



WEDNESDAY SLIDE CONFERENCE 2007-2008

# Conference 22

16 April 2008

Moderator:

Dr. Thomas Van Winkle, DVM, Diplomate ACVP

## CASE I – 3148216 (AFIP 3063505).

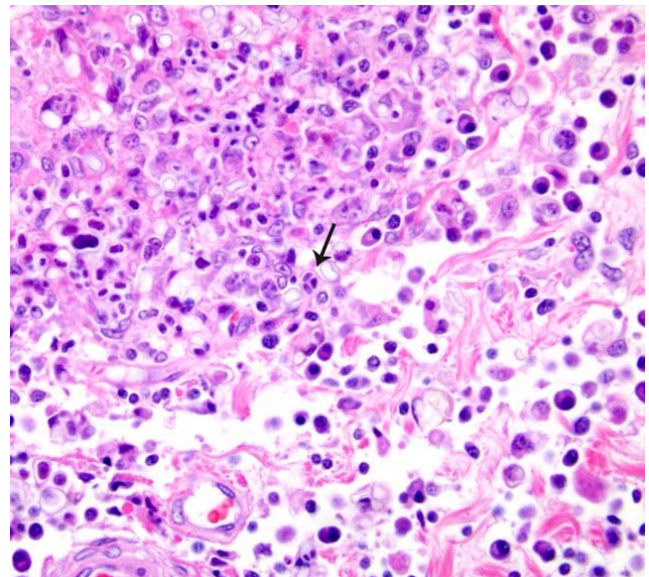
**Signalment:** This was a 2 years of age, male, Collie dog (*Canis familiaris*) that weighed 55 pounds.

**History:** This dog was reported to have inappetence and neurologic signs for 3 weeks duration. The neurologic signs included inability to stand, nystagmus, torticollis, and fine tremors when moving the head.

**Gross Pathology** (Per submitting veterinarian): The lungs had a diffusely gritty consistency. The other reported gross findings were considered to likely be insignificant: the kidneys were slightly softer than normal, the brainstem was slightly softer than normal, and the valve leaflets of the heart were slightly roughened.

**Histopathologic Description:** In sections of brain and brainstem, the meninges were thickened by multiple locally extensive infiltrates of mononuclear cells—which

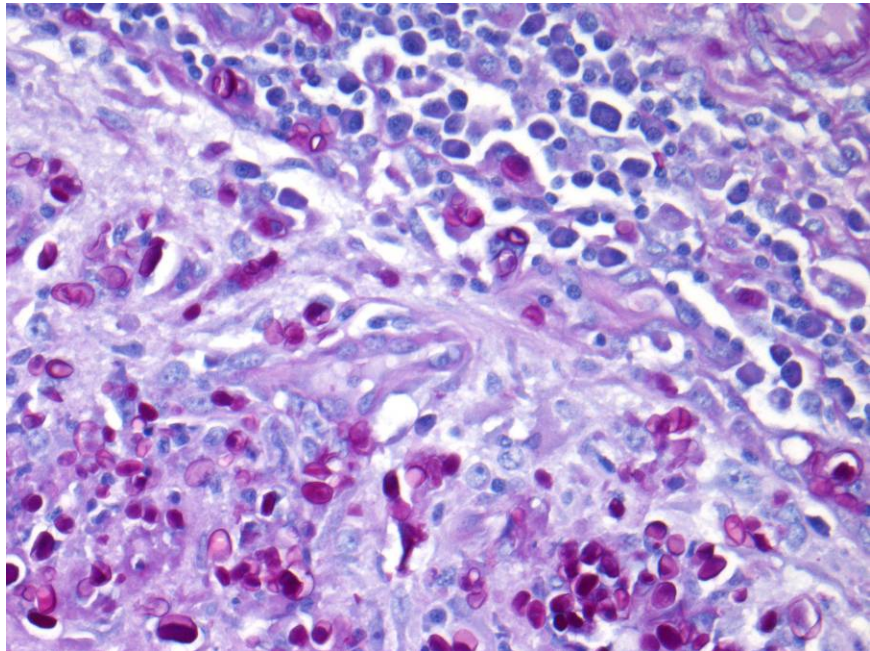
were predominately plasma cells, occasional foci of dense fibrin, scattered cellular debris, and numerous round to oval and angular, colorless and refractile organisms measuring 5-15 micrometers. The organisms had a thin, distinct, poorly staining cell wall and contained a nucleus. There were morulae with 2-6 internal round daughter cells measuring 3-8 micrometers and containing 1 nucleus (**Fig. 1-1**). The organisms were free, as well as phagocytized by macrophages. These organisms were



*1-1. Cerebellum, meninges, Collie. Admixed within the inflammatory cell infiltrate and necrotic debris there are numerous algal forms in different stages of development. Occasional organism is in the intermediate form of development characterized by nuclear and cytoplasmic cleavage (arrow). (HE 400X).* →

PAS positive (Fig. 1-2) and consistent with *Prototheca* sp. Variably sized multifocal aggregates of these organisms and identical nonsuppurative inflammatory reactions and necrosis were occasionally within the neuropil and extending into the neuropil from the meninges.

Sections of pancreas (not submitted) contained multifocal to coalescing areas of necrosis with predominate histiocytes and occasional plasma cells, lymphocytes and neutrophils. Round to oval and angular, 5-15 microns, colorless, refractile organisms were phagocytized in macrophages and free within these necrogranulomatous aggregates.



1-2. Cerebellum, meninges, Collie. Numerous PAS-positive algal organisms. (Periodic acid-Schiff stain). Photomicrograph courtesy of Diagnostic Center for Population and Animal Health, Michigan State University.

**Contributor's Morphologic Diagnosis:** 1. Brain: Severe, locally extensive and multifocal, granulomatous meningoencephalitis with intralesional organisms consistent with *Prototheca* sp.

(Following tissues not included in submitted histologic sections)

2. Pancreas: Moderate to severe, multifocal to coalescing, necrogranulomatous pancreatitis with intralesional organisms consistent with *Prototheca* sp.

3. Lung: Moderate, multifocal, pyogranulomatous interstitial pneumonia

**Contributor's Comment:** *Prototheca* is a saprophytic achlorophyllic algae related to the green algae *Chlorella*. *Prototheca* thrives in moist environments, and is commonly contracted from lakes and environments with organic debris. The two species associated with disease are *Prototheca wickerhamii* and *Prototheca zopfii*. In animals, protothecosis most commonly develops as a systemic infection with or without cutaneous involvement. The organism often has a predilection for the brain and the eyes with resulting neurologic signs or blindness be-

ing a common clinical presentation.<sup>2</sup> Infection usually develops in immunocompromised patients, and Collie dogs are over-represented in reported cases of natural infections. It is believed that with disseminated protothecosis, the organism typically infects the gastrointestinal tract with rapid spread to other organs.<sup>3</sup>

*Prototheca* sp. are round, oval, or angular cells that are 8-20 micrometers in diameter, have a refractile wall and contain granular cytoplasm. The organism reproduces by endosporulation and can be seen histologically as morula of 2-20 daughter cells within a single organism. The morula ruptures and releases the individual daughter cells. The cell wall of *Prototheca* stains poorly with hematoxylin-and-eosin, but stains strongly positive to stains

Table extracted from Stenner et al.<sup>4</sup>

	Shape	Sporangia	Sporangiospores
<i>Prototheca zopfii</i>	oval or cylindrical	14-25 µm	up to 20
<i>Prototheca wickerhamii</i>	round	7-13 µm	up to 50

for carbohydrate, such as PAS, Gridley, Bauer, and GMS.

*Prototheca* sp. resembles *Chlorella* sp., but can be distinguished by PAS-positive starch granules in the cytoplasm of *Chlorella*. In fresh smears of lesions that contain *Chlorella*, the organisms are green due to the presence of chlorophyll.<sup>2</sup>

**AFIP Diagn osis:** Brain, cerebrum and cerebellum: Meningoencephalitis, granulomatous, multifocal, moderate, with algae, etiology consistent with *Prototheca* sp., Collie dog (*Canis familiaris*), canine.

**Conference Comment :** *Prototheca* sp. are saprophytic organisms that are ubiquitous in the environment, and have been identified on 4 continents.<sup>3</sup> They grow in moist environments including sewage, animal feces, slime flux of trees, soil, standing and flowing water, and food.<sup>3,4</sup>

Infection is thought to occur through traumatic inoculation, ingestion, or wound contamination. The immune status of an individual appears to play a role in acquiring the infection as well as lesion extent and distribution, although infections have been found in both immunocompetent and immunosuppressed patients.<sup>4</sup>

Clinical manifestation of protothecosis in mammals is to a certain extent species specific. In cattle, mastitis due to *P. zopfii* is the most common presentation of protothecosis in cattle<sup>1,4</sup> with occasional spread into adjacent lymph nodes, while protothecosis in the dog, also primarily due to *P. zopfii*<sup>4</sup>, is principally a systemic disease with multi-organ involvement and a predilection for the eyes and brain.<sup>2,3</sup> Protothecosis in cats, due to *P. wickerhamii*, localizes in the skin, and may be successfully treated by wide surgical excision.<sup>3,4</sup>

In dogs the most consistent presenting clinical sign of systemic protothecosis is hemorrhagic colitis.<sup>4</sup> In fact, the colon and the rectum appear to be primary sites of replication even without clinical evidence of colitis.<sup>4</sup> A proposed pathogenesis includes initial colonization of the colonic mucosa following ingestion of large numbers of infective organisms, followed by penetration of the gut wall and systemic spread via blood vessels and lymphatics.<sup>4</sup>

**Contributor:** Diagnostic Center for Population and Animal Health, Michigan State University

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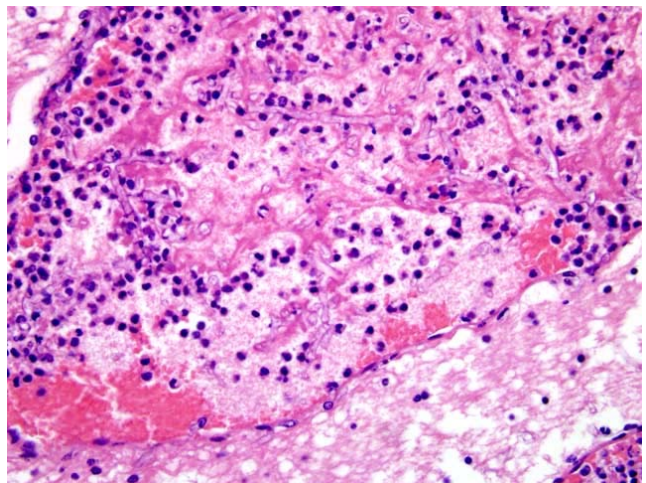
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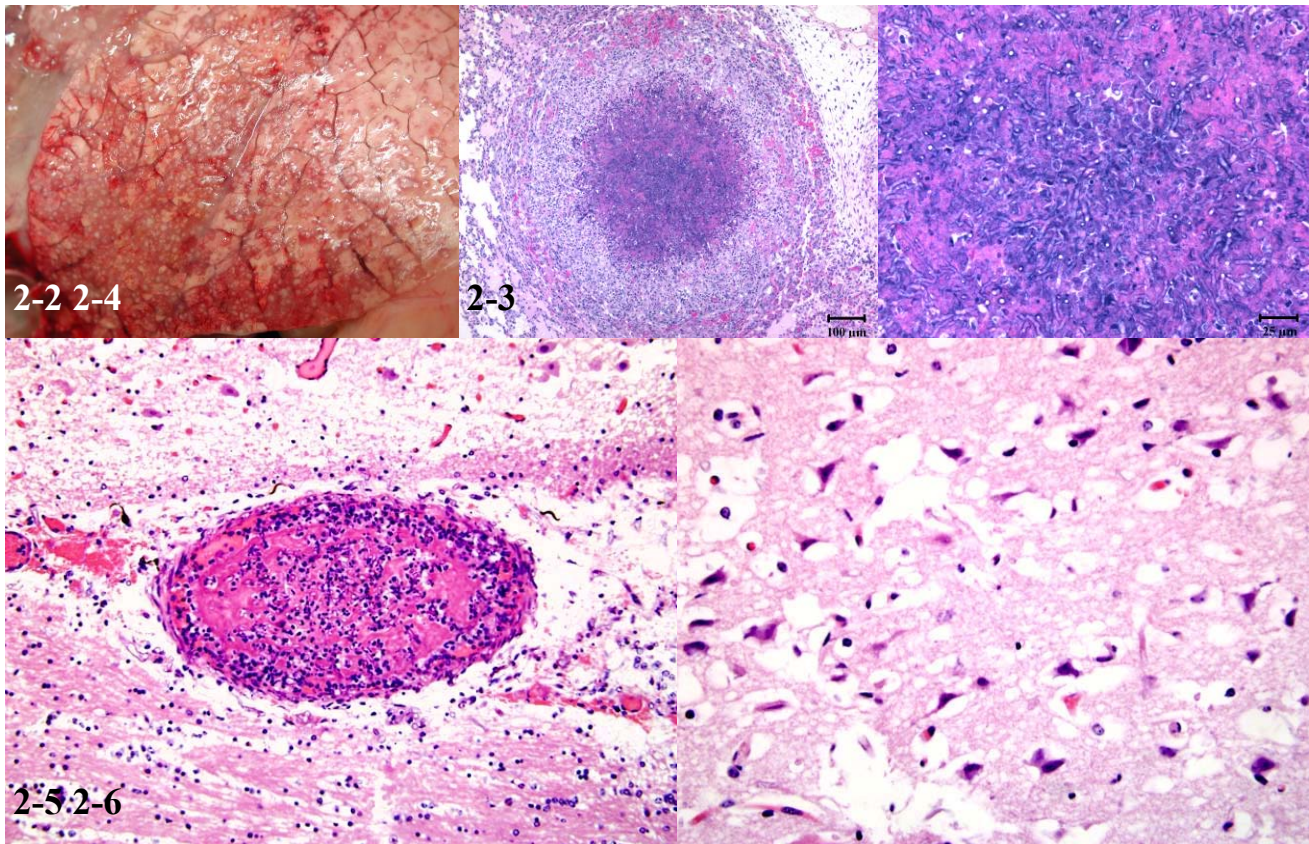
#### CASE II – NADC MVP-1 2007 (AFIP 3065886).

**Signalment:** White-tailed deer (*Odocoileus virginianus*), female, 10-days-old

**History:** This fawn had been removed from the dam 24-48 hrs after birth and moved inside a barn for bottle-feeding. The fawn was kept in a pen, bedded with oat straw. The fawn appeared normal until 9 days of age when there was an acute onset of respiratory signs including elevated respiratory rate, clear nasal discharge,



2-1. Cerebrum, deer. Thrombi contain numerous parallel-walled, dichotomous branching hyphae. (HE 400X)



2-2. Lung, deer. Multifocal to coalescing pyogranulomatous pneumonia.

2-3. Lung, deer. Pyogranuloma containing numerous hyphae consistent with *Aspergillus* sp.

2-4. Lung, deer. Higher magnification of Fig. 2-3 showing myriad hyphae admixed with necrotic debris.

2-5. Cerebrum, deer. Multifocally within many vessels there are thrombi occluding the vessels and there is a vasculitis characterized by disruption and fragmentation of the vessels wall with replacement by numerous inflammatory cells and eosinophilic fibrillar material. (HE 200X).

2-6. Cerebrum, deer. Within the neural parenchyma there are areas of infarction with numerous shrunken, eosinophilic neurons with pyknotic nuclei (neuronal necrosis). (HE 400X).

Photomicrographs (Fig. 2-2, 2-3, 2-4) courtesy of National Animal Disease Center, ARS, USDA, 2300 Dayton Avenue, Ames, IA 50010, [www.nadc.ars.usda.gov](http://www.nadc.ars.usda.gov)

anorexia, and fever (103°F). The fawn was treated with antibiotics and antipyretics. There was no response to treatment and within 24 hrs of onset the animal died.

**Gross Pathology:** The lung had multifocal areas of consolidation and failed to deflate. There were multiple fibrinous adhesions to the internal thorax and numerous pin-point (2-4 mm) white to translucent nodules visible on the pleural surface. Nodules were surrounded by a narrow hyperemic border. The renal cortex contained several variable sized wedge-shaped areas of pallor (infarcts). Within the brain there was a gray to white, soft area (3 cm in size) in the cortex of the right frontal

lobe that was especially pronounced after formalin fixation.

**Laboratory Results:** Lung submitted for bacteriological culture: No bacteria isolated, heavy pure growth of *Aspergillus flavus*.

**Histopathologic Description:** Within the section of brain there is accentuation of the vasculature and meninges due to edema, accumulation of fibrin and infiltrates of moderate to large numbers of inflammatory cells including neutrophils, macrophages and lymphocytes. Numerous vessels are characterized by infiltrates of neutro-

phils, lymphocytes and macrophages within the vessel wall, fibrinoid degeneration and partially occluding fibrinocellular thrombi. Associated with the inflammatory infiltrate and especially prominent within vessels are numerous intralumenal fungal hyphae (**Fig. 2-1**). Hyphae have parallel sides, are 3-6 microns in width and are characterized by frequent septation and dichotomous, progressive branching. Inflammatory cells and fungal hyphae are also found within the neuropil.

Within the lung (**Fig. 2-2**) (not submitted) are multifocal to coalescing nodular infiltrates (**Fig. 2-3**) of macrophages and lymphocytes surrounding cores of numerous neutrophils, necrotic debris and aggregates of fungal hyphae with morphology similar to that seen in the brain (**Fig. 2-4**). Similar lesions were present in the kidney (not submitted).

**Contributor's Morphologic Diagnosis:** Brain: Meningoencephalitis, pyogranulomatous, focally extensive, subacute, moderate, with thrombosis and intralumenal, angioinvasive fungal hyphae consistent with *Aspergillus* sp.

**Contributor's Comment:** Organisms of the genus *Aspergillus* are ubiquitous in the environment and opportunistic pathogens. Although *A. fumigatus* is most commonly associated with infection in mammals, infections with *A. flavus*, *A. terreus*, *A. nidulans* and *A. niger* have also been described. Fungal spores may be inhaled from moldy bedding or feed and implant on the mucous membranes of the upper or lower respiratory tract. Although most often a respiratory disease, dissemination of infection can occur, with the meninges and kidneys being most commonly involved.

Disseminated aspergillosis, due to *A. fumigatus*, with involvement of the lungs, brain and kidneys has been previously described in an adult white-tailed deer.<sup>10</sup> Pulmonary aspergillosis has been reported in fallow deer (*Dama dama*) due to *A. fumigatus* and *A. corymbifera*.<sup>4</sup> Disseminated aspergillosis is often associated with debilitation, immunologic suppression or prolonged antibiotic or corticosteroid administration. In the present case, debilitation, or prolonged use of antibiotics or corticosteroids were not factors; however, an unknown immunologic deficiency cannot be ruled out.

The two main portals of entry for fungal spores that cause systemic aspergillosis in cattle are the respiratory and gastrointestinal tracts. Mycotic placentitis in cattle can lead to abortion. Systemic aspergillosis in 4-day-old calves where lesions included well developed hepatic granulomas with intralumenal hyphae also suggests that a

local or transitory mycotic placentitis could lead to calves that are born alive and survive.<sup>2</sup> In mature cows it is suggested that the gastrointestinal tract is almost exclusively the portal of entry for *A. fumigatus* and that placentitis and pneumonia are secondary to hematogenous dissemination from the gastrointestinal lesions.<sup>8</sup>

The precise virulence factors of *Aspergillus* spp. are not well characterized. However, the common features of necrosis, angioinvasion and hematogenous dissemination may serve as clues to key factors in pathogenesis.

**AFIP Diagnosis:** Brain, cerebrum: Vasculitis (**Fig. 2-5**) and meningoencephalitis, necrotizing, subacute, multifocal, marked, with hemorrhage, edema, fibrin thrombi, focally extensive cortical coagulative necrosis (infarct) (**Fig. 2-6**), and numerous hyphae, etiology consistent with *Aspergillus* sp., white-tailed deer (*Odocoileus virginianus*), cervid.

**Conference Comment:** Histologically, *Aspergillus* sp. infections are characterized by vasculitis, often with numerous hyphae, and resultant thrombosis and infarction. More chronic lesions are granulomas with central cores of necrotic debris. Although hyphae are often present within the lesions and may be seen as negative images with hematoxylin and eosin stains, special stains, such as periodic acid-Schiff (PAS) or Gomori methenamine-silver (GMS), may be required to visualize their characteristic morphology.<sup>5</sup>

*Aspergillus* sp. can produce several virulence factors including adhesins, antioxidants, enzymes, and toxins. The role of these virulence factors has not been fully defined. Restrictocin and mitogillin are two ribotoxins produced by *Aspergillus* that degrade host mRNA, thereby inhibiting host-cell protein synthesis. In addition, melanin pigment, mannitol, catalases, and superoxide dismutases are all antioxidant defenses produced by *Aspergillus*.<sup>7</sup>

Aspergillosis, primarily caused by *A. fumigatus*, is commonly encountered in birds.<sup>6,9</sup> Captive penguins, turkeys, raptors and waterfowl appear to be particularly susceptible to infection. Certain physical and immunological characteristics of avians may make them more susceptible to infection. Birds lack an epiglottis to prevent particulate matter from being inhaled. Also, they are not able to produce a strong cough reflex due to their lack of a diaphragm. Avian heterophils use cationic proteins, hydrolases and lysosomes to kill fungal hyphae, which may be less effective than mammalian myeloperoxidase and oxidative destruction mechanisms. A unique feature of avian aspergillosis is the presence of reproductive phases of the fungus in tissue. This unique finding

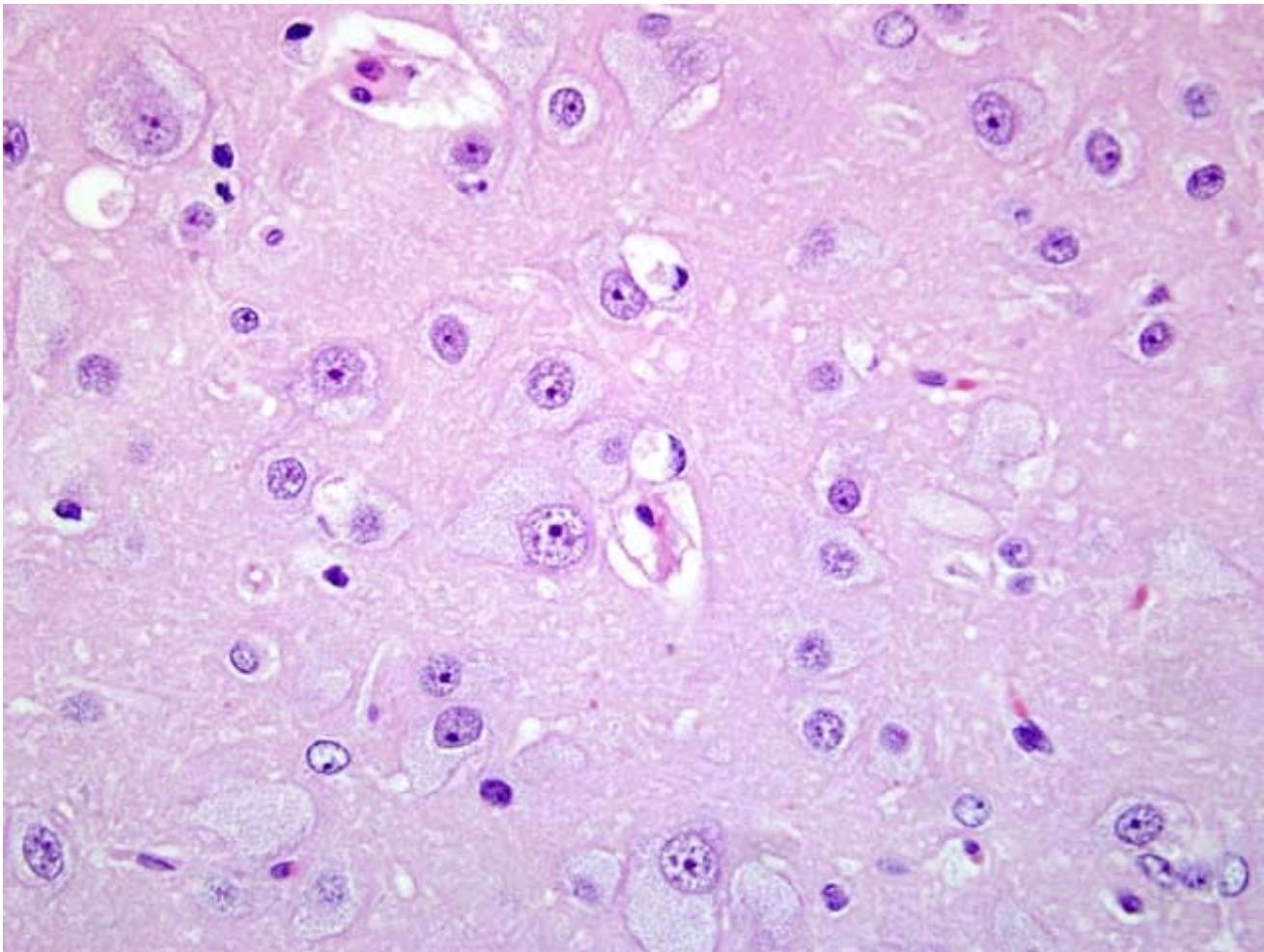
maybe due to the presence of cavernous air sacs, a warm core body temperature, or birds' sensitivity to gliotoxin, which results in tissue necrosis and thus produces a nutrient rich environment for fungus growth.<sup>9</sup>

**Contributor:** National Animal Disease Center, ARS, USDA, 2300 Dayton Avenue, Ames, IA 50010  
www.nadc.ars.usda.gov

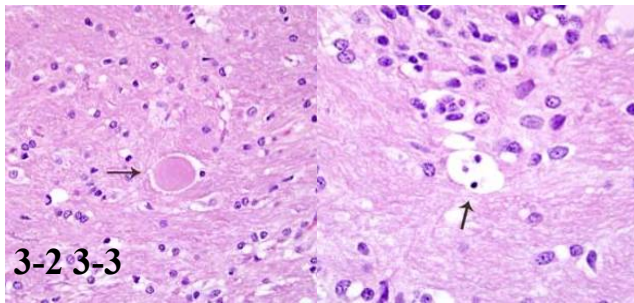
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3-1. Cerebrum, cat. Diffusely, neurons are moderately swollen with finely granular to microvacuolated cytoplasm. Multifocally, glial cells often contain large, discrete, clear vacuoles. (HE 400X).



3-2. Cerebrum, cat. Rarely within the white matter there are spheroids characterized by swollen, hyper-eosinophilic axons (arrow). (HE 400X).

3-3. Cerebrum, cat. Within the white matter there are rare digestion chambers characterized by swollen axon sheaths containing gitter cells and cellular debris (arrow). (HE 400X).

#### **CASE III – 05-26548 (AFIP 3027410).**

**Signalment:** An approximately 14-week-old, male, European Burmese kitten (*Felis catus*) presented for euthanasia.

**History:** Nervous signs including ataxia, dysmetria, head and limb tremors and constant loud purring

**Gross Pathology:** Necropsy was unremarkable with no gross lesions observed.

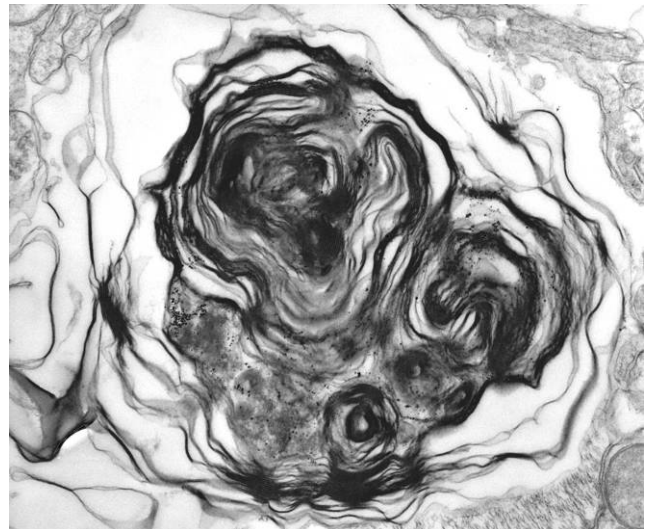
**Laboratory Results:** Enzyme assays for total hexosaminidase,  $\alpha$ -hexosaminidase and control lysosomal enzymes,  $\beta$ -galactosidase and mannosidase and molecular analysis was conducted under Dr. Henry J. Baker at Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University. The tests revealed a deficiency in total hexosaminidase activity, identifying the disease as a variant of GM2 gangliosidosis. Further molecular analysis identified a deletion in the  $\beta$  subunit of the hexosaminidase gene as the cause of disease. Though virology was not conducted on samples from this kitten, no virus could be isolated from a previously submitted kitten from the same litter with similar clinical signs and lesions.

**Histopathologic Description:** Histologically neurons throughout the brain are diffusely swollen with abundant finely granular foamy cytoplasm which often displaces the nucleus to the periphery (Fig. 3-1). Small numbers of individual neurons are shrunken, with deeply basophilic clumped chromatin material and with variable degree of cytoplasmic hyper-eosinophilia. Many glial cells

contain a single, large, clear discrete vacuole which displaces the nucleus to the periphery. Additional lesions include many hyper-eosinophilic and swollen axons with dilated myelin sheaths (Fig. 3-2). The fragmented myelin sheath of a few axons form ellipsoids arranged in a row which contain small amounts of eosinophilic granular necrotic debris and macrophages (digestion chambers) (Fig. 3-3). Electron microscopy revealed the presence of characteristic electron-dense concentric lamellar membranous whorls, separated by clear spaces (membranous cytoplasmic bodies) within the neuronal somata (Fig. 3-4).

**Contributor's Morphologic Diagnosis:** Cerebrum: Neuronal vacuolation, cytoplasmic, diffuse, severe, with neuronal degeneration and necrosis and axonal degeneration, European Burmese, feline

**Contributor's Comment:** Lysosomal storage disorders are a diverse group of mainly autosomal recessive inherited diseases that can result from the lack of any protein that is necessary for the normal functioning of lysosomal degradation pathways.<sup>5</sup> Lysosomal acid hydrolases are important for the catabolism of a variety of macromolecules. Faulty breakdown of these macromolecules results in their accumulation within the lysosomes, which progressively enlarge and lead to interference with normal cell functions. Although initially described as disorders that are caused by the deficiency of lysosomal enzymes, lysosomal storage disorders can also occur through de-



3-4. TEM, cerebrum, cat. Intraneuronal concentric lamellar membranous whorls. Transmission electron micrograph courtesy of Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, <http://www.cvm.uiuc.edu/path/>

fective posttranslational modification of lysosomal enzymes, lack of enzyme activator, lack of substrate activator, or the lack of transport proteins required for the removal of digested material from the lysosomes.<sup>5</sup> The organs affected by particular lysosomal storage diseases are determined by the tissue in which the substrate to be degraded is found and where it is degraded.<sup>5</sup>

Biochemically, the inherited lysosomal storage diseases can be broadly divided into sphingolipidoses, cholesterol ester storage disease, glycoproteinoses, glycogenoses, mucopolysaccharidoses, mucopolipidoses and ceroid-lipofuscinoses.<sup>3</sup> Sphingolipidoses comprise diseases in which there is a failure to properly catabolize various complex lipids derived from ceramide.<sup>3</sup> These include GM1 and GM2 gangliosidosis, galactocerebroside, glucocerebroside, sphingomyelin lipidosis and galactosialidosis.<sup>3</sup> GM2 ganglioside is a cell membrane glycolipid which is catabolized by the action of N-acetylhexosaminidase and GM2 activator (GM2A) protein.<sup>6,7</sup> N-acetylhexosaminidase exists as a dimer in two forms,  $\alpha\beta$  (hexosaminidase A) and  $\beta\beta$  (hexosaminidase B). Improper functioning of either one of these subunits ( $\alpha$  or  $\beta$ ) or GM2A results in accumulation of GM2 ganglioside in the lysosomes of neurons leading to a progressive deterioration of the central nervous system (GM2 gangliosidosis).<sup>6,7</sup> Tay-Sachs disease of humans is a GM2 gangliosidosis caused by a mutation in the  $\alpha$  subunit resulting in the deficiency of hexosaminidase A, whereas Sandhoff disease is caused by mutation of  $\beta$  subunit which affects both hexosaminidase A and B.<sup>3</sup> GM2 gangliosidosis has been previously described in German shorthaired pointer and Japanese Spaniel dogs, domestic short haired and Korat cats, and Muntjak deer.<sup>1,3</sup>

GM1 gangliosidosis (generalized gangliosidosis) is caused by the deficiency of  $\beta$  galactosidase which cleaves the terminal galactose from GM1 ganglioside. GM1 gangliosidosis has been described in dogs, cats, cattle and sheep.<sup>3</sup> Galactocerebroside (globoid cell leukodystrophy, Krabbe disease of humans) is primarily a leukodystrophy and has been described in dogs, cats and sheep. Galactocerebroside is found in myelin sheaths and the deficiency of galactocerebroside causes accumulation of the lipids in the macrophage-like 'globoid cells' and in oligodendroglial cells.<sup>3</sup> Glucocerebroside (Gaucher disease of humans) is caused by a deficiency in the lysosomal enzyme glucocerebroside, a  $\beta$ -glucosidase, which results in the accumulation of the lipid glucocerebroside in neurons and in macrophages. The disease has been described in Sydney Silky dogs.<sup>3</sup> Sphingomyelin lipidosis (Niemann-Pick disease of humans) is caused by a deficiency of sphingomyelin phosphodiesterase (sphingomyelinase) which results in the accumulation of

sphingomyelin in neurons and macrophages. The disease has been described in cats and a single case in miniature Poodle dog.<sup>3</sup>

**AFIP Dia gnosis:** Brain, cerebrum, neurons and glia: Cytoplasmic vacuoles, diffuse, moderate, European Burmese (*Felis catus*), feline.

**Conference Comment:** The contributor gives an excellent overview of the sphingolipidoses. GM2 gangliosidosis results from a defect in only one of three gene products, the  $\alpha$ -subunit, the  $\beta$ -subunit or the GM2 activator. Three variant forms of GM2 gangliosidosis have been described and are based on the specific subunits that retain functionality: Variant B ( $\alpha$ -subunit deficiency), variant 0 ( $\beta$ -subunit deficiency), and variant AB (GM2 activator deficiency).<sup>7</sup>

Neurons of the central and autonomic nervous system and retina are primarily affected.<sup>5</sup> Typical histopathologic findings in GM2 gangliosidosis include swollen neurons with cytoplasmic vacuoles consisting of lysosomes distended with accumulated gangliosides. Oil red O and Sudan black B may stain storage material in astrocytes and macrophages.<sup>3,5</sup> With electron microscopy (EM), the cytoplasmic inclusions are visualized and consist of membrane layers forming "onion-skin" whorls within the lysosomes.<sup>5</sup>

Lysosomal storage bodies visualized on EM are not considered characteristic of a particular storage disorder. In general, GM1 and GM2 may form similar concentric lamellations, whereas in mucopolysaccharide storage diseases, membranous stacks (zebra bodies) are more common.<sup>3</sup> Abnormal twisted tubular structures are often seen in glucocerebroside and galactocerebroside, while glycogen particles may be seen in the glycogen storage diseases.<sup>3</sup>

**Contributing Institution:** Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign  
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#### **CASE IV - S0610573 (AFIP 3071896).**

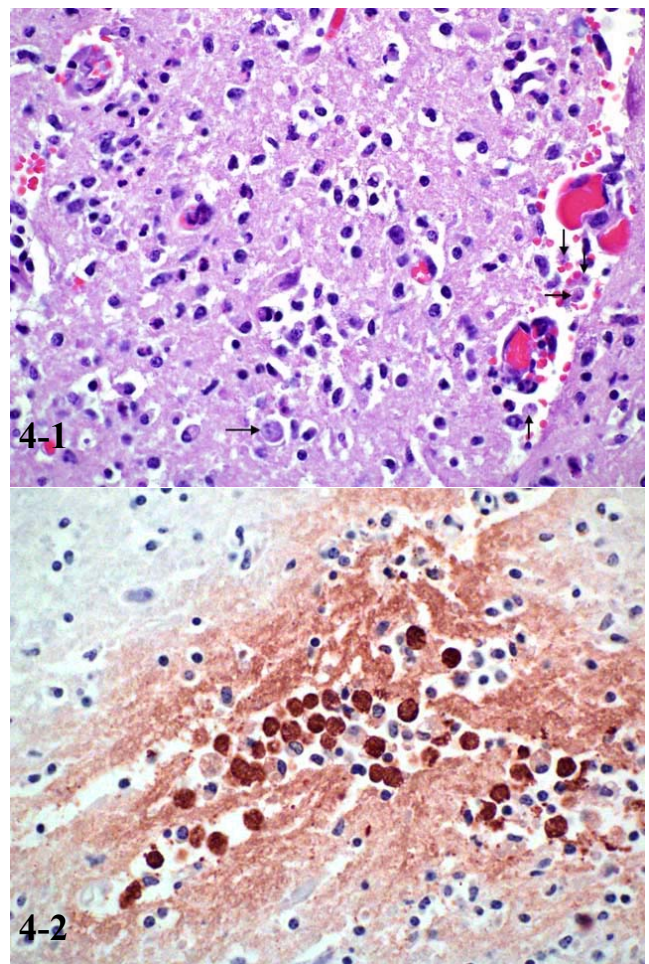
**Signalment:** Holstein heifer, unknown age, bovine.

**History:** Between July 30 and September 28, 1998 and 1999, specimens from nine 10-20-month-old Holstein

heifers with a history of acute CNS disease were submitted to our laboratory. All submissions were from one large heifer-raising operation located in southern California in an arid but highly productive agricultural area irrigated by Colorado River water supplied through a system of canals and ditches. The ranch relied on untreated canal water for livestock drinking water. These heifers had been diagnosed with fatal meningoencephalitis caused by *Naegleria fowleri*. Sporadic specimens from animals with similar clinical signs have been submitted in the successive years, including the formalin-fixed brain we are now examining.

**Gross Pathology:** No gross lesions.

**Laboratory Results:** Immunohistochemistry: Positive



4-1. Cerebrum, bovine. Multifocally, often perivascularly, there are low number of amoebic trophozoites with visible nuclei and karyosomes (arrows).

4-2. Cerebrum, bovine. Positive immunostaining for *Naegleria fowleri* within focus of necrosis and inflammation..

Photomicrographs courtesy of the California Animal health and Food Safety laboratory, U.C.Davis

for *Naegleria fowleri* (Fig. 4-2).

**Histopathologic Description:** Multifocal necrosuppurative hemorrhagic encephalitis, predominating within neuropil bordering the cerebral aqueduct, includes foci of malacia or necrosis, populated by degenerative neutrophils, hemorrhage, interspersed with neuropil populated by scattered lymphocytes and macrophages, and rare multinucleated giant cells. Numerous blood vessels are mildly (1-2 cell thickness) to markedly (9-10 cells thick) cuffed by lymphohistiocytic cells. Clustered and individual amoebic trophozoites (Fig. 4-1) are observed within and surrounding areas of necrosis/malacia and perivascularly. The amoebae are round to oval, approximately 5-10 µm in diameter, and have a pale eosinophilic, finely granular cytoplasm. The nuclei are small (approximately 2-3 µm in diameter), poorly delineated, mostly eccentric and weakly basophilic with a prominent basophilic karyosome.

**Contributor's Morphologic Diagnosis:** Encephalitis, hemorrhagic, multifocal, necrosuppurative, severe, with presence of multifocal amoebic trophozoites.

**Contributor's Comment:** Primary amoebic meningoencephalitis is a fulminant infection of the human central nervous system caused by *Naegleria fowleri*, a free-living amoeba that thrives in artificially or naturally heated water. The infection usually is acquired while bathing or swimming in such waters. There are 4 genera of amoebae that cause CNS disease in mammals, namely *Acanthamoeba* (several species), *Naegleria fowleri*, *Balamuthia mandrillaris*, and the recently described *Sappinia diploidea*.<sup>9</sup> *Acanthamoeba* and *Naegleria* are ubiquitous in soil and fresh water, including lakes, streams, and hot springs. These amoebae have also been isolated from various artificial water sources, such as swimming pools, tap water, heating and ventilation units, air conditioners, cooling water, sewage, contaminated cell cultures, and contact lens-storing fluid.<sup>1,9</sup> Their cysts have even been demonstrated in dust during dust storms.<sup>1</sup>

Primary amoebic meningoencephalitis (PAM) is the term for the human disease caused by *N. fowleri*. It is an acute, usually fatal, necrotizing, and hemorrhagic meningoencephalitis.<sup>9</sup> Although CNS infections due to *Acanthamoeba* and *Balamuthia* have been recorded in animals, such as dogs, sheep, cattle, primates, and horses, there has been only 1 report of naturally acquired PAM in animals, namely in a South American tapir at a zoo in Arizona.<sup>1-3,7,8,10</sup> Primary amoebic meningoencephalitis has been experimentally induced in mice, sheep, and monkeys.<sup>1,10</sup> *Naegleria fowleri* is thermophilic and tolerates temperatures of up to 45° C; hence, the frequent association of PAM with a history of contact with naturally

warm or artificially heated waters.<sup>1</sup> The portal of entry is the olfactory mucosa. The parasite migrates to the brain via olfactory nerves. Incubation period is short, with onset of clinical signs several days following exposure. The disease rapidly progresses and usually culminates in death within 5-7 days.<sup>5</sup>

The life cycle of *N. fowleri* includes a trophozoite stage (amoebic form), a temporary flagellate stage, and, in unfavorable environments, a cyst stage. The cyst stage is susceptible to desiccation.<sup>1</sup> Only the trophozoite stage of *N. fowleri* has been detected in CNS lesions. This is in contrast with other free-living opportunistic amoebae, where identification of cyst stages aids in their differentiation from *Naegleria*.<sup>8</sup>

Gross lesions vary and may or may not be present. Findings may include multifocal meningeal hemorrhages and tan-gray thickening of meninges, tan-gray deposits obscuring cerebellar cortical surface, and light brown and malacic brain parenchyma. Lesions may be bilateral and symmetrical.

Histopathology shows mainly a multifocal, necrosuppurative, and hemorrhagic meningoencephalitis. Most severely affected areas were the anterior cerebra, olfactory bulbs, and cerebella. Amoebae are not easily detected microscopically because they resemble degenerated macrophages and may be few in number. The tendency of the amoebae to accumulate in aggregates or in perivascular spaces within areas of necrosis is most helpful, as is the small, weakly basophilic karyosome within a poorly delineated, small nucleus. There are very few or no multinucleated giant cells or eosinophils to direct the pathologist toward parasitic etiology. Some amoebae form cysts, visualized by periodic acid-Schiff stain, but *N. fowleri* does not, possibly because death occurs during the acute stage of the disease. Routine special stains to identify bacteria, such as Giemsa, Brown-Brenn-modified Gram, and Steiner silver stain were not helpful in identifying amoeba. Immunohistochemistry of the brain is an important tool in the confirmation of the diagnosis and also the isolation of the organism from animal tissues and suspected sources can be attempted. Finally, it is advantageous to know that the disease is seasonal, occurs in areas with high ambient summer temperatures, and may be associated with the use of consumption of untreated surface water.<sup>1</sup>

**AFIP Diagnosis:** Brainstem: Encephalitis, necrotizing, subacute, multifocal, moderate, with hemorrhage and amoebic trophozoites, Holstein (*Bos taurus*), bovine.

**Conference Comment:** The contributor gives an excellent overview of *Naegleria fowleri* infection. The full pathogenesis and virulence factors of the organism are not completely understood. These infections are believed to be transmitted when water containing the organisms comes in contact with the host nasal mucosa. The amoebic trophozoites then migrate along the olfactory nerves and pass through the cribriform plate to enter the brain.<sup>4,9</sup> *N. fowleri* contains a surface protein, similar to the human integrin-like receptor, that facilitates adhesion to fibronectin, an extracellular matrix glycoprotein found at the basal lamina and surrounding cells.<sup>4</sup> Two pore-forming proteins termed naegleriapores A and B, as well as proteases and phospholipases have all been implicated in host cell destruction.<sup>4,9</sup>

Most animals infected with *N. fowleri* die before mounting a detectable adaptive immune response. The innate immune response, consisting of complement, neutrophils, and macrophages, appears to be a frequently inadequate defense mechanism. In vitro studies have shown that *N. fowleri* is able to evade complement damage by either expressing complement-regulatory proteins (CD59-like protein), shedding the MAC complex (C5b-C9) on surface membrane blebs, internalizing and degrading the MAC complex, or preventing the insertion of the MAC into the amoebic membrane.<sup>4</sup> Although neutrophils play a major role in anti-amoebic activity, their exact function is not clearly defined. TNF- $\alpha$  does not have a direct effect on *N. fowleri*, but it appears that organism destruction by neutrophils cannot occur without TNF- $\alpha$  being present.<sup>4</sup> Production of high levels of proinflammatory cytokines such as IL-1 $\alpha$ , IL-1b, IL-6, and TNF- $\alpha$  do not appear to provide protection against infection.<sup>4</sup> The roles of humoral and cell mediated immunity are also uncertain.

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<http://cahfs.ucdavis.edu>

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**Notes:**