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Veterinary Resource Program
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Bethesda, MD 20892-5230

CASE I – CP01-095 (AFIP 2788783)

Signalment: Adult nude mouse

History: Several nude mice died which had been injected with a transplantable prostatic tumor. The mice had become emaciated over 2-3 months. The tumor cell line was not tested for rodent pathogens prior to injection. Several dead mice were submitted for necropsy.

Gross Pathology: The mice were very thin and emaciated. Internal organs were within normal limits, with the exceptions of depleted fat stores and the liver containing multiple grey-yellow pinpoint foci.

Laboratory Results: Sentinel serology obtained from this colony was positive for mouse hepatitis virus (MHV) and negative for *Mycoplasma pulmonis*, Sendai virus, Epizootic diarrhea of infant mice (EDIM), pneumonia virus of mice (PVM), ectromelia, lymphocytic choriomeningitis virus (LCM), reovirus type 3, parvovirus and Theiler's murine encephalomyelitis virus.

Contributor's Morphologic Diagnosis: Mouse, liver, marked subacute, multifocal necrotizing hepatitis. Etiology: Coronavirus, Mouse Hepatitis Virus.

Contributor's Comment: The liver demonstrates multifocal, coalescing hepatic necrosis. The necrotic hepatocytes are eosinophilic and have indistinct nuclei. Karyorrhectic nuclei are in the necrotic areas. Neutrophils and lymphocytes infiltrate the necrotic regions. Also evident are syncytial cells.
Mouse hepatitis virus belongs to the family Coronaviridae and genus Coronavirus. It is a pleomorphic, single stranded RNA virus that is enveloped with surface projections or peplomers.

Mouse Hepatitis Virus (MHV) is recognized as adversely affecting research results, especially in multipurpose, multi-user facilities. *Mus musculus* is the natural host. Mice with active infection shed the virus in feces, as well as, oral and nasal secretions. MHV is extremely contagious and widespread. Transmission is through contact, aerosol, fomites, feces, bedding, and possibly transplacental.

After infection, the virus replicates in nasal mucosa and is disseminated from the nose via the blood and lymphatics. The majority of natural MHV infections in immunocompetent mice are subclinical with no discernible lesions. In athymic nudes, MHV presents as a chronic, progressive emaciation or wasting syndrome with a persistent infection of the intestinal tract or nasal mucosa. The infection is often fatal.

MHV alters lymphocytic differentiation, immunoglobulin responses, phagocytosis, tumor growth, interferon production and the level of hepatic enzymes. Immunodeficient mice can develop a chronic, nodular hepatitis and splenomegaly due to compensatory hematopoiesis.

A diagnosis of MHV is made using clinical history, necropsy and histopathology. Histopathologic features are syncytial giant cells in the nasal mucosa, liver, absorptive epithelium of the ascending colon, cecum, or small intestine. Differential diagnoses include Salmonellosis, Tyzzer's disease and mousepox in adult mice, as well as reovirus, cytomegalovirus, and adenovirus in infant mice.

MHV is a common contaminant of transplantable tumor and cell lines. Mouse antibody production (MAP) testing is recommended on all transplantable tumors and cell lines.

AFIP Diagnoses: Liver: Hepatitis, necrotizing, subacute, multifocal, random, moderate, with syncytial cells, nude mouse.

Conference Comment: Strains of Mouse Hepatitis Virus (MHV) either express tropism for the respiratory system with dissemination to multiple organs (polytropic) or are selective for the enteric system (enterotropic).

Polytropic strains initially replicate within the nasal mucosa. Dissemination occurs through viremia and via the lymphatics. Secondary replication occurs in endothelial cells and the parenchyma. In susceptible mice, hallmark lesions include necrosis and syncytia formation. Organs affected include, but are not limited to, the liver, brain, and lymphoid organs. Occasionally encephalitis occurs via direct extension along the olfactory nerves and tracts.

Enterotropic strains are more selective and lesions are generally confined to the intestines. The cecum, ascending colon, and distal small intestine are preferred sites. Villus blunting and fusion and formation of characteristic syncytial cells (balloon cells) are diagnostic in neonates; lesions are progressively milder in older animals. A syndrome of high mortality in infant mice was recognized and referred to as lethal intestinal virus of infant mice (LIVIM) before MHV was identified as the causative agent. Enterotropic infections may be complicated by coinfection with one or more secondary infectious agents such as *Escherichia coli* and *Spironucleus muris*.

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CASE II – MK99-1285 (AFIP 2679786)

Signalment: Male, Aotus monkey (*Aotus vociferans*), nonhuman primate

History: This monkey was captured from the Nanay River region of Peru in August 1995, and imported to the National Institutes of Health, Bethesda, MD, where he was used in a malarial vaccine research protocol. He was euthanized in November, 1998 due to ascites and poor appetite.

Gross Pathology: The spleen was moderately enlarged. The kidneys contained miliary 0.5 to 2 mm diameter white foci in the subcapsular cortex. The testes were 1/3rd expected size.

Laboratory Results: Dif-Quik stained touch impressions of the spleen demonstrated myriad 5-10 micron yeast, with narrow based budding.

Contributor's Morphologic Diagnosis: 1. Lung: Interstitial pneumonia, diffuse, moderate, histiocytic, with myriad yeast
2. Bone marrow: Myelitis, histiocytic, with phagocytized yeast

Contributor's Comment: This monkey's yeast infection was initially diagnosed in June, 1997 by bone marrow biopsy (see photomicrograph for appearance of the yeast cells in Dif-Quik stained bone marrow aspirates). He remained in good health until August, 1998, when poor appetite was noted.

Similar infections have been previously encountered in wild caught Aotus monkeys at the National Institutes of Health, United States Army Medical Research Institute of Infectious Disease, and Walter Reed Army Institute of Research. The diagnosis is generally made at necropsy, and attempts to culture the yeast have failed.

Assuming the infection is acquired in the wild, the incubation period can be several years in length. Clinical signs can include weight loss and anemia. Gross findings may include hepatosplenomegaly and pale mottled kidneys. Histologically, large numbers of yeast can be found in all organs, with the exception of eyes and CNS. Yeast occur extracellularly as well as within macrophages and occasionally hepatocytes; other inflammatory infiltrates are notably absent. Yeast cells, which range from 8 to 10 microns, are spherical to oval with a thick slightly refractile wall and exhibit narrow based budding. Yeast cell walls stain selectively with PAS, GMS, and Gridley fungus stain but are mucicarmine negative. This organism differs from other recognized pathogenic yeast in its combined light microscopic appearance, organ involvement and host response.

Yeast morphology was studied using transmission electron microscopy. The cell wall is multilayered, and the internal structure is markedly heterogeneous. In some cells, the cytoplasm is lightly electron-opaque, finely granular and lacks recognizable organelles or nuclei. A few of these contained complex membranous figures of unknown function. In other cells, the cytoplasm is electron dense and contains mitochondria, ribosome-like granules, and a multilobulated nucleus.

The exact classification of the yeast is unclear, but evidence suggests that it is a close relative of *Histoplasma capsulatum*. Samples from previous cases have been submitted to the Mycotic Diseases Branch of the Centers for Disease Control and Prevention (Atlanta, GA). Immunohistochemistry was consistent with *H. capsulatum*; serology, however, failed to demonstrate antibodies to *H. capsulatum*. Unfixed liver was sent to a group at the University of California at Berkeley (Drs. John Taylor, Fred Harbinski, Takao Kasuga). 18S rRNA sequence is consistent with *H. capsulatum*. The DNA sequences of several protein coding genes most

closely aligned with those of South American *H. capsulatum var. capsulatum*. Paraffin embedded sections from several infected monkeys were sent to Dr. Elizabeth Keath, St. Louis University, for PCR analysis. The tissues were PCR positive for *H. capsulatum*, using primers believed to be specific for *H. capsulatum var. capsulatum*.

AFIP Diagnoses: Lung: Pneumonia, interstitial, histiocytic, diffuse, mild, with numerous yeast, etiology consistent with *Histoplasma capsulatum* var. *capsulatum*, South American variant, Aotus monkey (*Aotus vociferans*), nonhuman primate.

Conference Comment: Histologically this organism most closely resembles *Histoplasma capsulatum* var. *duboisii*. In contrast, a striking feature of infection with *Histoplasma capsulatum* var. *capsulatum* South American variant is the lack of any significant inflammation. This feature, in conjunction with the histomorphology, is helpful in differentiating it from *Histoplasma capsulatum* var. *capsulatum* (histiocytic and lymphocytic inflammation, smaller size), *Blastomyces dermatitidis* (pyogranulomatous inflammation, broad-based budding, larger size), and *Cryptococcus neoformans* (minimal cellular response, prominent carminophilic capsule).

In additional cases, repeated attempts to grow this organism have been unsuccessful as well. This too is a helpful diagnostic feature in that *H. capsulatum* var. *duboisii*, *B. dermatitidis*, and *C. neoformans* grow readily in culture.

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CASE III – MVP-007 (AFIP 2740380)

Signalment: 1-year-old, female, White-tailed deer (*Odocoileus virginianus*)

History: Transported from central Wisconsin to Ames, IA (approximately 500 miles) with 12 other deer. There were no obvious injuries or illness upon arrival. Two days after arrival she was depressed and unable to stand. Treatment consisted of IV fluids and antiinflammatories. After 2 days of treatment the

recumbency persisted and was accompanied by extreme rigidity of the appendicular muscles. She was euthanized 5 days after arrival.

Gross Pathology: Multifocal streaks of pallor and hemorrhage in muscles of both hindlimbs, both forelimbs, epaxial, and sublumbar muscles. Affected muscles were dry. Multifocal black discoloration of the left kidney extending through the cortex. Urine in the bladder was brown.

Laboratory Results: None

Contributor's Morphologic Diagnosis: Skeletal muscle: myositis, histiocytic with necrosis, multifocal, subacute, moderate with multifocal hemorrhage and mineralization.

Contributor's Comment: Necrotic myocytes are contracted containing coagulated to vacuolated or flocculent eosinophilic cytoplasm. There is loss of cross-striations, with nuclear pyknosis and in many cases, finely granular basophilic cytoplasmic mineralization. Necrotic myocytes are separated by low to moderate numbers of macrophages, edema, and hemorrhage, which in some sections is focally extensive.

Capture myopathy (exertional myopathy) is associated with physiologic imbalances that accompany extreme stress, struggling, or pursuit. It is characterized by damage to skeletal and cardiac muscle and is commonly observed after immobilization, capture, or transport of most wild vertebrates including birds. Clinical signs can occur after any episode of extreme muscular exertion and include reluctance to move, ataxia, and myoglobinuria. Animals may exhibit clinical signs immediately after the exertional episode or hours, days, or weeks later. All ages and sexes are susceptible. Warm environmental conditions predispose to the development of myopathy. Under normal conditions wild animals are not subjected to prolonged muscular activity of maximal effort. However, in situations of pursuit and capture by humans such conditions may exist.

The underlying pathophysiology of exertional myopathy is similar to shock, with death due to acidosis. Capillary congestion and subsequent hypoxia result in hypotension, visceral pooling, decreased venous return, and decreased cardiac output. Thrombosis of small veins exacerbates hypoxia. Anaerobic glycolysis results in severe acidosis. Hyperthermia and acidosis are felt to be critical elements in the pathogenesis of myopathy and is most severe in animals chased intensely. Treatment to correct metabolic acidosis has resulted in clinical improvement. Metabolic acidosis decreases cardiac output and blood pressure, and also results in cardiac fibrillation and death. Hyperthermia and increased lactate generated locally in muscles contributes to myocyte degeneration and necrosis. Initial changes associated with exertional myopathy take place in type 2 myofibers and resemble

those of nutritional myopathy. A history of exertion, capture or transport can help differentiate nutritional myopathy from exertional myopathy. Treatment of affected animals with vitamin E or selenium has not resulted in the amelioration of clinical signs.

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AFIP Diagnosis: Skeletal Muscle: Degeneration and necrosis, focally extensive, with mineralization, white-tailed deer (*Odocoileus virginianus*).

Conference Comment: Acquired myopathies generally fall under one of three categories: toxic (Gossypol, ionophore, and *Cassia* sp. toxicities), nutritional (vitamin E/selenium deficiencies), and exertional.

During times of muscular exertion, stored ATP and the energy derived from phosphocreatine metabolism allows for only 10 to 15 seconds of maximal muscular contraction. Under extreme or prolonged circumstances aerobic respiration cannot meet the necessary demands, ATP stores are depleted, and anaerobic glycolysis occurs.

Concurrently, during the stress of capture, a vasogenic-neurological shock often ensues.   This is secondary to prolonged sympathetic stimulation of the microvasculature and its eventual exhaustion. Decreased capillary tone results in visceral pooling, decreased venous return, and decreased cardiac output. Inadequate nutrient delivery, intracellular lactic acid buildup, and hypoxia exacerbate the metabolic derangements already present in the muscle due to anaerobic glycolysis.   Reduced sodium pump activity (secondary to ATP depletion) allows sodium influx and a concurrent isosmotic gain of water resulting in cell swelling. Calcium influx activates numerous enzymes including phospholipases, proteases, ATPases, and endonucleases. Decreased pH results in chromatin clumping and release of lysosomal enzymes. If the condition persists, or is severe enough initially, irreversible cellular injury occurs followed by cell death.

Skeletal muscle lesions can be described as monophasic or multiphasic. Monophasic lesions are all in the same stage of degeneration, regeneration, or necrosis and imply a single insult (ie. a single dose of a toxin or a strenuous episode). Multiphasic lesions are in multiple stages of degeneration, regeneration, or necrosis and imply an ongoing insult such as chronic toxin exposure or vitamin E/selenium deficiency.

Contributor: National Animal Disease Center, Ames, IA 50010

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CASE IV – 01-020 (AFIP 2788788)

Signalment: Mature, male, baboon (*Papio anubis*), nonhuman primate

History: The mass was found during a necropsy which followed an anesthetic death. No clinical signs could be attributed to the mass.

Gross Pathology: A large, round to oval (approximately 3.0 X 2.0 X 4.0 cm) mass involved the mid-left hemisphere of the cerebrum. On section the mass was homogenous gray-pink and contained two approximately 0.4 cm diameter hemorrhagic foci which were separated by approximately 2.0 cm. The mass slightly distorted the surface of the brain and compressed surrounding tissues.

Laboratory Results: None

Contributor's Morphologic Diagnosis: Glioblastoma multiforme, left cerebrum, *Papio anubis*.

Contributor's Comment: This is a vaguely circumscribed, highly cellular mass composed of pleomorphic cell types, scattered foci of necrosis and rudimentary vascular structures. The mass affects both white and gray matter. The principal neoplastic cell types have small, round to polygonal, hyperchromatic nuclei and poorly defined cytoplasm. Although in some locations, such as adjacent gyri which are minimally and maximally affected (consistent with secondary structures of Scherer), the predominant neoplastic cells have a spindle shape and cytoplasmic outlines are more evident. Scattered throughout the neoplasm are many multinucleated cells. Bizarre mitotic figures are also common. Foci of coagulative necrosis, sometimes rimmed by crudely palisaded cells, are common in deeper portions of the neoplasm. Portions of the periphery of the lesion are highlighted by poorly formed vascular structures composed of dense accumulations of endothelial cells. (Not all of these features are necessarily present in all slides).

Glioblastoma multiforme can develop spontaneously or occur as a complication of exposure to ionizing radiation in humans and nonhuman primates. This tumor is believed to arise from astrocytes although other glial lineages are possible. It is considered to be the highest grade of a malignant glioma (Grade IV).

The complex morphologic presentation of these tumors makes biopsy diagnosis difficult. The presence of coagulative necrosis is of singular importance in arriving at a diagnosis.

AFIP Diagnosis: Brain: High-grade astrocytoma (glioblastoma multiforme), Baboon (*Papio anubis*), nonhuman primate.

Conference Comment: The medical literature on humans defines four grades of astrocytic tumors while the World Health Organization International Histological Classification of Tumors of Domestic Animals defines three grades. According to the latter classification, low-grade (well-differentiated) astrocytomas are moderately cellular, well differentiated, have little cellular atypia, and have a low mitotic rate. Medium-grade (anaplastic) astrocytomas have prominent cellular atypia and increased mitotic activity. High-grade astrocytomas (glioblastoma multiforme) have anaplastic features consistent with medium grade astrocytomas and characteristic areas of necrosis and vascular proliferation. A distinctive feature of high-grade astrocytomas, although difficult to discern in these sections, is pseudopalisading of neoplastic cells around areas of necrosis.

In this case, areas of necrosis and vascular proliferation fulfill the criteria for the specific diagnosis of high-grade astrocytoma. Unfortunately, because of variation among sections, some slides lack these prerequisite features. In these slides, the diagnosis of medium-grade astrocytoma would be appropriate.

Immunohistochemically, astrocytic processes of neoplastic cells and gemistocytic (reactive) astrocytes expressed positivity for glial fibrillary acidic protein (GFAP). Interestingly, high-grade astrocytomas and gemistocytic astrocytes are immunoreactive for cytokeratin as well.

Contributor: University of Washington, Department of Comparative Medicine, School of Medicine, Seattle, WA 98195

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