The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
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CONFERENCE 4
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Washington, DC 20306-6000

CASE I – 02-0171 (AFIP 2839286)

Signalment: Adult male Sprague-Dawley rat (*Rattus norvegicus*)

History: This animal is one of a group that was treated with a seizure inducing dose of Soman (pinacolyl methylphosphonofluoridate). Euthanasia, perfusion and necropsy were performed 24 hours after exposure.

Gross Pathology: None reported.

Laboratory Results: None.

Contributor's Morphologic Diagnoses: 1. Brain: Neuronal necrosis and degeneration, diffuse, severe.
2. Heart: Necrosis and degeneration, multifocal, moderate, with neutrophilic and histiocytic myocarditis (neurogenic cardiomyopathy).

Contributor's Comment: Soman (pinacolyl methylphosphonofluoridate) is an organophosphorous nerve agent that exerts its effects by inhibiting the enzyme cholinesterase. This inhibition results in a rapid accumulation of acetylcholine and hyperstimulation of both central and peripheral cholinergic sites. Clinically organophosphate toxicity results in lacrimation, salivation, apnea, bronchoconstriction, miosis, muscle fasciculations, and seizures. Acute death is generally the result of respiratory failure, but animals surviving the acute effects may develop neural and cardiac lesions.

Neural lesions in rats 24 hours post-exposure are characterized by multifocal vacuolation of the neuropil in the neocortex, sector CA3 of the hippocampus and the piriform cortex; neuronal necrosis and loss; liquefactive necrosis; gliosis and early neovascularization. Three possible causes of neural lesions have been suggested: direct neurotoxicity by organophosphates, prolonged elevated acetylcholine leading to accumulation of excitatory amino acids (glutamate), and hypoxia induced by prolonged seizures. Though multiple factors are likely involved, the theory of hypoxic neuronal

necrosis is strongly supported by the facts that prolonged seizure activity is required to produce the neural lesions and controlling seizure activity virtually eliminates brain lesions.

Neuronal necrosis is often difficult to discern. This slide demonstrates both artifactually hypereosinophilic neurons and truly necrotic neurons. The following table is a useful guide for discerning artifact and necrosis.

	NECROSIS	ARTIFACT
NEUROPIL	**VACUOLATED, +/- EDEMA AND HEMORRHAGE**	NORMAL
CELL PROCESSES	ABSENT OR FRAGMENTED	**SWOLLEN**
CYTOPLASM	PERIPHERAL VACUOLATION, HYPEREOSINOPHILIC-PINK	HYPEREOSINOPHILIC
NUCLEUS	ENLARGED, KARYOLYSIS, LOSS OF NUCLEOLI	SMUDGY NUCLEI, DARK NUCLEOLI
DISTRIBUTION	SYMMETRIC +/-, SPECIFIC LOCATIONS	ALONG SURFACE, NEAR CUTS IN SECTION

(also: Virtually every section has an internal control of artifactually red neurons, if you can only find what you think are necrotic neurons and no artifact, you are probably **only** seeing artifact.)

Myocardial lesions 24 hours post-exposure are characterized by multifocal degeneration and necrosis, edema, hemorrhage, and neutrophilic and histiocytic infiltrates. These lesions generally affect the left ventricular free wall and papillary muscles and the interventricular septum. The right ventricle and atria are rarely affected.

The mechanism of action for the myocardial necrosis is also highly debated. The two most prominent theories are: excessive catecholamine release and seizure induced hypoxia. Once again, both features may be important but, histologically similar lesions attributed to catecholamine release are reported with multiple etiologies, including: cocaine toxicity; pheochromocytomas; and increased intracranial pressure caused by head trauma, infection, subdural hemorrhage, cerebral edema, etc. Catecholamine induced necrosis has been attributed to both calcium overload within myocytes and myocardial vasoconstriction.

AFIP Diagnoses: 1. Brain, cerebral cortex: Neuronal necrosis, extensive, bilateral, with minimal neutrophilic encephalitis, Sprague-Dawley, (*Rattus norvegicus*), rodent. 2. Heart, left ventricle: Myocardial degeneration and necrosis, multifocal, moderate, with neutrophilic and histiocytic myocarditis.

Conference Comment: Conference participants discussed the differential diagnosis for neuronal necrosis (including intoxications, hypoglycemia, hypoxia, and anoxia); the importance of anatomical location of lesions in both brain and heart (recognizing the presence or absence of symmetry in brain lesions); and the type of inflammatory cell infiltration in the temporal staging of brain lesions. The contributor has provided a concise summary of the lesions associated with soman intoxication. Acetylcholine is an important neurotransmitter of both the sympathetic and parasympathetic autonomic nervous system. When released from the presynaptic membrane in the sympathetic nervous system, it stimulates either muscarinic or nicotinic postsynaptic receptors. When released in the parasympathetic nervous system, it acts at the somatic

neuromuscular synapse and autonomic ganglia. The enzyme acetylcholinesterase destroys acetylcholine in the postsynaptic cleft, which prevents hyperstimulation.

Contributor: U.S. Army Medical Institute of Chemical Defense, 3100 Ricketts Point Rd., Aberdeen Proving Ground, MD 21010-5400

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CASE II - 02-2546 (AFIP 2839321)

Signalment: Two-week-old, male, Nubian goat.

History: The kid developed severe dyspnea 24 hrs prior to examination. Euthanasia was performed. Formalin fixed heart, liver and lung was submitted from a field necropsy performed by the referring veterinarian.

Gross Pathology: The referring veterinarian reported finding hepatomegaly and ascites. No gross lesions of the lung were reported.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Pneumonia, granulomatous, multifocal to coalescing, with spherules of *Coccidioides immitis*.

Contributor's Comment: The lungs contained multifocal to confluent granulomas centered on spherules of *Coccidioides immitis*. The liver had diffuse centrolobular necrosis consistent with anoxic change. There was mild, multifocal myocardial fibrosis in the heart but no granulomas or spherules were seen.

Coccidioidomycosis is a deep fungal infection caused by the dimorphic soil fungus, *Coccidioides immitis*. Infection occurs by inhalation of arthrospores from the environment followed by the establishment of a pulmonary infection. The fungus develops in the lungs as a spherule, a spherical structure with a refractile wall within which develops numerous endospores. The spherules rupture releasing endospores into the adjoining tissue, spreading the infection. The infection is common in humans and dogs. The infection may be self-limiting and restricted to the lung, or spread hematogenously to lymph nodes, bone, liver, kidney, spleen, heart and brain. Dissemination is fairly common in the dog. Infection in other species is uncommon, except for some exotics such as non-human primates and llamas. Cats, horses and various exotics are susceptible but clinical disease is not common. Infection in the bovine and ovine is asymptomatic and restricted to the bronchial lymph nodes or small pulmonary granulomas.

Infection of the neonate by *Coccidioides immitis* is rarely reported but has been documented in humans and the equine. The infection may be obtained in the early post-partum period by inhalation of arthrospores from the environment. This is usually the case in neonates diagnosed after two weeks of age since the incubation period of the infection is considered to be approximately 10 to 16 days. Cases of in-utero infection have been documented. In humans, in-utero infection is typically associated with disseminated infection of the mother. Lesions in the fetus include granulomatous placentitis and disseminated lesions in multiple organs. In a report of abortion due to *Coccidioides immitis* in an Arabian mare, the mare was reported to be in good health. The fetus had a granulomatous placentitis and pneumonia with spherules. This caprine neonate was reported to be about two weeks of age. The infection could have been established post-partum. However, the presence of chronic changes such as cardiac fibrosis and ascites suggest a longer disease course consistent with an in-utero infection.

AFIP Diagnosis: Lung: Pneumonia, pyogranulomatous, diffuse, severe, with numerous fungal spherules, etiology consistent with *Coccidiodes immitis*, Nubian goat, caprine.

Conference Comment: *Coccidioides immitis* can be diagnosed on routine hematoxylin and eosin (H&E) stained sections based on spherule size (10-80 um), the double contoured birefringent wall, and the presence of 2-5 um in diameter endospores. Periodic Acid-Schiff (PAS) staining may demonstrate the internal structures more distinctly than H&E and should be included in the routine histological workup of pyogranulomatous inflammation. Endospore walls and the granular contents of small spherules are PAS-positive. *C. immitis* is a soil inhabitant in the southwestern United States, Mexico, Central America, Argentina, Paraguay, and the northern and western states of South America.

Contributor: Arizona Veterinary Diagnostic Laboratory, 2831 N. Freeway, Tucson, AZ 85705

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CASE III –Abbott/Abbott-01 (AFIP 2595748)

Signalment: 14-month-old male Crl:CD(SD)BR rat (*Rattus norvegicus*)

History: This rat was a control animal on a 2-year carcinogenicity study. He became pale, stopped eating, and lost weight for several weeks before he was euthanized and necropsied.

Gross Pathology: The thymus was replaced by a tan, irregular, solid, mass that filled much of the thoracic cavity. Tan tissue partially lined the ventral subpleural surface of the spinal column. The mesenteric lymph nodes were enlarged, and the wall of the stomach was focally thickened.

Laboratory Results: None.

Contributor's Morphologic Diagnoses: 1. Histiocytic sarcoma, multifocal, kidney, rat.
2. Hyaline droplets, multifocal, marked, proximal tubules, kidney, rat.

Contributor's Comment: Histiocytic sarcoma of the rat is a multicentric lesion commonly affecting the liver, spleen, skin, lung, or female reproductive tract. Many other organs may be involved in advanced cases. The cell of origin has not been definitively identified, however, evidence suggests that this entity arises from macrophages and is not of lymphoid origin. Hyaline droplet accumulation in the proximal renal tubules commonly accompanies histiocytic sarcoma in the rat. In this rat, the thymus, vertebral column, lung, thoracic and mesenteric lymph nodes, stomach, and pancreas were partially or completely replaced by histiocytic sarcoma.

AFIP Diagnoses: 1. Kidney: Histiocytic sarcoma, intravascular and interstitial, Crl:CD(SD)BR rat, rodent.
2. Kidney, proximal tubule epithelium: Hyaline droplets, diffuse, marked.
3. Kidney: Glomerulosclerosis, multifocal, segmental, moderate, with chronic interstitial nephritis, tubular ectasia and protein casts (chronic progressive nephropathy).

Conference Comment: Older Sprague-Dawley rats are predisposed to development of histiocytic sarcoma, which also occurs in Osborne-Mendel and Wistar strains. In the rat, there is often a prominent increased fibroblastic component accompanying the neoplastic cells. This neoplasm is also common in many strains of mice, including B6 and SJL strains. By immunohistochemistry, rat histiocytic sarcoma cells are positive for vimentin, ED1 (rat monocyte/macrophage-specific antibody), alpha 1-antitrypsin, and lysozyme. The differential diagnosis may include malignant lymphoma, malignant fibrous histiocytoma, fibrosarcoma, granulomatous inflammation and osteosarcoma. Sections vary in the extent and distribution of the histiocytic sarcoma within the kidney, and in the extent and severity of the chronic progressive nephropathy lesions.

Contributor: Abbott Laboratories, D-469 AP13A, 100 Abbott Park Road, Abbott Park, IL 60064-3500

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CASE IV - N01-347 2 (AFIP 2834685)

Signalment: 21-year-old, adult, female, Killer Whale, *Orcinus orca*

History: This captive killer whale had a fairly uneventful medical history. The month before she died, she had received three lacerations across the end of the rostrum from another killer whale. These lesions healed normally and uneventfully. Her appetite and behavior remained normal during that entire period. A sudden change of behavior was noted by the animal handlers on the day of death. The killer whale was slow to respond to trainers and to eat, although her total food consumption was normal. In response to negative changes in her clinical laboratory parameters, she was started on antibiotics. Her condition appeared improved later in the day but took a sudden turn for the worse. She died later that evening.

Gross Pathology: The caudal-dorsal surface of the right cerebral hemisphere is hemorrhagic. An 8 to 10 cm in diameter area of hemorrhage with malacia is apparent in the cortex of the right caudal-dorsal cerebral hemisphere. A 5 cm blood clot is observed in the central portion of the lesion. The surface of the left cerebral hemisphere appears slightly hyperemic. The cerebellum appears grossly normal. There is a single small, approximately 2.5 cm in diameter, firm, red, slightly-raised subpleural nodule in the right anterio-ventral lung.

Laboratory Results: Pending. Samples were submitted to the Centers for Disease Control laboratory for speciation of the fungus.

Contributor's Morphologic Diagnoses: 1. Brain, cerebrum: Necrosis and hemorrhage, multifocal, acute, severe, with necrotizing vasculitis and fungal hyphae, Killer whale, *Orcinus orca,* cetacean.
2. Lung (not submitted): Pneumonia, necro-hemorrhagic, focal, chronic, severe, with necrotizing vasculitis and fungal hyphae.

Contributor's Comment: Based on gross and microscopic findings, the cause of death in this animal was determined to be severe necrosis and hemorrhage of the cerebrum caused by a systemic fungal infection. The likely pathogenesis of the observed lesions was a primary fungal pneumonia followed by vascular invasion and hematogenous spread to the brain. The pathogenic fungus could not be specifically identified histologically but is morphologically consistent with a zygomycete-type fungus. A literature search failed to reveal a single reported case of fungal encephalitis in killer whales, however, discussions with cetacean experts revealed that there have been at least eight known cases of fungal encephalitis in killer whales.

AFIP Diagnosis: Brain, cerebrum: Vasculitis, necrotizing, acute, multifocal, moderate, with necrosuppurative encephalitis and fungal hyphae, etiology consistent with class Zygomycetes, Killer whale (*Orcinus orca*), cetacean.

Conference Comment: Fungi of the class Zygomycetes are noncontagious, saprophytic, opportunistic pathogens which gain entry via inhalation or wound contamination and include two orders: Mucorales and Entomophthorales. In tissue section, Zygomycetes hyphae are broad, up to 10 um; infrequently septate; nonparallel and thin walled, with nondichotomous branching. These fungi are best demonstrated

with H&E, Grocott's methenamine silver (GMS), and PAS staining. The differential diagnosis includes *Aspergillus sp.* and *Pythium sp.*

Contributor: Air Force Research Lab/HEDV, 2509 Kennedy Circle, Brooks Air Force Base, TX 78235

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