculty of Veterinary Medicine, Clinical Skills Building, 11877 85th St NW, Calgary, AB T3R 1J3 http://vet.ucalgary.ca

Infection in adult dogs can result in polymyositis, polysystemic infection, or multifocal central nervous system involvement.[8,10] Several reports describe necrotizing cerebellitis and cerebellar atrophy as a significant lesion associated with Neospora encephalitis in adult do [3,11,12] This particular case is unique in that significant the necrotizing cerebellar lesions were found in a puppy. Transmission of *N. caninum* in dogs may

occur both vertically and horizontally. Vertical transmission is well recognized in the dog with data suggesting that transmission from the dam to puppies occurs transplacentally, during the terminal stages of gestation, or postnatally via



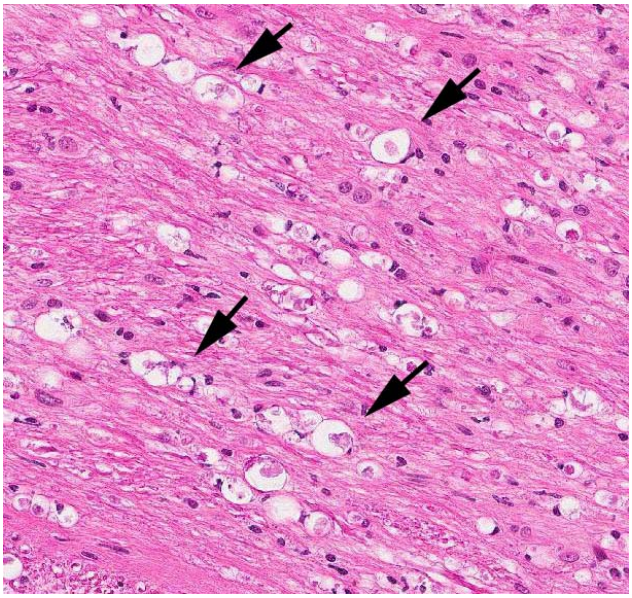
Ruptured Neospora cysts are replaced by glial nodules (HE, 320X)

milk.[9] Horizontal transmission also occurs in the dog through ingestion of infected tissues.[9] Further communications with the breeder revealed that the dam of this puppy, as well as 10 other dogs from the same kennel, was fed a diet of raw beef and deer. Additional serological testing revealed that dam, as well as 4 other dogs, tested positive for *Neospora caninum.* The diet of raw tissues were the likely source of infection in the dam with subsequent horizontal transmission this puppy. Given that most puppies do not develop clinical signs until over 4 weeks of age,[8] it is difficult to determine if transplacental or postnatal infection occurred in this case.

JPC Diagnosis: Cerebellum and brainstem: Necrotizing polioencephalitis, subacute, multifocal to coalescing, moderate with lymphocytic and neutrophilic meningitis and apicomplexan schizonts.

Conference Comment: Central nervous system (CNS) changes in the slide due to *Neospora* infection were profound and conference participants noted the protozoal schizonts were often found in less affected areas of the section; the precise reason for this discordant finding is unclear but not uncommon. Conference participants agreed that the most prominent change in the cerebellum was necrosis and loss of cerebellar grey matter extending into the adjacent white matter. The molecular and granular cell layers were reduced in thickness and there was loss of Purkinje cells with occasional empty baskets in the most severely affected areas. Glial nodules and prominent spheroids were described, as well as inflammatory infiltration of the meninges with extension into, and prominent expansion of, Virchow-Robin spaces. The inflammatory infiltrate was primarily mononuclear, dominated by lymphocytes, plasma cells and gitter cells; the latter being most noticeable in areas of necrosis and neuroparenchymal loss.

Differential diagnosis considered for this case included *Toxoplasma gondii* as well as *Sarcocystis* spp. Many features of toxoplasmosis and neosporosis are similar including the presence of the proliferative tachyzoite and tissue cyst phases. However, *N. caninum* does not develop in a parasitophorus vacuole and has a thicker cyst wall than *T. gondii.* The differences cannot be reliably differentiated by light microscopy, and require the use of electron microscopy or immunohistochemistry in many cases.[14] Ultrastructural differences include



Multifocally, within the folial white matter, there is axonal damage with swelling and formation of digestion chambers. (HE, 320X)

greater number of micronemes and rhoptries with *N. caninum*, in addition to a thicker cyst wall.[12] *N. caninum* is more commonly reported in the CNS where the tissue cysts are most commonly found; although *T. gondii* has a CNS form causing similar histologic changes. *N. caninum* seems to have an affinity for cells of the monocyte macrophage system, although many cell types can be infected, and it likely spreads to the CNS via leukocyte trafficking.[14] Toxoplasmosis seems to affect a wider variety of mammalian species

Although very uncommon, both *Sarcocystis canis* and *Sarcocystis neurona* have been documented to cause CNS disease in dogs,[12] In one documented case of *S. neurona* the affected dog was receiving immunosuppressive therapy and developed widespread encephalitis, predominantly in the grey matter, with brainstem, cerebellum and cerebrum being involved. The lesions also consisted of intense areas of inflammation, which was most pronounced in the cerebellum. [4] The dividing schizonts of *S. neurona* form distinct rosettes of merozoites, arranged around a prominent residual body and their schizonts differ from other protozoa in that the merozoites lack rhoptries.[14] Encephalomyelitis due to *S. neurona* infection has also been described in cats, which along with many other mammals including harbor seals and nonhuman primates, can serve as intermediate hosts for *S. neurona*.[12]

Contributing Institution:
University of Calgary Faculty of Veterinary Medicine (vet.ucalgary.ca)

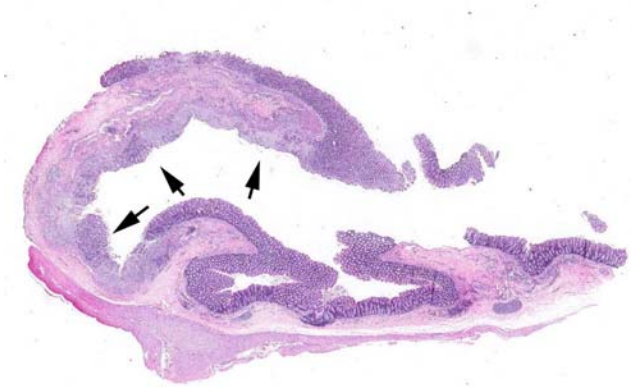
References:

1. Barber JS, Trees AJ. Clinical aspects of 27 cases of neosporosis in dogs. *Vet Rec.* 1996; 139(18)**:** 439-443.
2. Bjerkas I, Mohn SF, Presthus J. Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. *Z Parasitenkd.* 1984; 70(2)**:** 271-274.
3. Cantile C, Arispici M. Necrotizing cerebellitis due to Neospora caninum infection in an old dog. *J Vet Med A Physiol Pathol Clin Med.* 2002; 49(1)**:**47-50.
4. Cooley AJ, Barr B, Rejmanek D. Sarcystis neurona encephalitis in a dog. *Vet Pathol*. 2007;44:956-961.
5. Dubey JP. Recent advances in Neospora and neosporosis. *Vet Parasitol.* 1999; 84(3-4)**:** 349-367.
6. Dubey JP: Review of Neospora caninum and neosporosis in animals. *Korean J Parasitol.* 2003; 41(1)**:** 1-16.
7. Dubey JP, Carpenter JL, Speer CA, et al.: Newly recognized fatal protozoan disease of dogs. *J Am Vet Med Assoc.* 1988; 192(9)**:** 1269-1285.
8. Dubey JP, Lindsay DS: A review of Neospora caninum and neosporosis. *Vet Parasitol.* 1996; 67(1-2)**:** 1-59.
9. Dubey JP, Schares G, Ortega-Mora LM: Epidemiology and control of neosporosis and Neospora caninum. *Clin Microbiol Rev.* 2007; 20(2)**:** 323-367.
10. Gaitero L, Anor S, Montoliu P, et al. Detection of Neospora caninum tachyzoites in canine cerebrospinal fluid. *J Vet Intern Med.* 2006; 20(2)**:** 410-414.
11. Garosi L, Dawson A, Couturier J, et al. Necrotizing cerebellitis and cerebellar atrophy caused by Neospora caninum infection: magnetic resonance imaging and clinicopathologic findings in seven dogs. *J Vet Intern Med.* 2010; 24(3)**:** 571-578.
12. Greene CE. *Infectious diseases of the dog and cat*. 4th ed. St. Louis, MO: Elsevier Saunders; 2012:825, 836-838.
13. Lorenzo V, Pumarola M, Siso S. Neosporosis with cerebellar involvement in an adult dog. *J Small Anim Pract.* 2002; 43(2)**:** 76-79.
14. Zachary JF. Nervous system. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby Elsevier; 2012:809, 841.

CASE III: 1423851 (JPC 4065318).

Signalment: 8 year old male neutered domestic short-haired cat *(Felis catus).*

History: This cat lived in a colony used for long term nutritional studies. It was noted to be icteric and inappetant, and subsequently euthanized.



There is a focally extensive area of ulceration and necrosis within the colonic wall extending into the muscular tunics. (HE, 4X)

Gross Pathology: No gross lesions were noted by the prosector, who submitted multiple fixed tissues for microscopic examination, including colon.

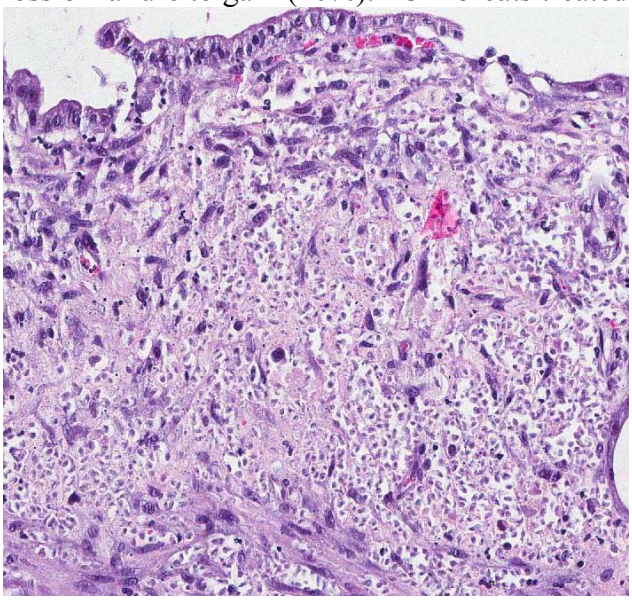
Laboratory Results: The spleen was positive for feline coronaviral antigens. Fungal stains on the lesion in the colon were negative.

Histopathologic Description: Segments of colonic crypts contain abundant small protozoal organisms that are teardrop to crescent-shaped, have eosinophilic cytoplasm and round hyperchromatic nuclei. Cellular debris is associated with their presence. The mucosal surface is altered by erosions and ulcerations, while neighboring glands are lined by hypertrophic and hyperplastic enterocytes exhibiting increased mitotic activity and loss of goblet cells. The neighboring lamina propria and submucosa also contains numerous organisms, associated with moderate numbers of macrophages, neutrophils, lymphocytes and fewer plasma cells. Mixed inflammation extended into the muscularis in a few areas, but with fewer protozoa. Similar organisms were found in the ileal crypts.

In lesions not shown, this animal also had an exocrine pancreatic adenocarcinoma, with surrounding inflammation. Vacuolar change was discovered in the liver, along with bile stasis. Pyogranulomas were found in the spleen and were immunohistochemically positive for FIP antigens.

Contributor's Morphologic Diagnosis: Colon: Colitis, necrotizing and suppurative, locally transmural, with numerous protozoal organisms consistent with *Tritrichomonas foetus.*

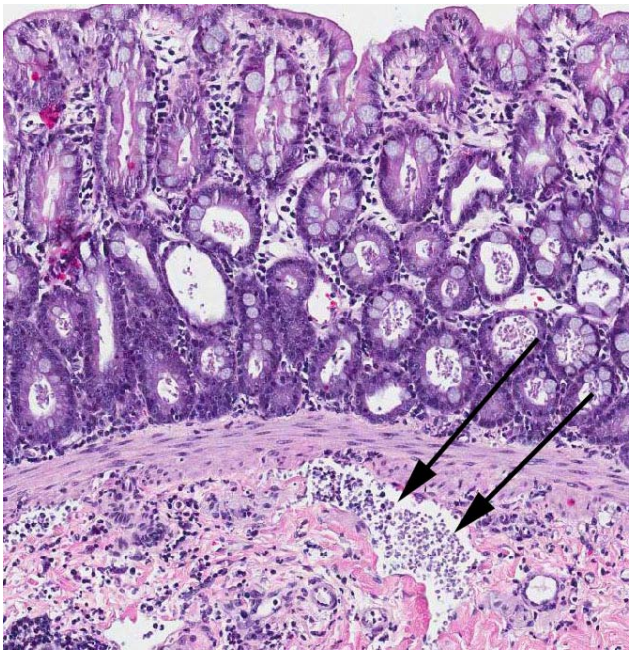
Contributor's Comment: *Tritrichomas foetus* is an important cause of chronic large bowel diarrhea in cats, as well as an important venereal pathogen of cattle, causing early abortion and infertility. It is a commensal inhabitant of the nasal and GI tract of pigs. Diarrhea generally affects young, densely housed animals, and has been described as a frequent pathogen of purebred animals.[9] Catteries with purebred cats positive for *Tritrichomonas foetus* infection have fewer square feet per cat.[2] Cats infected with this organism had diarrhea for mean duration 135 days (range 1-288 days). Other signs included anorexia (22%), depression (24%, and weight loss or failure to gain (20%).[1] Of 45 cats treated



3Ulcerated areas of the mucosa contain innumerable trichomonads within the mucosa and submucosa. (HE, 92X)

with ronidazole, the only antibiotic effective against this organism, 36% had partial or no improvement or relapsed, but some animals had concurrent *Giardia*.[10]

PCR is the test capable of identifying the greatest number of cats (34/36), versus culture (in pouch 24/36 or Diamond's media (5/36).[2] Other tests can include wet mount smears of loop samples from the rectum and chromogenic in situ hybridization.[4] A confounding factor is the presence of *Pentatrichomonas hominis.*[3]



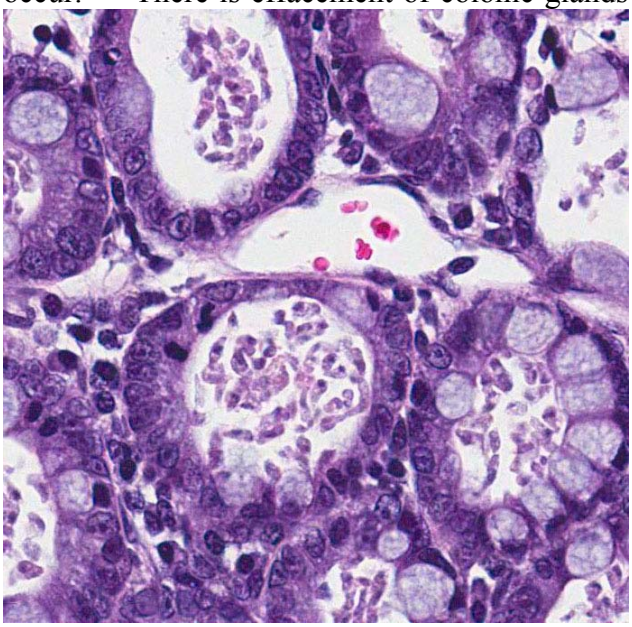
Numerous trichomonads are present within glands in the adjacent, less affected mucosa, as well as in the submucosa, as well as within submucosal lymphatics (black arrows). (HE, 120X).

In sections from spontaneously infected cats, lesions included an increase in lymphocytes and plasma cells in the lamina propria as measured across the width of the villi, and a few infiltrating neutrophils. Trichomonads were tear-shaped to crescentic organisms; flagella were not visible. Organisms were on the mucosa and less frequently in crypts. Organisms were visible only in 56% of sections in colon and 6 sections were needed to have 95% confidence of seeing them. Some cats, such as this one, had ulcers containing trichomonads as well.[10]

Initial infection of naïve cats with a feline isolate of *T. foetus* produced persistent infection in all cats and resulted in diarrhea that resolved after 7 weeks.[7] Experimental infection of cats with 2 bovine isolates resulted in colonization of the intestine and lesions that varied in severity with the isolate.[6] Conversely heifers inoculated with a feline organism developed similar endometritis to that caused by a bovine isolate and organisms were recovered over an 11 week period.[3] Some authors believe the bovine and feline pathogens are separate species.[5,8]

JPC Diagnosis: Large intestine: Colitis, necrotizing, multifocal to coalescing, severe with numerous intra- and extracellular tritrichomonads.

Conference Comment: The sheer number of organisms present in the sections was impressive with tritrichomonads filling colonic gland lumina in the superficial and deeper mucosa, both intra- and extracellular, as well as being present in dilated submucosal lymphatics. The deep invasion in this case is unusual in that organisms are reported to be most consistently found in close association with the mucosal surface, although invasion of deeper layers is known to occur.[10] There is effacement of colonic glands



Higher magnification of numerous elliptical trichomonads which are present within colonic glands. There is a mild decrease in goblet cells as well as a lymphoplasmacytic colitis which separates and surrounds colonic glands. (HE, 360X)

and crypts with attenuation and crypt abscesses in the remaining crypts. The presence of mitotic figures provide evidence of regeneration. Conference participants commented on the goblet cell hyperplasia in some areas in contrast with the loss of goblet cells in regenerative areas.

Two trichomonads have been identified as inhabiting the intestinal tract of cats, *Tritrichomonas foetus* and *Pentatrichomonas hominis*, with *T. foetus* being considered the cause of large bowel diarrhea and *P. hominis* being considered a commensal.[4] Although most commonly considered an infection of kittens, older cats may be asymptomatically infected with *T. foetus* as well.[9] Generally thought to be the same organism which causes diarrhea in cats as well as venereal disease in cattle, recent studies have suggested the organisms may indeed be different species. A new species, *Tritrichomonas blagburni* n.sp., has been suggested as the cause of intestinal tritrichomonas in cats.[8]

Giardia sp. trophozoites can be difficult to distinguish from those of *T. foetus*. Differentiating features of *T. foetus* includes a distinct undulating membrane, lack of cyst formation and being refractory to treatment with common antiprotozoals used for treatment of giardia. Pathogenicity of *T. foetus* may be related to alterations in normal host flora, epithelial adherence and elaboration of cytotoxins. An important consideration in obtaining biopsy samples for evaluation is sampling from multiple locations, as literature indicates organisms may not be present in all samples.[10]

Contributing Institution:
Veterinary Medical Diagnostic Laboratory, University of Missouri
vmdl.missouri.edu

References:

1. Gookin JL, Levy MG, Law JM, et al. Experimental infection of cats with *Tritrichomonas foetus*. *Am J Vet Res.* 2001;62:1690-1897.
2. Gookin JL, Stebbins ME, Hunt E, et al. Prevalence of and risk factors for feline *Tritrichomonas foetus* and *Giardia* infection. *J Clin Microbiol*. 2004;42:2707-2710.
3. Levy MG, Gookin JL, Poore M, et al. *Tritrichomonas foetus* and not *Pentatrichomonas hominis* is the etiologic agent if feline trichomal diarrhea. *J Parasitol.* 2003; 89:99-104.
4. Mostegl MM, Wetscher A, Richter B, et al. Detection of *Tritrichomonas foetus* and *Pentatrichomonas hominis* in intestinal tissue specimens of cats by chromogenic in situ hybridization. *Vet Parastitol.* 2012;183:209-214.
5. Šlapeta J, Muller N, Stack CM, et al. Comparative analysis of *Tritrichomonas foetus* (Riedmuller 1928) cat genotype, *T. foetus* (Riedmuller 1928) cattle genotype and *Tritrichomonas suis* (Davaine, 1875) at 10 DNA loci. *Int J Parasitol.* 2012;42:1143-1149.
6. Stockdale HD, Dillon AR, Newton JC, et al. Experimental infections of cats (*Felis cattus*) with *Tritrichomonas foetus* isolated from cattle. *Vet Parasititol*. 2008;154:156-161.
7. Stockdale HD, Rodning S, Givens M, et al. Experimental infection of cattle with a feline isolate of *Tritrichomonas foetus*. *J Parasitol.* 2007;93:1429-1434.
8. Walden HS, Dykstra C, Dillon A, et al. A new species *Tritrichomonas* (Sarcomastigophora: Trichomonida) from the domestic cat (*Felis catus*). *Parasitol Res.* 2013;112: 2227-2235.
9. Xenoulis PG, Lopinski DJ, Read SA, et al. Intestinal *Tritrichomonas foetus* infection in cats: a retrospective study of 104 cases. J Feline Med Surg 2013; 15: 1098-1103.
10. Yaeger MJ, Gookin JL. Histologic features associated with *Tritrichomonas foetus*-induced colitis in domestic cats. *Vet Pathol 2005;42: 797-804.*

CASE IV: D15-6096 (JPC 4066091).

Signalment: 4-month-old, intact female, Rottweiler mix dog (*Canis familiaris*).
History: The puppy had a recent history of increased respiratory rate and effort progressing to dyspnea. A bronchointerstitial pattern on chest radiographs was noted. There was a lack of response to antifungals and antibiotics. The dog had a one week history of prednisone use for

suspected protein-losing enteropathy. The dog had a longer history of pyoderma, chronic diarrhea, ascites, and microhepatica. The puppy had been administered two DHPP vaccines sometime prior to 2 months of age. The puppy died at home two days after being discharged.

Gross Pathology: The puppy was moderately thin with muscle wasting. There was periocular dermatitis, inflammation centered on the mucocutaneous junction, and ventral abdominal pyoderma. The lungs were mottled pale pink to dark pink with multifocal to coalescing, white to beige, flat to slightly raised, semi-firm areas in a branching pattern. The lesions were most prominent in the hilar and mid lobe regions intermixed with slightly sunken, non-crepitant, dark pink to red irregular areas of parenchyma (atelectasis). There were multifocal, fairly well-demarcated, dark red, irregular foci, 1-3 mm in diameter, throughout the lung parenchyma (hemorrhage). On cut section the lungs were mottled dark red to red to pink to beige and semi-firm. The liver was friable and mottled pale pink to dark pink. A delicate fibrin membrane was adherent to the liver capsule. The intestines were moderately dilated with watery to viscous contents. The lymphatics overlying the pancreas and associated with the duodenum and proximal jejunum were prominent.

Bacteriology

Lung (2 specimens) - No growth and no significant growth, respectively

Liver - No growth

Spleen- Staphylococcus intermedius group (mixed culture, 1+)

Immunohistochemistry (IHC)

Canine adenovirus type 2 – lung (respiratory epithelium, pneumocytes, alveolar macrophages), exocrine pancreas, and labial glands in the oral mucocutaneous junction - **positive**

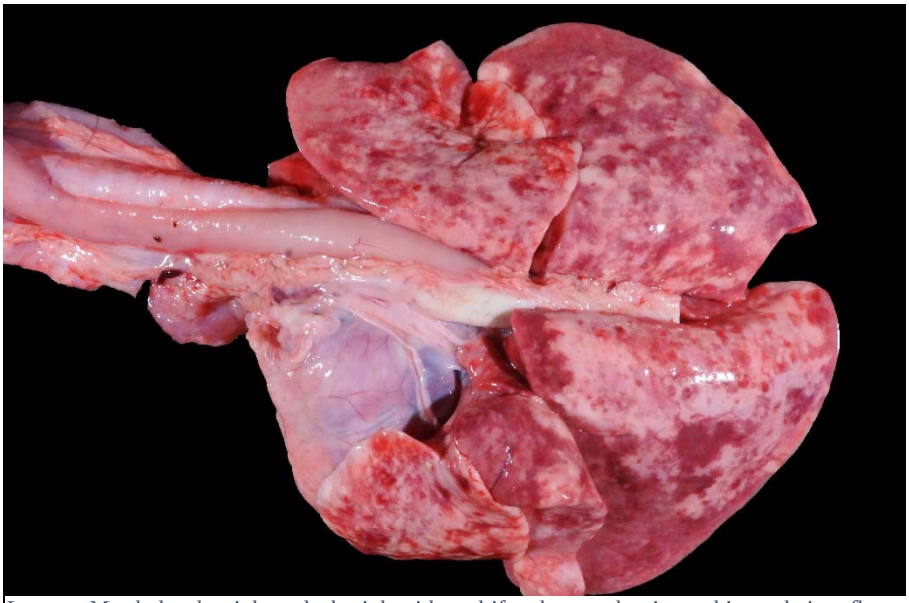
Canine adenovirus type 2 – small intestine - inconclusive

Canine distemper virus – lung - negative

Canine parvovirus – small intestine - negative

Virology

Virus isolation – lung - **Canine adenovirus isolated**



Lungs - Mottled pale pink to dark pink with multifocal to coalescing, white to beige, flat to slightly raised, semi-firm areas in a branching pattern admixed with areas of atelectasis. (Image courtesy of: University of Minnesota Veterinary diagnostic Laboratory, http://www.vdl.umn.edu)

Electron Microscopy

Negative stain – lung - **Adenovirus**

Molecular Diagnostics

Canine Respiratory Panel (PCR) – Lung - Canine distemper virus - **positive**

Canine influenza virus - negative

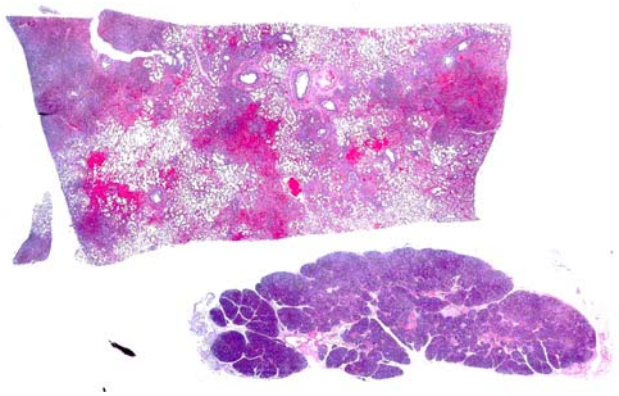
Canine parainfluenza virus - negative

Canine respiratory coronavirus - negative

Canine Adenovirus 2 - positive

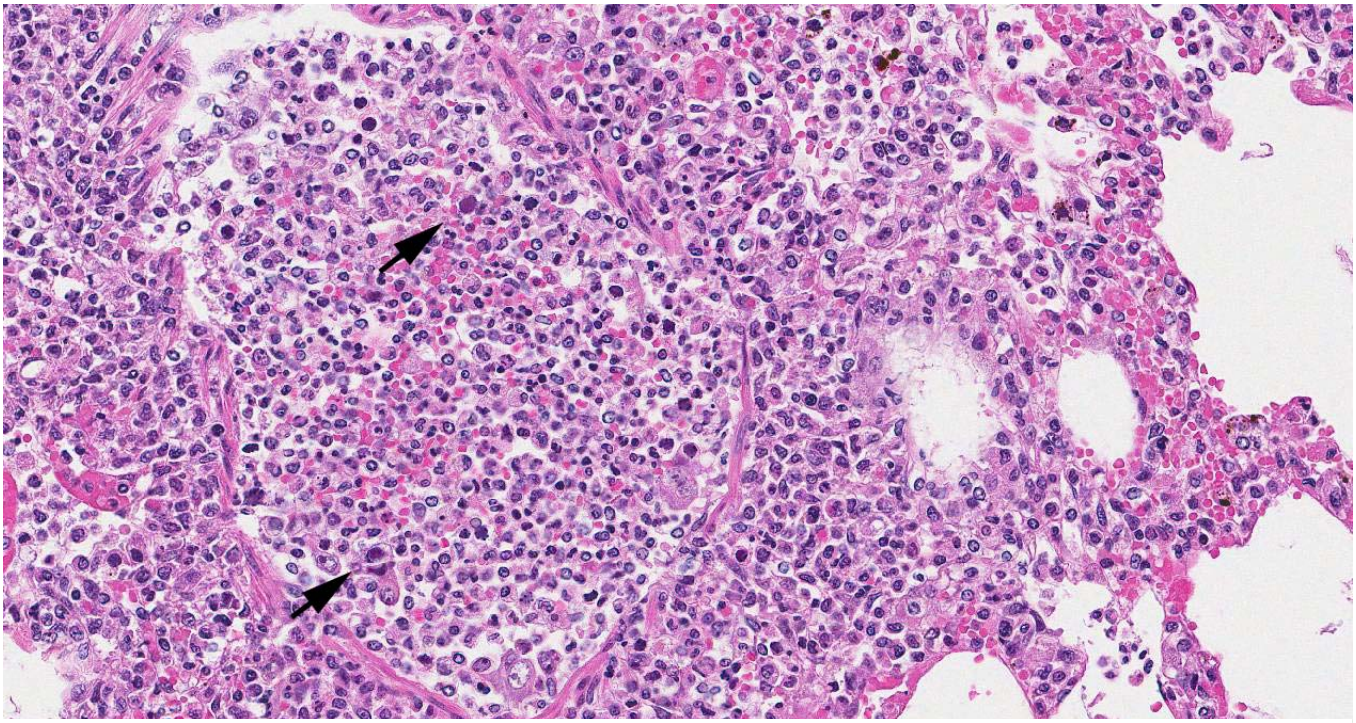
Canine *Bordetella bronchiseptica* - negative

Streptococcus equi subsp zooepidemicus – negative



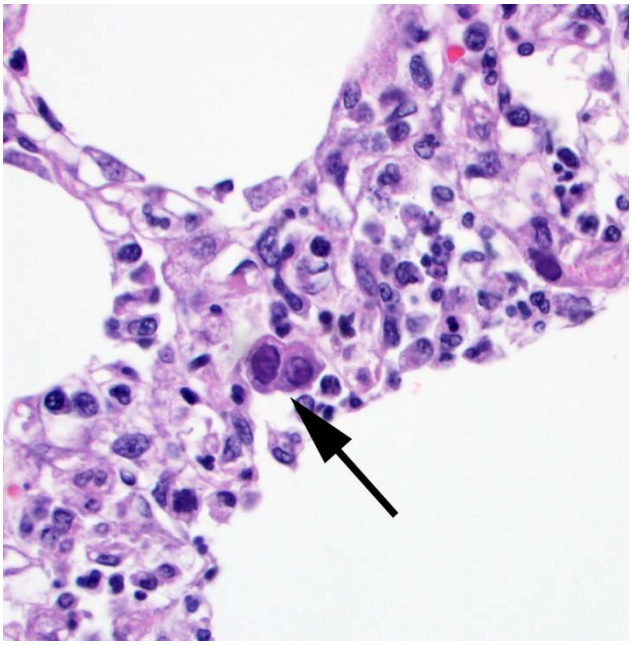
Submitted tissues include lung and liver. The lung contains multiple confluent foci of hemorrhage and necrosis centered on airways. (HE, 4X)

Histopathologic Description: Lung – Multi-focally affecting ~50% of the section there are geographic areas of alveoli filled with neutrophils and alveolar macrophages admixed with accumulations of fibrin, hemorrhage, and siderophages centered around bronchi and bronchioles. The bronchi and bronchioles are lined by mildly to moderately hyperplastic respiratory epithelium and are filled with degenerate and non-degenerate neutrophils and sloughed epithelial cells. Occasionally the bronchial and bronchiolar walls are disrupted by inflammatory infiltrates as previously described and necrotic debris. Within these bronchi and bronchioles, a small to moderate number of scattered epithelial cells, sloughed epithelial cells, bronchial gland epithelium, and rarely pulmonary macrophages have swollen, enlarged nuclei that contain a large, 5-15um, ovoid to round, smudgy, basophilic to amphophilic intranuclear inclusion that typically completely fills the nucleus but occasionally has a thin clear rim (halo) between the inclusion and marginated chromatin. There are moderate to large numbers of lymphocytes and plasma cells, neutrophils and macrophages within the bronchial submucosa and surrounding bronchial glands with mild edema. There are infrequent thrombi within adjacent vessels. There is moderate perivascular edema around large caliber vessels within these areas. The alveolar septa multifocally are mildly expanded by neutrophils, macrophages, lymphocytes, infrequent fibrin, and rarely lined by few plump type II pneumocytes. Within affected alveoli, there are scattered pneumocytes, which are often sloughed, as well as alveolar



Higher magnification of an affected bronchiole. There is extensive necrosis and sloughing of airway epithelium; within the luminal exudate, sloughed epithelial cells contain adenoviral intranuclear inclusions. (HE, 110X)

macrophages that contain intranuclear inclusion bodies as described in the bronchial epithelium.
Pancreas – The exocrine pancreatic cells in randomly distributed foci are pale with loss of zymogen granules, occasionally vacuolated (degeneration), or faded and shrunken. Nuclei of these cells are frequently absent and the cells are admixed with amorphous cellular debris (necrosis). There are low numbers of neutrophils, macrophages, and lymphocytes and plasma cells within these areas and in the interstitium. Moderate numbers of acinar cells in these areas have an enlarged nucleus with marginated chromatin and an eosinophilic to basophilic inclusion similar to that described in the bronchial epithelial cells in the lung. Multifocally large areas of the surrounding adipose tissue are hypereosinophilic with absent nuclei and concentric flocculent to amorphous eosinophilic material within the adipocytes or between them (fat necrosis). There are small numbers of macrophages, neutrophils, and lymphocytes along the periphery of these areas.



4Lung. (H&E) There are large basophilic intranuclear inclusion bodies in two adjacent pneumocytes lining the alveolar septa. The septa wall is thickened by infiltrates of macrophages, lymphocytes, and neutrophils. (HE, 400X) (Image courtesy of: University of Minnesota Veterinary diagnostic Laboratory, http://www.vdl.umn.edu)

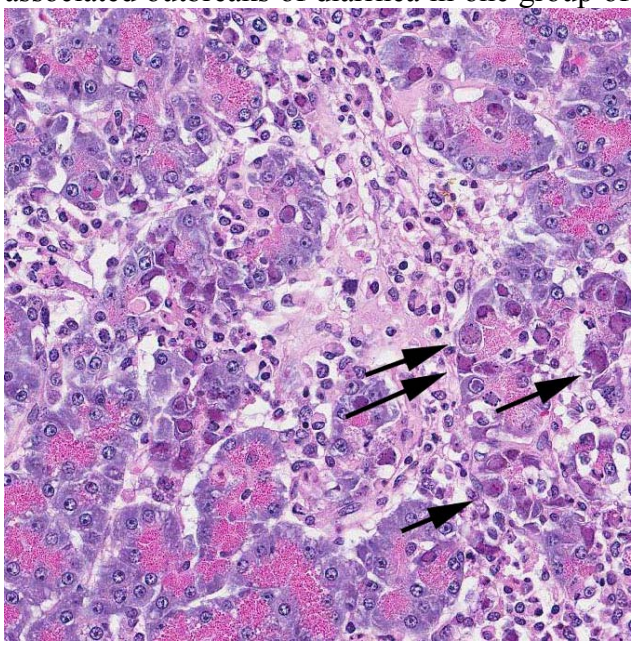
Mucocutaneous junction (not included): Multifocally there is mild parakeratotic hyperkeratosis, acanthosis, infrequent spongiosis, and occasional areas of ulceration. There are multifocal neutrophilic intracorneal pustules and superficial serocellular crusts composed of serum proteins and degenerate and non-degenerate neutrophils. There are multifocal areas in some sections of glabrous skin, with moderate numbers of lymphocytes and plasma cells, macrophages and neutrophils surrounding the labial glands. The labial glands are often absent, degenerative, or replaced by necrotic debris and sloughed cells with few intraluminal neutrophils and debris. Rarely within these glands the nuclei are enlarged with marginated chromatin and contain a large ovoid basophilic inclusion body.

Contributor's Morphologic Diagnosis:
1. Lung: Necrosuppurative and lymphoplasmacytic bronchitis/bronchiolitis and bronchointerstitial pneumonia, multifocal to coalescing, moderate to marked, subacute with mild bronchial epithelial hyperplasia and intraepithelial intranuclear inclusion bodies (consistent with canine adenovirus type 2).

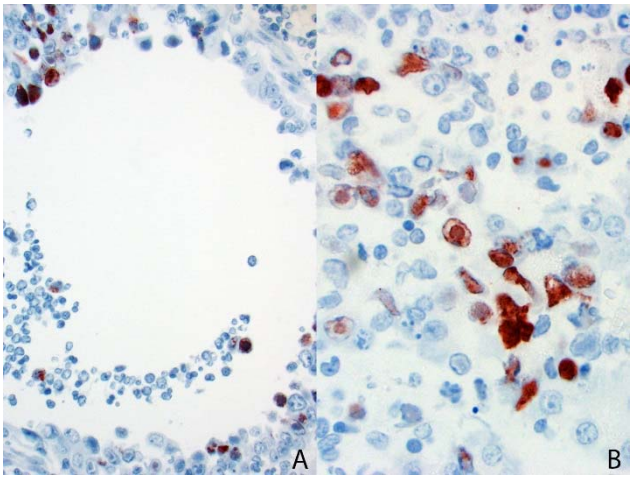
2. Pancreas: Necrotizing and lymphohistiocytic pancreatitis, multifocal, moderate, subacute with intraepithelial intranuclear inclusion bodies (consistent with canine adenovirus type 2).

Contributor's Comment: Canine adenovirus (CAV) is a non-enveloped icosahedral double stranded DNA virus found worldwide that belongs to the genus *Mastadenovirus* and family *Adenoviridae*. In addition to affecting domestic dogs, other carnivores such as foxes, coyotes, otters, and wolves are susceptible to CAV infection and antibodies have been detected in fishers, polar and black bears, sea lions, and walruses.[4,8] There are two serotypes of adenovirus, canine adenovirus type 1 (CAV1) and canine adenovirus type 2 (CAV2), that are genetically and antigenically similar yet divergent enough to allow differentiation with various diagnostic methods. The similarities allow vaccination with a parenteral modified live attenuated CAV2 in dogs to provide cross-protection against CAV1 while avoiding the

well-known side effects of using a modified live CAV1 vaccine.[8,13] In countries with established systematic vaccination in the domestic dog population, circulation of the virus is limited and thus prevalence of disease is low. In unvaccinated and wild canid populations, seroprevalence is variable but significant (between 30-70% worldwide) indicating exposure to this virus is common.[5,6,8,15] CAV1 is a systemic infection that targets hepatocytes and endothelial cells and mostly affects young dogs with mortality rates between 10-30%. In contrast, CAV2 is typically limited in extent and virulence resulting in a respiratory infection that is often asymptomatic in otherwise healthy dogs. This virus is one of the organisms involved in canine infectious respiratory disease (CIRDC) complex (also called canine infectious tracheobronchitis or kennel cough) that typically occurs after exposure to other dogs (boarding, shelter, dog parks, etc.). CAV2 replicates most readily in non-ciliated bronchiolar and bronchial epithelium as well as the epithelial cells of the tonsillar crypts, pharynx, nasal mucosa, mucus cells of the trachea and bronchi, and occasionally type 2 pneumocytes.[13] In addition, replication can occur to a limited extent in the intestinal epithelial cells and CAV2 has been previously associated outbreaks of diarrhea in one group of dogs.[6]



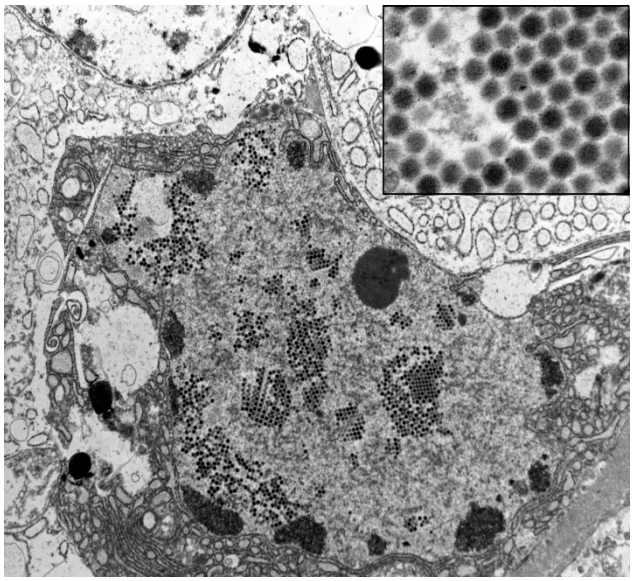
Within the pancreas, there is multifocal degeneration and necrosis of acinar tissue. Numerous acinar epithelial cells contain intranuclear viral inclusions (arrows). (HE, 100X)



Immunohistochemistry - Canine adenovirus type 2. A. Lung, bronchiole. B. Pancreas. (Image courtesy of Univ. of Minnesota Veterinary Diagnostic Lab, http://www.vdl.umn.edu)

The extensive pulmonary infection with CAV2 resulting in dyspnea and respiratory failure in this puppy in the absence of a secondary bacterial infection is unusual. Additionally the extent of infection in this puppy including the oral labial glands at the mucocutaneous junction and exocrine pancreatic acinar cells is rare. The pattern of tissue involvement is reminiscent of that described in some immunodeficient Arabian foals with equine adenoviral infection[10] and, to the authors' knowledge, involvement of the pancreas with CAV2 infections in the dog has not been previously described in the literature. However, less typical locations to identify adenoviral inclusions have been noted previously in the splenic macrophages and hepatocytes in a bulldog puppy with systemic CAV2 infection.[1] In a separate report, detection of CAV2 via in situ-hybridization in the liver and spleen and via polymerase chain reaction in the brain was noted in a puppy without inclusion bodies present in any of these tissues.[3] The exact cause of the histologic pattern in this puppy is unclear and although mutations have been noted to occur in CAV[3], this virus is generally considered genetically stable[8] while the significance of mutations in terms of virulence or cell tropism is unknown.

Clinical and severe pulmonary infection with CAV2 is typically attributed to an immunocompromised status or complication with other viruses or bacteria.[1] Co-infections with CIRDC are common of which infections with multiple respiratory viruses appears to be more common than previously believed.[12,14] In this puppy, both canine distemper virus (CDV) and adenovirus were detected by PCR of lung tissue but interestingly there was no immunoreactivity noted in the lung on CDV IHC and a lack of viral inclusions consistent with CDV. Although PCR and IHC testing of CDV has not been directly compared, the higher sensitivity of PCR over IHC has been noted in another veterinary infectious disease[2] and inclusion bodies were only found in 25% of CDV cases confirmed by other methods in another report[7] which may explain the findings in this case. The contribution of CDV in this case is unknown and assuming that the lack of histologic or immunohistochemical detection suggests a low viral load, one of two following scenarios is likely: 1. Early infection prior to significant viral replication and cytopathic effects, or 2. Late infection after significant but incomplete clearance of the virus. With either of these scenarios, the degree of virus-induced immunosuppression can be questioned as the former is before the lymphoid destruction that induces immunosuppression and the latter would imply enough recovery of the immune system to allow significant clearance of the virus. Other reasons for possible immunosuppression also existed in this puppy including recent steroid usage and others conditions including marked lymphangiectasia with hypoproteinemia and evidence of an intrahepatic portosystemic shunt. The occurrence of clinical infection in this vaccinated puppy is likely attributable to interference of maternal antibodies which typically persist until 12-14 weeks of age (2-4 weeks beyond this puppies last vaccine) rather than emergence of a variant strain.[8,13]



Transmission electron microscopy of pancreatic acinar cell (15000x). There are numerous intranuclear adenoviral particles forming paracrystalline arrays. Inset (100000x): Magnified view of adenoviral particles. (Image courtesy of the University of Minnesota Veterinary Diagnostic Laboratory, http://www.vdl.umn.edu)

JPC Morphologic Diagnosis:

1. Lung: Pneumonia, bronchointerstitial, necrotizing, multifocal to coalescing, severe with numerous intraepithelial intranuclear viral inclusion bodies, Rottweiler mix, canine.
2. Pancreas: Pancreatitis, necrotizing, multifocal to coalescing, moderate with numerous intraepithelial intranuclear viral inclusion bodies.
3. Adipose tissue, peripancreatic and mesenteric: Fat necrosis, multifocal to coalescing, with saponification.

Conference Comment:

In the lung, conference participants readily identified intranuclear inclusion bodies within intact and sloughed epithelial cells, both bronchiolar and type II pneumocytes. Along with expansion of alveolar septa with inflammatory cells and foci of alveolar septal necrosis, and megakaryocytes are also occasionally found in alveolar capillaries. Multifocally, alveoli contain a macrophage-rich exudate and there is type II pneumocyte hyperplasia. Participants also described segmental necrosis of bronchiolar epithelium, epithelial attenuation, and sloughing of epithelial cells into the lumina. Changes identified by participants in the pancreas mirrored those of the contributor and include degeneration and necrosis of pancreatic acinar epithelium; loss of zymogen granules; expansion of the interlobular

areas with fibrin and edema; and presence of intranuclear inclusion bodies in the acinar epithelial cells. Other changes include necrosis and mineralization (saponification) of the peripancreatic fat.

Canine adenovirus type 2 is most often associated with mild respiratory disease, although more severe fatal disease has been documented.[1] This dog's history included vaccination only up to 8 weeks, as well as administration of corticosteroids, both of which were suggested as playing a role in the pathogenesis in this case. The differential diagnosis for viral pneumonia in the dog discussed by participants included: canine morbillivirus, canine influenza virus, canine coronavirus and canine parainfluenza virus. In this case, the intranuclear viral inclusion bodies within epithelial cells are considered distinctive for canine adenovirus type 2, making other viral etiologies less likely. Some participants speculated that a secondary bacterial infection also may have contributed to the pulmonary changes in this dog.

Viruses are well-known to play a role in the pathogenesis of bacterial pneumonias by damaging the respiratory epithelium, inhibiting bacterial clearance and facilitating bacterial adhesion. Other participants commented that a contributing bacterial etiology likely would have resulted in a more neutrophil-rich inflammatory component in the lung. Bacterial agents of canine infectious respiratory disease include *Bordetella bronchiseptica, Mycoplasma* spp., and *Streptococcus equi* sp. *zooepidemicus.*[14]

The pancreatic involvement in this case is uncommon in typical infections with canine adenovirus. Pancreatitis due to adenovirus infection is well documented in nonhuman primates; adenoviral infection in a number of non-human primate species can result in pancreatic necrosis and fibrosis with chronic-active inflammation. Pancreatic involvement typically occurs in more clinically severe cases, typically with underlying immunosuppression; other lesions also include hepatitis and nephritis. Adenovirus infection in immunocompetent nonhuman primate hosts usually presents as self-limiting respiratory and gastrointestinal infections.[9] Fowl adenovirus, a common disease in chickens, results in necrotizing pancreatitis, gizzard erosion, and inclusion body hepatitis.[11]

We appreciate the excellent supporting materials with the submission, including laboratory data, gross, immunohistochemistry, and electron microscopy images. These additional materials greatly enhances the teaching value of this case.

Contributing Institution:
University of Minnesota Veterinary Diagnostic Laboratory. http://www.vdl.umn.edu

References:

1. Almes KM, Janardhan KS, Anderson J, Hesse RA, Patton KM. Fatal canine adenoviral pneumonia in two litters of Bulldogs. *J Vet Diagn Invest.* 2010;22: 780–784.

2. Baszler TV, Gay LJ, Long MT, Mathison BA. Detection by PCR of Neospora caninum in fetal tissues from spontaneous bovine abortions. *J Clin Microbiol.* 1999;37: 4059–4064.

3. Benetka V, Weissenbock H, Kudielka I, Pallan C, Rothmüller G, Möstl K. Canine adenovirus type 2 infection in four puppies with neurological signs. *Vet Rec.* 2006;158: 91–94.

4. Brown CC, Baker DC, Barker IK. Alimentary system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals.* 5th ed. Vol. 2. Philadelphia, PA: Elsevier Saunders; 2007:166.

5. Bulut O, Yapici O, Avci O, Simsek A, Atli K, Dik I, Yavru S, Hasircioglu S, Kale M, Mamak N. 2013The Serological and Virological Investigation of Canine Adenovirus Infection on the Dogs. *The Scientific World Journal* 2013 Sep 24;2013:587024. doi: 10.1155/2013/587024. eCollection 2013.

6. Buonavoglia C, Martella V. Canine respiratory viruses. *Vet Res* 2007:38; 355–373.

7. Damián M, Morales E, Salas G, Trigo FJ. Immunohistochemical Detection of Antigens of Distemper, Adenovirus and Parainfluenza Viruses in Domestic Dogs with Pneumonia. *J Comp Pathol* 2005:133; 289–293.

8. Decaro N, Martella V, Buonavoglia C. Canine Adenoviruses and Herpesvirus. *Vet Clin North Am Small Anim Pract* 2008:38; 799–814.

9. Mansfield KG, Sasseville VG, Westmoreland SV. Molecular localization techniques in the diagnosis and characterization of nonhuman primate infectious diseases. *Vet Pathol.* 2014:51(1);1101-26.

10. McChesney AE, England JJ, Adcock JL, Stackhouse LL, Chow TL. Adenoviral infection in suckling Arabian foals. *Vet Pathol* 1970:7; 547–564.

11. Ono M, Okuda S, Yazawa Y et al. Adenoviral gizzard erosion in commercial broiler chickens. *Vet Pathol*. 2003:40; 294-303.

12. Rodriguez-Tovar LE, Ramírez-Romero R, Valdez-Nava Y, Nevárez-Garza AM, Zárate-Ramos JJ, López A. Combined distemper-adenoviral pneumonia in a dog. *Can Vet J.* 2007:48; 632–634.

13. Sykes JE. Canine Viral Respiratory Infections. In: Sykes JE, ed. *Canine and Feline Infectious Diseases*. *1st ed.* Philadelphia, PA: Elsevier Saunders; 2013:170-181.

14. Viitanen SJ, Lappalainen A, Rajamäki MM. Co-infections with Respiratory Viruses in Dogs with Bacterial Pneumonia. *J Vet Intern Med.* 2015:29; 544–551.

15. Wright N, Jackson FR, Niezgoda M, Ellison JA, Rupprecht CE, Nel LH. High prevalence of antibodies against canine adenovirus (CAV) type 2 in domestic dog populations in South Africa precludes the use of CAV-based recombinant rabies vaccines. *Vaccine.* 2013:31; 4177–4182.