



WEDNESDAY SLIDE CONFERENCE 2009-2010

# Conference 2

16 September 2009

*Conference Moderator:*

Sarah Hale, DVM, Diplomate ACVP

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**CASE I: A07-194 (AFIP 3133675).**

**Signalment:** 12-year-old, male, intact Rhesus macaque (*Macaca mulatta*).

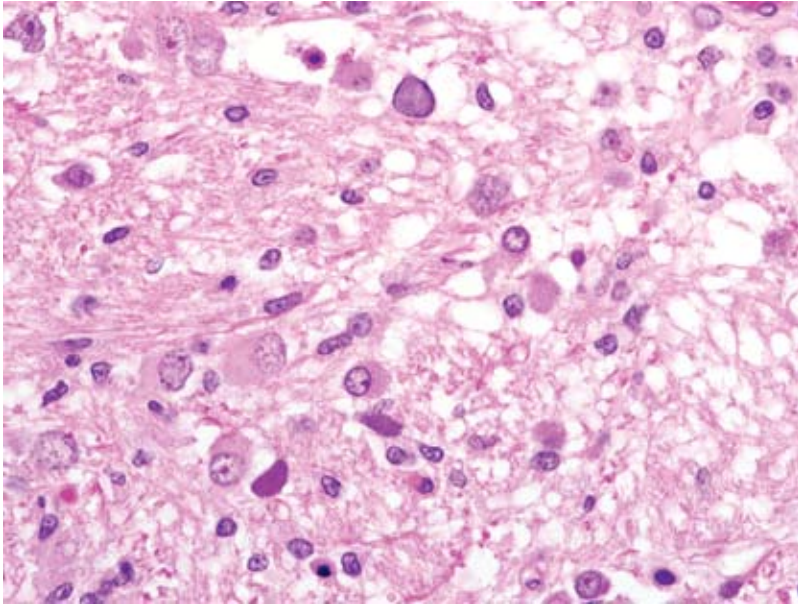
**History:** This monkey was inoculated with SIVmac251 and had undergone routine phlebotomies. Several months after inoculation, the animal developed diarrhea and loss of appetite. The animal was treated with Baytril<sup>®</sup>, with partial resolution of the animal's diarrhea and improvement in appetite. Following discontinuation of Baytril<sup>®</sup>, the overall condition of the animal continued to decline with increased respiratory effort at rest and mucoid nasal discharge. Complete blood count and blood chemistry were within normal limits. A weight loss of 5 kilograms (from 21 to 16 kilograms) was recorded over a three month period. At the end of this period, the animal still had marked diarrhea and pneumonia. A cardiac ultrasound at this time revealed a thrombus attached to the right atrioventricular valve. The animal was placed on amoxicillin treatment; however, due to failure to respond to treatment, the animal was euthanized.

**Gross Pathology:** The animal was markedly obese. The axillary and inguinal lymph nodes were mildly enlarged. The lungs were mottled with cranioventral consolidation.

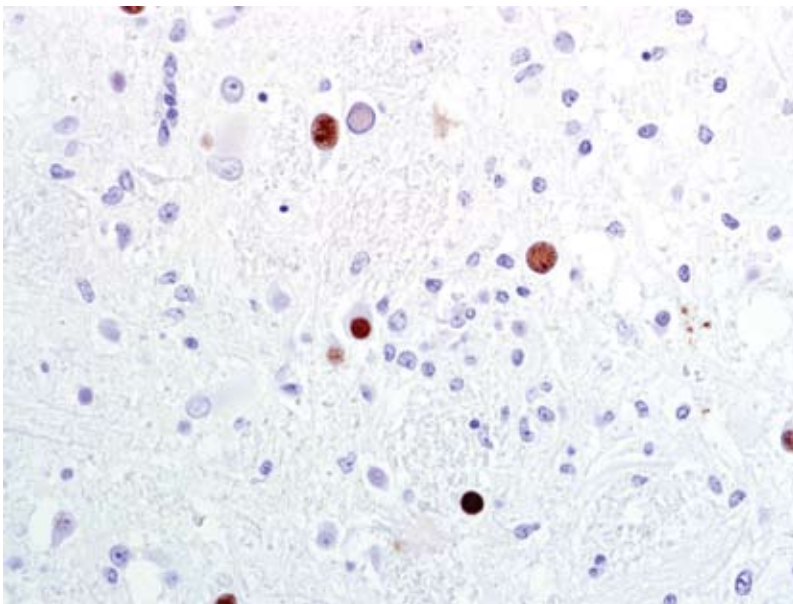
There was an approximately 2 cm diameter thrombus attached to the right atrioventricular valve. The small and large intestines were filled with fluid digesta and gas. The gallbladder contained a pasty white fluid. There were no gross lesions noted on examination of the brain or spinal cord.

**Laboratory Results:** CBC and blood chemistry were within normal limits.

**Histopathologic Description:** Brain (telencephalic/diencephalic junction- including thalamus, lateral ventricle, and substantia nigra; some sections are composed of mesencephalon): Replacing extensive regions of the white matter and multifocally spreading into the adjacent gray matter are variably sized foci of liquefactive necrosis. These foci contain large numbers of macrophages that contain abundant, intracytoplasmic, degenerate myelin (gitter cells) and are admixed with fewer lymphocytes and reactive astrocytes (some of which are multinucleate). The adjacent neuroparenchyma is loosened (edema) and contains large numbers of reactive astrocytes (gemistocytes) and rod-shaped microglia. Both within the foci of liquefactive necrosis and in the adjacent neuroparenchyma are large numbers of astrocytes that are characterized by large, swollen nuclei that contain a single, round, 10-20µm, amphophilic, glassy inclusion



*1-1. Cerebrum, rhesus macaque. Within areas of liquefactive necrosis are numerous gemistocytic astrocytes which occasionally contain large, pale basophilic 15um intranuclear inclusion bodies which peripheralize the chromatin. Photomicrograph courtesy of New England Primate Research Center, Harvard Medical School, One Pine Hill Drive, Southborough, MA 01772, Andrew\_miller@hms.harvard.edu.*



*1-2. Cerebrum, rhesus macaque. Intranuclear inclusion bodies are strongly immunohistochemically positive for SV40 antigen. Photomicrograph courtesy of New England Primate Research Center, Harvard Medical School, One Pine Hill Drive, Southborough, MA 01772, Andrew\_miller@hms.harvard.edu.*

body (fig. 1-1). There are multiple foci of gliosis in the adjacent neuropil and occasional perivascular cuffs of lymphocytes are present within the gray matter. A small section of lateral ventricle is present in this section and the ependyma lining the lateral ventricle is irregular, proliferative, and broken in multiple sites. The ependymal cells have abundant cytoplasm and large, reactive nuclei and there are small numbers of lymphocytes within the ependyma. Blood vessels throughout the lesions are reactive and branched and typically are lined by reactive, hypertrophied endothelium. Immunohistochemistry for

SV40 confirmed CNS infection (fig. 1-2).

**Contributor's Morphologic Diagnosis:** Brain (thalamus/substantia nigra): Severe, multifocal, chronic necrotizing encephalitis with intranuclear polyomaviral inclusions, marked astrocytosis and gliosis.

**Contributor's Comment:** Simian virus 40 (SV40) is a non-enveloped, oncogenic, double-stranded, DNA virus belonging to the Papovaviridae family and the Polyomavirinae subfamily. The viral genome consists of

early- and late-coding regions and a non-coding regulatory region. The early region encodes DNA binding proteins, known as large or small T (tumor) antigens, which bind to the regulatory region of the genome to facilitate replication of late region viral DNA, which encodes three capsid proteins (VP1, VP2, VP3) that display genus-specific antigenic determinants. Polyomaviruses cause tumors when inoculated into species (e.g. hamsters) unrelated to their natural host.<sup>1,2</sup> Other primate polyomaviruses are: JC virus and BK virus, which infect humans; SA12 isolated from a South African vervet monkey kidney culture; B-lymphotropic polyomavirus (LPV) isolated from an African green monkey lymphoblast cell line; baboon polyomavirus isolated from baboon kidney cell culture; and cynomolgus polyomavirus (CPV) isolated from immunosuppressed cynomolgus monkeys. Other non-primate mammalian polyomaviruses include Murine polyomavirus (MPyV), Hamster polyomavirus (HaPyV), Bovine polyomavirus (BPvV), Rabbit kidney vacuolating virus (RKV), Murine pneumotropic virus, and finally various avian polyomaviruses including budgerigar fledgling disease virus (BFDV).<sup>2</sup>

Polyomaviruses are virtually ubiquitous and harmless in healthy hosts, but can cause severe disease in immunocompromised individuals. In immunocompetent natural hosts, polyomaviruses remain latent within renal tissue and likely the central nervous system, with periodic asymptomatic reactivation of latent virus during pregnancy, diabetes, or old age. In immunocompromised SIV-infected macaques with simian AIDS, primary infection or reactivation of a latent infection results in interstitial nephritis, interstitial pneumonia, and either meningoencephalitis (ME) or progressive multifocal leukoencephalopathy (PML). In human AIDS patients, PML occurs in up to 5% of affected individuals and is considered an AIDS-defining lesion. It is characterized by multifocal demyelination primarily of the white matter due to infection of oligodendrocytes. The ME variant is diffuse and distinct from PML, affecting cerebral gray matter without demyelination.<sup>1,3</sup> PML is characterized by variably sized foci of necrosis that start in the white matter and merge into the adjacent gray matter. Reactive astrocytosis is a common finding around the lesions. Inclusion bodies are typically numerous and are most often found in astrocytes.

Cases of PML and ME are rare in the primate literature. The ME manifestation is typically seen in animals with concurrent nephritis, as tubular nephritis has been reported to be a feature of primary SV40 infection but not SV40 reactivation in SIV infected macaques. The current animal had mild interstitial nephritis; however, no SV40 antigen could be found by immunohistochemistry. In addition

to polyomavirus, this Rhesus macaque had several other AIDS defining opportunistic infections, including biliary, pancreatic and tracheal cryptosporidiosis and adenoviral enteritis. Other AIDS defining lesions that were not present in this animal include *Pneumocystis* sp. pneumonia and Cytomegalovirus infection.

A spontaneously occurring disease with characteristics similar to PML has been described in eight macaques. This observation was published in 1975, years before the discovery of SIV, and immunological competence was not determined in the monkeys reported.<sup>4,5</sup> Other virally-induced CNS lesions with inclusion bodies in non-human primates include CMV-associated meningoencephalitis; poliomyelitis virus-induced poliomyelitis; and measles virus-induced encephalitis. CMV-associated meningoencephalitis is differentiated from SV40-induced PML by the characteristic feature of cytomegaly with prominent intranuclear inclusion bodies. In addition, CMV associated encephalitis is not associated with necrosis and is typically more localized to the meninges. Poliomyelitis infection in non-human primates is rare and is restricted to the gray matter. Measles virus can affect the gray and white matter; however, the inclusion bodies are found both in the nucleus and the cytoplasm and are similar to other morbilliviruses.<sup>5,6</sup> Finally, SIV-associated encephalitis has frequently been described in literature; however, the lesions in SIV encephalitis are present in both gray and white matter and composed of multifocal glial nodules, perivascular cuffs, and perivascular/meningeal aggregates of giant cells that lack inclusion bodies.<sup>5,7</sup>

**AFIP Diagnosis:** Brain, thalamus and substantia nigra: Encephalitis, necrotizing, multifocal, marked, with gliosis and amphiphilic intranuclear inclusion bodies.

**Conference Comment:** The contributor provided a comprehensive synopsis of this entity and the differential diagnosis for CNS lesions with viral inclusion bodies in nonhuman primates. Conference participants discussed the importance of recognizing that the severity of lesions in this case suggests underlying immunocompromise and raises the index of suspicion for concomitant infection with other opportunistic pathogens. This case provided conference participants with excellent examples of classic polyomaviral inclusions, gemistocytic astrocytes, and gitter cells.

**Contributor:** New England Primate Research Center, Harvard Medical School, One Pine Hill Dr., Southborough, MA 01772

<http://www.hms.harvard.edu/nerprc/>

**References:**

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**CASE II: 09-456, 09-457 (AFIP 3133950).**

**Signalment:** Adult, mixed breed, beef cow (*Bos taurus*).

**History:** This is a 25-cow herd of adult mixed breed beef cows in late gestation. Several animals developed a staggering gait over weeks and were euthanized.

**Gross Pathology:** No gross lesions.

**Histopathologic Description:** Scattered neurons in the brain stem around the cerebral aqueduct and in the pons contain green-brown granular material in the cytoplasm. Many neurons contain golden pigment consistent with lipofuscin. Occasional neuronal necrosis is seen. Some

sections have axonal degeneration of nerves along the ventral surface of the brain stem.

**Contributor's Morphologic Diagnosis:** Neuronal pigmentation, brain stem, mild, with axonal degeneration and neuronal necrosis.

**Contributor's Comment:** The pigment in neurons is consistent with that seen in poisoning by plants of the genus *Phalaris*. Ultrastructurally, the granules within the neuronal cytoplasm are membrane-bound and composed of concentric membranous lamellae that may be intermingled with fine granular material. They are considered to be lysosomal in nature.<sup>1</sup> The toxic principle is a mix of methyl tryptamine and beta carboline indoleamines that are related to the neurotransmitter serotonin. The experimental administration of these alkaloids to sheep has induced the acute syndrome, presumably by interfering with the function or metabolism of serotonin.

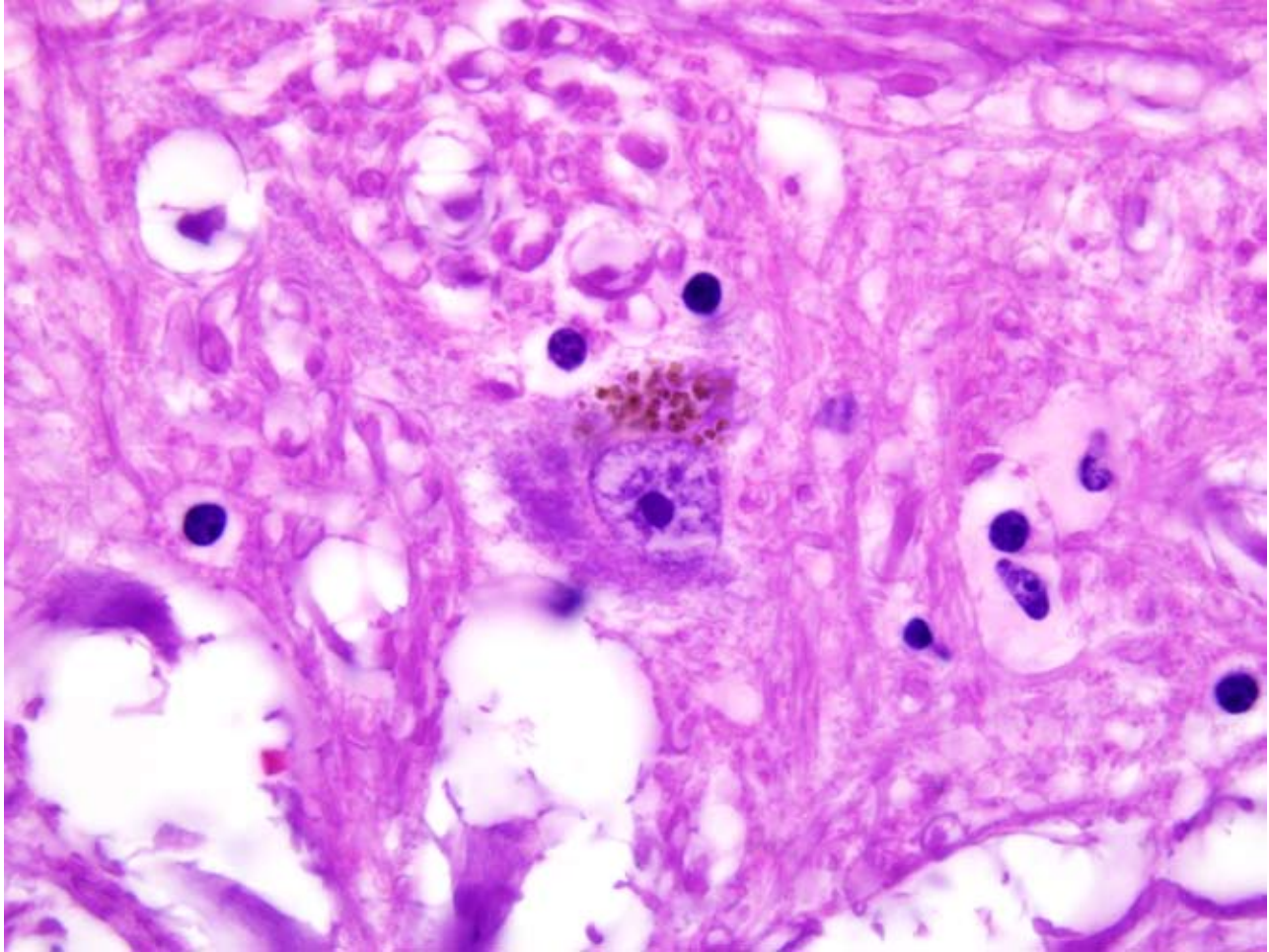
*Phalaris* toxicosis is typically a disease of sheep causing staggers. Onset of staggers may be rapid following ingestion of the plant or delayed by several months. Most animals recover from the staggering syndrome but some do not. Cattle are occasionally poisoned by *Phalaris* and develop staggers from which they usually do not recover. Sudden death is another less common manifestation of *Phalaris* poisoning seen in sheep and occasionally horses.<sup>3</sup>

The *Phalaris* species likely involved in the poisoning of these cattle is *Phalaris arundinacea* or reed canarygrass. This plant was found in the pasture grazed by the affected cattle.

**AFIP Diagnosis:** Brainstem: Neuronal pigmentation, multifocal, with mild gliosis.

**Conference Comment:** Conference participants discussed the tinctorial and morphologic differences between lipofuscin and the pigment imparted by *Phalaris* toxicosis. The distinction is important, because although the "wear and tear" pigment of lipofuscin indicates free radical and lipid peroxidation of polyunsaturated lipids of subcellular membranes, it does not generally cause injury to the cell.<sup>2</sup> In *Trachyandra* intoxication, lipofuscinosis may be intense in central and peripheral neurons; however, the relationship between this storage process and clinical signs is uncertain. The pigment in many of the "ceroid lipofuscinoses" is not lipofuscin, but rather protein subunit c of mitochondrial ATP synthase.<sup>3</sup>

In this case, tan, globular lipofuscin is predominantly concentrated in the axon hillock of motor nuclei. The



2-1. Mesencephalon, ox. Multifocally neurons contain small amounts of brown granular cytoplasmic pigment. (HE 1000X)

storage granules resulting from *Phalaris* toxicosis are more granular, brown to green, and have a predominantly perinuclear distribution (**fig. 2-1**). Similar granules may occur in the renal tubular epithelium of affected animals, and in severe cases the granules impart a grossly visible green discoloration to kidneys and gray matter.<sup>3</sup>

*Phalaris* toxicosis is one of several induced storage diseases caused by plants. Others include the aforementioned lipofuscinosis associated with *Trachyandra* toxicosis; *Solanum*-induced cerebellar neuronal degeneration and loss; Gomen disease, a suspected toxicosis of horses in New Caledonia; and alpha-mannosidosis induced by the indolizadine alkaloid swainsonine, found in a variety of plants, including locoweed (*Astragalus* sp., *Oxytropis* sp.), poison pea (*Swainsona* sp.), and the shrubby morning glory (*Ipomoea* sp.).<sup>3</sup>

The term “staggers,” as applied to the clinical manifestation

of *Phalaris* toxicosis, should not be confused with “staggering disease,” caused by Borna disease virus, or “ryegrass staggers,” which includes both perennial and annual ryegrass staggers. Annual ryegrass staggers is caused by a corynetoxin produced by the bacterium *Corynebacterium rathayi* in nematode-induced seed head galls; host plants include annual ryegrass (*Lolium rigidum*), annual beardgrass (*Polypogon monspeliensis*), chewings fescue (*Festuca nigrescens*), and Pacific bent grass (*Agrostis avenacea*). The corynetoxin inhibits lipid-linked N-glycosylation of glycoproteins, compromising membrane integrity, and causing increased vascular permeability in multiple organs, including the brain.<sup>3</sup> Perennial ryegrass staggers is caused by tremorgenic mycotoxins (lolitremes) produced by the endophytic fungus *Neotyphodium lolii* in perennial ryegrass (*Lolium perenne*); disease is milder than in annual ryegrass staggers and is often reversible.<sup>4</sup>

**Contributor:** Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Duck Pond Drive, Blacksburg, VA 24061  
<http://www.vetmed.vt.edu/>

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**CASE III: TP0950 CASE 1 (AFIP 3134515).**

**Signalment:** 10- to 11-month-old, male and female, Beagle dogs (*Canis familiaris*).

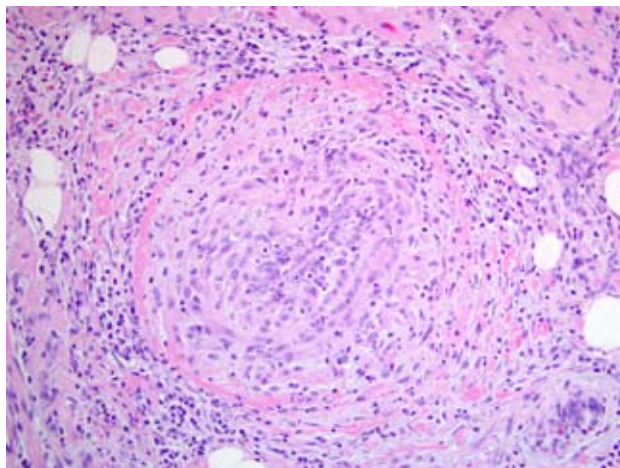
**History:** These dogs were part of the high dose group involved in a 7-day study with an orally administered

experimental drug. Dogs were dosed once daily and euthanized on day 8. The animal care and experimental procedures of this study were conducted in compliance with the U.S. Animal Welfare Act and were performed in accordance with the standards of the Institute of Laboratory Animal Resources (ILAR) Guide (1996). The Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited the facility in which this study was conducted.

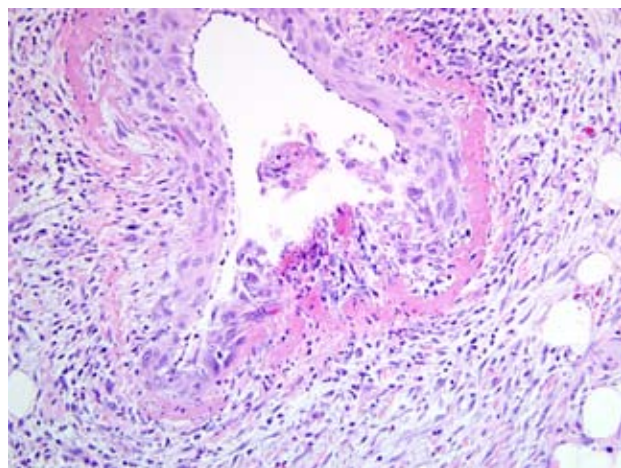
**Gross Pathology:** In the heart of a few animals in the high dose group, there were multifocal areas of red or pale discoloration.

**Laboratory Results:** Hematologic alterations in the high dose group included decreased red blood cell count, hemoglobin, and hematocrit with an increased white blood cell count due to elevated neutrophils and monocytes. Clinical chemistry perturbations in the high dose group included increases in troponin and total bilirubin.

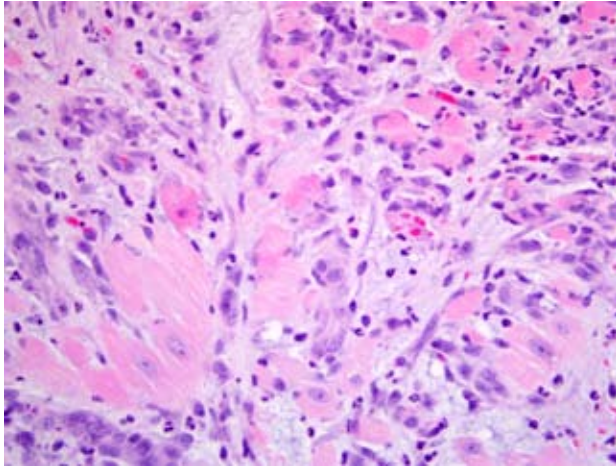
**Histopathologic Description:** The tunica media of multiple small- to medium-sized arteries in the epicardium and myocardium is segmentally to circumferentially replaced by brightly eosinophilic, homogenous material (fibrinoid necrosis), with small to moderate numbers of degenerate neutrophils and histiocytes, scattered karyorrhectic debris, and occasionally hemorrhage (**figs. 3-1 and 3-2**). Smooth muscle fibers of the tunica media in some arteries contain plump, vesicular nuclei. There is moderate expansion of the tunica adventitia by edema and inflammatory cells. Multifocally in the myocardium,



3-1. Heart, dog. The tunica media and adventitia of numerous coronary vessels is markedly expanded by numerous myofibroblasts, histiocytes, and fewer neutrophils, occasionally occluding vascular lumina. (HE 200X)



3-2. Heart, dog. Multifocally tunics of coronary arteries are disrupted by eosinophilic cellular and karyorrhectic debris admixed with viable and degenerate neutrophils (necrotizing vasculitis). Endothelium lining these vessels is often hypertrophied and protrude into the vascular lumen. (HE 200X)



3-3. Heart, dog. Multifocally cardiac myocytes surrounding affected vessels are necrotic, and the interstitium is expanded by a moderate amount of edema and low numbers of neutrophils, lymphocytes, and histiocytes. (HE 400X)

particularly the atria, there are foci of interstitial expansion by edema with loose infiltration by small numbers of neutrophils, histiocytes and lymphocytes. Scattered cardiomyofibers in affected areas are shrunken and hypereosinophilic, and often surrounded by cells with large, vesicular nuclei (**fig. 3-3**). Capillaries in affected areas are lined by plump endothelial cells. In some sections, there is multifocal subendocardial and/or subepicardial hemorrhage.

**Contributor's Morphologic Diagnosis:** Heart: 1) Severe, multifocal, subacute, necrotizing arteritis with myocardial degeneration and necrosis. 2) Minimal, multifocal, subendocardial and subepicardial hemorrhage (some sections).

**Contributor's Comment:** This case represents an example of drug-induced vascular injury (DIVI) due to administration of a phosphodiesterase inhibitor in the dog. DIVI is currently a topic of debate in regulatory circles, as several drugs are associated with vascular injury in laboratory animals without concordant vascular injury in humans. In laboratory beagles, compounds that cause DIVI often have a predilection for the coronary vascular bed. This vascular bed is also a common area for spontaneous arterial lesions; therefore, care must be taken when interpreting vascular changes.<sup>1,5</sup> Manifestations of DIVI in laboratory beagles include vasoactive arteriopathy (produced by some vasodilator/positive inotropic and vasoconstrictor compounds), toxic vasculitis, and hypersensitivity vasculitis.<sup>1</sup>

Vasodilator and/or positive inotropic drugs (e.g. minoxidil, phosphodiesterase inhibitors, theobromine, others)

produce distinctive, time-dependent, arterial changes affecting primarily extramural and intramural, small to medium-sized arteries. Gross changes include epicardial and possibly myocardial petechial and ecchymotic hemorrhages. At 7 days of treatment or less, microscopic changes include necrosis of the tunica media and hemorrhage, with inflammation and perivascular edema. Intimal and adventitial proliferative changes are observed with more chronic treatment.<sup>1,4</sup> Distinct changes associated with administration of phosphodiesterase III inhibitors to dogs include coronary arteriopathy generally restricted to extramural coronary arteries, myocardial necrosis with endocardial hemorrhage, and atrial hemorrhage with formation of granulation tissue.<sup>1,3</sup>

Vasoconstrictive agents (e.g. endothelin-1, digoxin, others) induce arterial changes consisting of medial thickening and necrosis, with hyalinization after longer-term treatment, and tend to affect small caliber arteries in a variety of organs. Some compounds (e.g. allylamine, systemic administration of monocrotaline and mitomycin C) may act directly on the arterial wall (endothelium and/or smooth muscle) causing toxic necrotizing vasculitis characterized by changes varying from acute necrosis of the tunica media with infiltration by neutrophils to medial scarring and intimal/adventitial fibrosis. Some drugs have been reported to cause immune-mediated disease due to Type III and, more commonly, Type IV hypersensitivities. Arterial changes associated with the Type IV hypersensitivity are not confined to coronary vascular beds, and are characterized by infiltration with mononuclear cells, possibly mixed with eosinophils, without medial necrosis.<sup>1,4,5</sup>

The pathogenesis of DIVI due to vasoactive compounds is still not clear. Vasodilator/positive inotropic agents produce their pharmacologic effects via a variety of mechanisms, including: direct relaxation of arterial smooth muscle, opening of potassium channels, inhibition of vascular smooth muscle phosphodiesterase, or blockage of endothelin receptors. The most frequently cited mechanism for the pathologic changes incurred by vasodilator/positive inotropic agents is vasodilation -> increased blood flow -> turbulence, altered shear stress -> homeostatic imbalance of endothelial cells -> injury.<sup>2</sup> Arterial changes due to vasoconstrictor agents are attributable to local and/or systemic hypertension produced by these compounds.<sup>5</sup>

**AFIP Diagnosis:** Heart, epicardial and myocardial arteries: Arteritis, proliferative and necrotizing, multifocal, marked, with multifocal myocardial degeneration and necrosis.

**Conference Comment:** Because the submitted slides

are from multiple dogs, there is some section variation. Some slides feature reactive mesothelium overlying the epicardial surface and/or serous atrophy of pericardial fat.

The contributor provides an excellent synopsis of this entity. Of immense practical concern is the differentiation of drug-induced from spontaneous vascular injury in general, and idiopathic polyarteritis of beagle dogs in particular. While many features overlap, there are some diagnostically useful differences between DIVI and idiopathic polyarteritis. The clinical signs in DIVI are variable and likely unrelated to vascular effects, while in idiopathic canine polyarteritis there is generally fever, weight loss, cervical neck pain (thus the clinical term, "beagle pain syndrome"), and neutrophilic leukocytosis. In DIVI, vascular lesions are generally restricted to coronary arteries, while in idiopathic canine polyarteritis multiple organs are affected. While the histologic lesions of the vasculature may be similar in these entities, idiopathic canine polyarteritis generally exhibits mononuclear periarterial to transmural inflammation, a feature which is absent or only mild in DIVI. Finally, atrial hemorrhage is characteristic of DIVI, but not idiopathic canine polyarteritis.<sup>1</sup>

**Contributor:** Pfizer Inc. Global Research and Development, Eastern Point Rd., Groton, CT 06340  
<http://www.Pfizer.com>

#### References:

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#### CASE IV: 47888 (AFIP 3135957).

**Signalment:** 7-year-old, castrated male domestic shorthair cat (*Felis catus*).

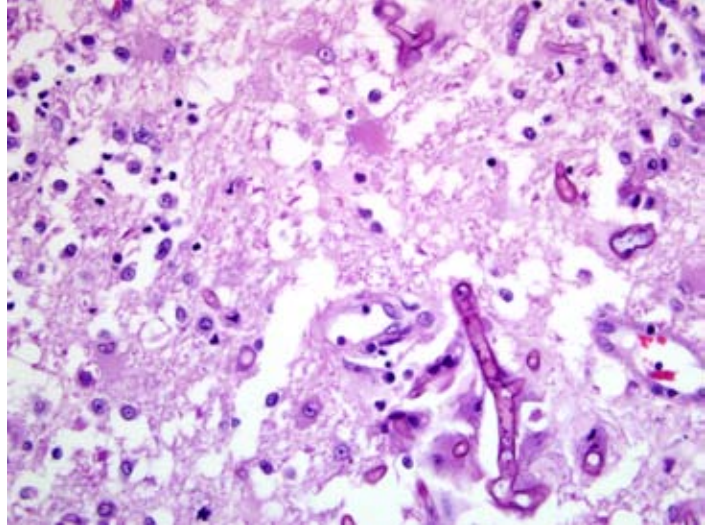
**History:** The cat presented for decreased activity, inappetance, dyspnea and tachypnea of 10 days duration. A markedly enlarged tracheobronchial lymph node, which was compressing the trachea, was noted on thoracic radiographs. Sternal lymph nodes were also enlarged. The patient returned after 4 days with progressive lethargy and inappetance. Neurological exam revealed signs consistent with a left supratentorial lesion (circling to the left, right conscious proprioceptive deficits, absent menace on the right side, and decreased facial sensation on the right side). The patient was euthanized 5 days after initial presentation, and a complete necropsy performed.

**Gross Pathology:** A 5.5 X 5.0 X 2.6 cm, soft, multinodular, mottled tan to pale tan mass (presumed tracheobronchial lymph node) is present at the tracheal carina. Adjacent epicardium and lung lobes are adhered to the mass. The sternal lymph nodes are soft, mottled pale tan to red and enlarged (up to 1.4 X 1 X 0.7 cm). The apex of the heart is mildly rounded. There are two, 0.3 cm diameter, well-demarcated, coalescent nodules in the myocardium at the apex. The left lung lobes are ~85% dark red, non-collapsing and slightly rubbery. The right lung lobes contain multifocal, tan to dark red, slightly rubbery foci predominantly at the periphery of the lobes. Both sides exude a moderate amount of blood when sectioned. There are multifocal, up to 0.5 cm diameter, well-demarcated, pale tan, soft nodules protruding from the endothelial surface of multiple pulmonary veins, partially or completely occluding the vascular lumina. In the left frontal gyrus, there is a poorly demarcated, 0.9 X 0.5 X 0.5 cm, tan mass, with multifocal pinpoint red foci, that is slightly more firm than surrounding cerebral parenchyma.

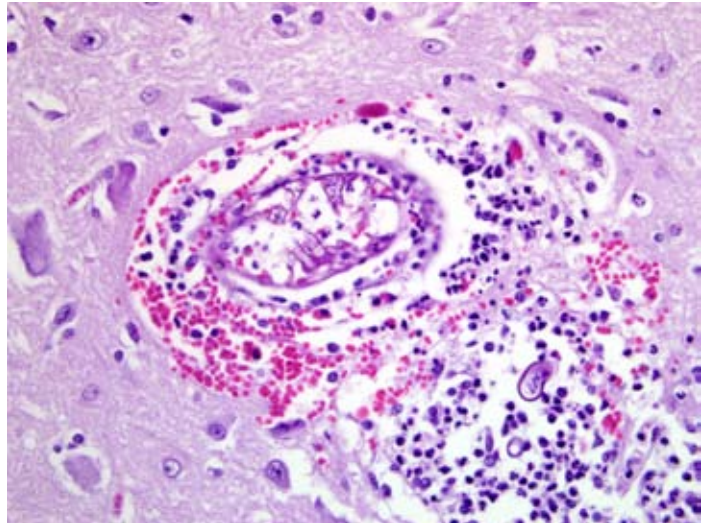
**Laboratory Results: Relevant serum chemistry:** Total protein 10.1 g/dL (5.9-8.5); Albumin 3.4 g/dL (2.5-3.9); Globulin 6.7 g/dL (2.4-5.3). **Protein electrophoresis:** Total protein 9.8 g/dL (5.9-8.5); Albumin 4.09 g/dL (2.1-3.3); Alpha 1 globulins 0.06 g/dL (0.2-1.1); Alpha 2 globulins 0.82 g/dL (0.4-0.9); Beta globulins 1.72 g/dL (0.9-1.9); Gamma globulins 3.11 g/dL (1.7-4.3); A/G ratio 0.72. **Electrophoresis interpretation:** Consistent with polyclonal gammopathy, most often due to nonspecific chronic inflammation or infection. FeLV and FIV ELISA: negative. **Hematology:** No significant findings. PCR (via Washington Animal Disease Diagnostic Lab): A portion



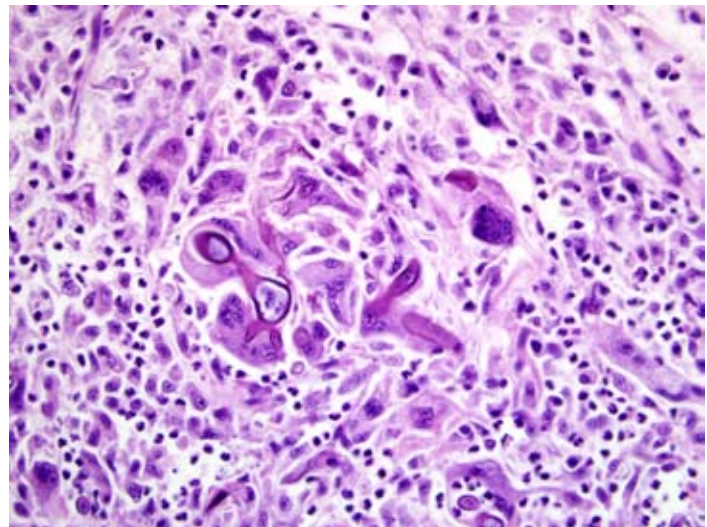
4-1. Cerebrum, cat. Within areas of liquefactive necrosis are numerous gemistocytic astrocytes, macrophages, and fewer neutrophils and multinucleated giant cells centered on fungal hyphae. Hyphae have 10-20um diameter non-parallel walls, are rarely septate, exhibit non-dichotomous right angle and acute angle branching, and frequently have bulbous dilatations. (HE 400X)



4-2. Cerebrum, cat. Multifocally vessels within the cerebral white matter are cuffed by many neutrophils, macrophages, lymphocytes, and hemorrhage. There is multifocal necrotizing vasculitis, and vessel lumens contain numerous previously described fungal hyphae. (HE 400X)



4-3. Heart, cat. Within areas of lytic necrosis are many neutrophils and macrophages and fewer multinucleated giant cells and eosinophils which surround the previously described fungal hyphae. Occasionally hyphae are found in the cytoplasm of multinucleated giant cells. (HE 400X)



of 28S ribosomal RNA gene was amplified and sequenced. The sequence most closely matched *Alternaria alternata* or *Alternaria tenuissimi* (>99% sequence identity) when compared with sequences in GenBank. These two species are synonymous.

**Histopathologic Description: Brain:** Sections of left frontal (rostral cerebral) cortex reveal multifocal infiltration and effacement of both gray and white matter by numerous fungal organisms and inflammatory cells. Fungal hyphae range from 7-15 micron in width, with nonparallel walls and frequent bulbous swellings (up to 30 microns in diameter). The hyphae contain infrequent septae, with irregular, dichotomous and non-dichotomous, acute angle to right angle branching (fig. 4-1). Occasionally, granular eosinophilic or basophilic material is present within hyphae. The hyphae are present on a background of rarified and often hyper eosinophilic neuroparenchyma, which is multifocally infiltrated by moderate to large numbers of neutrophils, histiocytes and multinucleated giant cells with fewer lymphocytes and plasma cells. Occasionally, fragments of fungal hyphae are present within the cytoplasm of multinucleated giant cells (fig. 4-3). Moderate populations of gitter cells, microglia and astroglia are scattered throughout the affected areas along with mild hemorrhage. Large astroglial cells with abundant eosinophilic cytoplasm (gemistocytic astrocytes) are multifocally observed. Multifocally, small to moderate populations of lymphocytes, plasma cells and neutrophils are present within Virchow Robin spaces (perivascular cuffs) of the adjacent unaffected cortex. There is fibrinoid degeneration of vessel walls, with fibrin exudation, necrosis, leukocytoclasia, and vascular infiltration by neutrophils and fungal hyphae (fig. 4-2). Small numbers of similar inflammatory cells are multifocally present within the meninges.

**Heart:** A section of the heart reveals multifocal to coalescing nodules within the myocardium composed of fungal hyphae and inflammatory populations similar to those described above. Inflammatory populations include numerous histiocytes and neutrophils with fewer multinucleated giant cells, and lymphocytes and plasma cells on a background of loose connective tissue stroma. Histiocytes multifocally contain abundant, granular, brown cytoplasmic pigment (hemosiderin). Entrapped myofibers are often hyper eosinophilic and shrunken with loss of cross striations, rarely vacuolated sarcoplasm and pyknotic nuclei (degenerative change). There is mild interstitial edema and few Anitschkow cells characterized by elongated, undulating nuclei. Multifocally, there are few foci of interstitial fibrosis in the remaining, unaffected myocardium.

**Tracheobronchial lymph node (tissue not included):**

The tracheobronchial lymph node is diffusely effaced by pyogranulomatous inflammation containing fungal hyphae similar to those described above. This inflammation is often partially surrounded by fibrous connective tissue. Inflammatory cells extend to the adjacent pulmonary parenchyma and vessels, which are partially to completely occluded by the granulomas. Vascular changes, including fibrinoid degeneration and inflammation similar to that described in the brain, is also multifocally present.

**Contributor's Morphologic Diagnosis:** 1. Brain, cerebral cortex: Severe, multifocal to coalescing, chronic ongoing pyogranulomatous encephalitis with intralesional fungal hyphae and multifocal fibrinonecrotizing vasculitis.

2. Heart: Severe, multifocal to coalescing, chronic ongoing, pyogranulomatous myocarditis with intralesional fungal hyphae, myocardiocyte degeneration and loss.

3. Tracheobronchial lymph node (tissue not included): Severe, diffuse, chronic-ongoing pyogranulomatous lymphadenitis with invasion into adjacent pulmonary parenchyma, multifocal fibrinonecrotizing vasculitis and intralesional fungal hyphae.

**Contributor's Comment:** Prior to PCR identification, other fungi, namely zygomycetes such as *Rhizopus*, *Absidia*, *Mucor* or *Mortierella* spp. were considered, especially in light of the fungal morphology and lack of visible melanin on H&E.

*Alternaria alternata* is a dematiaceous (pigmented), saprophytic fungus that is ubiquitous in the environment (indoors and outdoors) and can be isolated from the skin or fur of healthy humans and animals.<sup>4,13</sup> It is an uncommon cause of phaeohyphomycosis in humans with reports of cases in cats, dogs, goats, horses and deer.<sup>4</sup> In humans, lesions are mainly cutaneous with cases of osteomyelitis, sinusitis, keratitis, onychomycosis and pulmonary granulomas.<sup>3,4,13</sup> In veterinary species, lesions are usually confined to the skin and subcutis with few reports of nasal involvement in cats and keratitis in a horse.<sup>4,5,9,11</sup> Human cases are associated with hypersensitivity syndromes such as "woodworker's lung," asthma and eosinophilic pneumonia.<sup>9,11,13</sup> Because of the ubiquitous nature of the fungus, immunosuppression is often suspected, if not proven, in clinical cases.<sup>11-13</sup>

Phaeohyphomycoses are usually opportunistic infections in which fungi generally gain entry via skin wounds.<sup>5,7</sup> Systemic or visceral phaeohyphomycoses are rare, but seem to have a predisposition for the central nervous system (CNS).<sup>1,3,6,10</sup> In these cases, the route of infection is thought to be inhalation with subsequent hematogenous

spread to the brain.<sup>3,6,10</sup> CNS phaeohyphomycoses are almost always attributed to *Cladophialophora bantiana* (previously known as *Torula bantiana*, *Cladosporium bantianum*, *Xylohypha bantiana*, *Cladosporium trichoides* and *Xylohypha emmonsii*).<sup>1,10</sup> This species exhibits marked neurotropism, which is related to the presence of introns at the 18S rDNA subunit.<sup>1</sup> There is a single report of fatal systemic illness in a cat due to *Cladophialophora bantiana* without CNS involvement.<sup>6</sup> Other species implicated in phaeohyphomycoses in cats include *A. infectoria*, *Exophiala jeanselmai*, *Dreschlera spicifera*, *Exophiala spinifer*, *Fonsecaea pedrosoi*, *Moniliella suaveolens*, *Scolecobasidium humicola*, *Staphylotrichum coccosporum*, and *Ochronic gallopavum*.<sup>1,11,12</sup>

Systemic illness in cats due to *Alternaria* species has not been reported. It is interesting that this cat also had CNS involvement, although the clinical progression suggests a tracheobronchial lymphadenitis that disseminated to the brain rather than primary brain lesion. No cutaneous lesions were noted in this case, and it is suspected that inhalation may be the route of infection. *Alternaria* spp. have small spores that may allow it to gain entry to lower airways and subsequently to draining lymph nodes.<sup>5</sup>

Identification of dematiaceous fungi requires culture and examination of fungal morphology or molecular techniques such as PCR. *Alternaria* spp. in culture are pigmented, with tapering of one end of the conidia to form a characteristic beak. The shape of the beak can help differentiate between different species. The conidia form long chains, which may or may not branch depending on the species.<sup>13</sup>

Dematiaceous fungi always form pigment in culture; however, pigment may not be readily visible in H&E tissue sections.<sup>3</sup> Often, melanin stains, such as Fontana-Masson, are recommended to highlight the presence of melanin in hyphae.<sup>3,5,12</sup> In this case, Fontana-Masson was performed on the tissue sections from the affected cat, as well as in a control case of non-pigmented fungus (morphology consistent with *Aspergillus* sp.), and revealed staining of the hyphae of both types of fungi. Fontana-Masson staining of fungal hyphae should be interpreted with caution, as there may be nonspecific uptake of silver stain in fungal hyphae as demonstrated in this case. A copper-sulfide-silver method<sup>2</sup> described for fungal cultures was modified for use in fixed tissue to try to find a more discriminatory staining technique. This method resulted in inconsistent staining of all fungal hyphae examined (presumed melanized as well as non-melanized).

**AFIP Diagnosis:** 1. Brain, cerebrum: Encephalitis, necrotizing and pyogranulomatous, multifocal to

coalescing, severe, with vasculitis and fungal hyphae.

2. Heart: Myocarditis, pyogranulomatous, multifocal to coalescing, marked, with myocardial degeneration, necrosis and loss, and fungal hyphae.

**Conference Comment:** There is some slide variation with respect to lesion distribution, particularly in the heart, where the lesion ranges from intramural to transmural and from focal to multifocal to coalescing.

The contributor provided an excellent review of phaeohyphomycosis in general, and alternariosis in particular. Most conference participants considered disseminated zygomycosis to be the most likely etiologic diagnosis, which is not surprising in light of the lack of pigmentation in this particular case. Zygomycosis refers to infection by fungi of the orders Entomophthorales (*Basidiobolus* sp. and *Conidiobolus* sp.) and Mucorales (*Mucor* sp., *Rhizopus* sp., *Rhizomucor* sp., and *Absidia* sp.). In both, hyphae are broad and poorly septate; however, in entomophthoromycosis they are characteristically bounded by brightly eosinophilic sleeves, which are lacking in this case.<sup>8</sup> For many participants, the differential diagnosis also included hyalohyphomycosis caused by nondematiaceous opportunistic fungi that also form hyphae in tissue. These differ from the hyphae in phaeohyphomycosis in that they generally have thinner walls and are not pigmented.<sup>8</sup> This case highlights the utility of molecular diagnostics in practice.

The incidence of phaeohyphomycosis is increasing, resulting in a dramatic increase in importance in human medicine in recent years.<sup>13</sup> As noted by the contributor, it is important to recall that while the production of pigment is characteristic of dematiaceous fungi in culture, it may not be visible on H&E sections. *Alternaria infectoria* is the most common clinically important species in human cases of alternariosis, but its lack of pigmentation and poor ability to sporulate in routine media make it difficult to diagnose. As a result, numerous cases have been erroneously attributed to *A. alternata* or *A. tenuissima*, when *A. infectoria* was the true etiology.<sup>13</sup>

In contrast to localized phaeohyphomycosis, which is only rarely associated with immunosuppression in dogs and cats, disseminated disease is usually associated with immunosuppression. The polyclonal gammopathy present in this case is common with this disease, indicating a persistent, but ineffective humoral immune response.<sup>8</sup>

**Contributor:** The Animal Medical Center, Department of Pathology, 510 East 62<sup>nd</sup> St., New York NY 10065  
<http://www.amcny.org/>

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