

**The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2003-2004**

**CONFERENCE 1
3 September 2003**

Conference Moderator: COL Dale Dunn, DVM, Diplomate ACVP
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Armed Forces Institute of Pathology
Washington, DC 20306

CASE I – 02RD1062 (AFIP 2888047)

Signalment: Ten-year-old neutered male Australian Shepherd dog (*Canis domesticus*).

History: One month prior to presentation, the owners noticed a reddened cornea in the right eye which later spread to the surrounding portions of the eye. Yellow discharge was also noted from the right eye, and one morning a few days before presentation the owners found the dog with both eyes “crusted shut”. The owner eased the eyes open after the use of a warm compress. The owners also reported that the dog would rub at the right eye, and appeared to have no vision in that eye. At presentation, no menace response was invoked in the right eye. Intraocular pressure in the right eye was recorded as 15 mmHg. The clinician noted buphthalmia, conjunctivitis, and an iris neoplasm filling the anterior chamber of the right eye. Enucleation of the right eye was then elected by the owner.

Gross Pathology: Dyscoria, wrinkled cornea, raised white tissue adherent to iris and obscuring pupil.

Laboratory Results: The iris morphology is distorted and thickened due to the presence of a spindle cell neoplasm extending from the iris base a short distance into the ciliary body and obliterating most of the iris stroma. There is broad posterior synechiae. The retina and optic nerves suggest chronic glaucoma. On one side of the globe the tissue within the tumor, the ciliary body, and the choroid are pigmented. On the other side of the globe the tumor, the ciliary body, and the choroid are nonpigmented.

Contributor’s Morphologic Diagnoses:

1. Partially amelanotic globe
2. Spindle cell iridal tumor of blue-eyed dogs
3. Secondary glaucoma

Contributor's Comment: This dog has a pleomorphic spindle cell tumor effacing much of the iris and extending into the ciliary body. The tumor varies considerably in morphology with areas that have elongate spindle cells and other areas with more myxomatous features. Karyomegalic cells and multinucleate cells are easily found. Neoplastic cells infiltrate well into the ciliary body. This is one of the more malignant appearing of these iridal spindle cell tumors that we have evaluated.

Careful evaluation of the pigmentation of this eye reveals that there is fairly abundant melanin pigment in the portion of the iris with the least tumor invasion, and, likewise, there is a normally pigmented choroid on the same side as the pigmented iris. The tumor itself has almost no areas of trapped melanin pigment and the choroid on the side of the eye with more tumor is devoid of melanin. This tumor almost always occurs in blue or partly blue eyes. For that reason it is most commonly found in Husky dogs and other breeds which have heterochromia, such as the Australian Shepherd ^{1,2}.

Melanomas and melanocytomas will occasionally present as spindle cell tumors. Other spindle cell tumors of the iris are extremely rare. These tumors stain positive for S100 about 50% of the time. They all stain positive for vimentin, and about 50% stain positive for GFAP. Occasional tumors have morphologic features which suggest a peripheral nerve origin such as Antoni A and Antoni B patterns, and one of two cases in which electron microscopy was performed had broadly deposited basal lamina at the cell membrane. The inconsistent S100 staining is difficult to interpret if this is, indeed, a tumor of peripheral nerve origin.

AFIP Diagnosis: Eye, iris: Spindle cell neoplasm, favor peripheral nerve sheath origin, Australian Shepherd, canine.

Conference Comment: Spindle cell tumor in blue-eyed dogs is a recently described neoplasm that occurs in dogs with poorly pigmented uveal tissue. The cell of origin has not been determined, but based on immunohistochemical stains and cellular morphology, peripheral nerve sheath origin is suggested. Metastatic spread has not been described.^{3,4}

This tumor is vimentin positive, GFAP positive, and S-100 negative. Conference attendees favored melanoma but did not have the benefit of the immunohistochemistry stains prior to the conference. The GFAP positivity eliminates melanoma as a possibility and supports the diagnosis of spindle cell tumor in blue-eyed dogs, as described by the contributor. Also, the nuclear regimentation and occasional herringbone pattern support a tumor of peripheral nerve sheath origin.

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CASE II - Seal-1 (AFIP 2888451)

Signalment: Adult male Harbor seal, *Phoca vitulina*.

History: A male 18.9 kg Harbor seal was found stranded at Barnegat Lighthouse State Park in New Jersey. At time of recovery the animal had labored breathing and what appeared to be blood on the abdomen. The animal was treated at the local marine mammal stranding center with 500 cc electrolyte solution, vitamins, levamisole, and Baytril. The animal was inactive and shivering. It died approximately 7 hours later and was submitted for necropsy.

Gross Pathology: At necropsy, the urinary bladder was severely distended with a translucent wall. It contained red, watery fluid with a strong odor of ammonia. The mucosa was red with a cobblestone appearance. The ureters were distended measuring 4 mm in diameter bilaterally, but both kidneys were grossly normal. The periurethral soft tissue was swollen and multinodular and contained a central 1 cm cavitation with a firm, green-black wall. It oozed a yellow opaque fluid when cut. Numerous crystals were present around the preputial opening but the urethra remained unobstructed.

A large nematode was found adjacent to the rectum in the retroperitoneal space. Other necropsy findings include numerous lice on the dorsal skin and gingival ulcers. On the surface of the lung, dilated lymphatic vessels are seen.

Laboratory Results: Aerobic bacterial culture of the fluid in the cavitation was negative.

Contributor's Morphologic Diagnoses:

1. Severe multifocal subacute to chronic granulomatous cellulitis with numerous intralesional nematode ova, periurethral soft tissue, Harbor seal.

2. Severe diffuse necrotizing cystitis with vascular fibrinoid necrosis and superficial bacterial colonization, urinary bladder, Harbor seal.

Contributor's Comment: The recovered nematode was female, brownish-red, and measured 22.3 cm long by 4.5 mm wide. The worm was alive when recovered (approximately 1 day after the death of the seal). The anus was terminal and the mouth was surrounded by 2 circles of 6 papillae. This led to its identification as *Dioctophyma renale*. Eggs seen in histological sections had a characteristic thick mammilated shell and were consistent with this identification. Ten eggs recovered from formalin were measured with an ocular micrometer, with a mean size of 41.3 ± 2.3 μ m wide by 68.9 ± 3.7 μ m long. Parasite identification was made by Dr. Thomas J. Nolan from the laboratory of Parasitology, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania.

Dioctophyma renale, the “giant kidney worm”, is one of the largest species of nematodes. The usual definitive host for *D. renale* is the mink. The adult worms are normally found in the right kidney. This is thought to occur when the larvae migrate through the stomach wall (or duodenum) and liver. They are blood-red, and females can measure up to one meter long and one centimeter in diameter. Males are somewhat smaller (less than 40 cm). Eggs are brownish and thick-walled, measuring 68 by 44 μ m. The eggs are passed out in the urine in the one- or two-cell stage and mature, in water, to the first larval stage in approximately 35 days. They are then infectious to *Lumbriculus variegates*, a fresh-water oligochaete annelid worm, and develop into the infective third larval stage. Fish or frogs that ingest the infected oligochaete may act as paratenic hosts. Life cycle of *D. renale* is completed when the infected oligochaete or paratenic host is ingested by a final host¹.

The giant kidney worms cause a widely distributed parasitic infection in mustelids, canids (mostly fish-eating carnivores), swine, and occasionally man. In human beings, *D. renale* infection has been reported to manifest as a hemorrhagic cyst at one pole of the right kidney, mimicking a retroperitoneal neoplasm². Liesegang rings (LRs) are periodic precipitation zones from supersaturated solutions in colloidal systems. In histologic sections, LRs are eosinophilic, acellular, laminated, sharply outlined and sometimes have a central nidus of pyknotic debris. They occur in the kidney, synovium, conjunctiva and eyelid of men and can be sometimes mistaken for eggs, larvae or adults of *D. renale*³.

AFIP Diagnoses:

1. Urinary bladder: Cystitis, necrotizing, fibrinosuppurative, diffuse, severe, with fibrinoid necrosis, and mixed population of bacteria, harbor seal (*Phoca vitulina*), pinniped.
2. Fibromuscular tissue: Cellulitis, pyogranulomatous, diffuse, moderate, with numerous mammilated nematode eggs.

Conference Comment: According to the literature, this is the first case where *Dioctophyma renale* has been definitively identified in a Harbor seal. *D. renale* was previously described in a Caspian seal (*Phoca caspica*).⁴ The case presented in the conference is especially interesting because the life cycle of *D. renale* is associated with fresh water fish as paratenic hosts. Although the exact history of this particular free-living seal is unknown, access to fresh water fish would have been necessary for this animal to become infected, unless the infective third stage larvae may now use salt or brackish water fish as paratenic hosts.

D. renale has a wide host range, including mink, weasels, river otters, coyote, wolves, fox, domestic dogs, raccoons, and occasionally cattle, horses, and swine.⁴

Conference attendees had difficulty in identifying the two tissues. Identification of urinary bladder was made based on the appearance of the outer musculature in a thin-walled tubular organ. Although the exact morphology of the inflammatory cells was difficult to discern, it is likely they are neutrophils based on the presence of abundant fibrin and, therefore, an acute process. Within the section of fibromuscular tissue, the presence of more loosely arranged, immature granulation tissue towards the central cavitory lesion and progression to more mature granulation tissue at the periphery helped to elucidate the pathogenesis of this lesion.

This case was reviewed in consultation with Dr. C. H. Gardiner, Parasitologist.

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CASE III – HN 1825 (AFIP 2892546)

Signalment: 10-year-old, male Miniature Schnauzer, canine.

History: This dog had neurological signs including torticollis, circling, mental confusion, convulsive seizures, and unconsciousness for about a month. The dog showed severe hypoglycemia and infusions of glucose were performed. The dog died 3 days after the onset of treatment.

Gross Pathology: Autopsy was performed seven hours post-mortem and a mass was found in the left lobe of pancreas. The mass was white and 2×1×1 cm in size. The surface of the mass was slightly granular and showed lobular patterns on cut surface. Small whitish nodules were also observed in liver and heart. The cortex of the cerebrum was mildly clouded on cut surface.

Laboratory Results: Serological exam revealed that this dog showed persistent hypoglycemia of 15-42 mg/dl. In contrast, insulin concentrations were at conspicuously high levels at 100 mIU/l (reference 10-25 mIU/l).

Contributor's Morphologic Diagnoses:

1. Pancreas: Islet cell carcinoma, Miniature Schnauzer, canine
2. Cerebrum: Cortical necrosis, severe, Miniature Schnauzer, canine

Contributor's Comment: The neoplastic cells observed in the pancreas showed invasive growth into the pancreatic parenchyma and through the fibrous capsule. The closely packed neoplastic cells were sometimes subdivided into small lobules by fine connective tissue. The individual neoplastic cells were well-differentiated and mitotic figures were seen infrequently. Histologically, the whitish nodules grossly observed in the liver and heart were metastases of the pancreatic neoplastic cells.

A number of intrinsic or acquired disorders in animals produce transitory or prolonged hypoglycemia¹, for example, hypoadrenocorticism (Addison's disease), severe hepatic or renal disease, and constitutional hypoglycemia of pups. Pancreatic beta-cell tumors are also one of the important causes of canine hypoglycemia. To show that primary and metastatic tumor cells are beta-cell origin, Gomori method, immunohistochemical reactions for insulin and ultrastructural evidence of secretory granules are useful. By immunohistochemical examination performed in our laboratory, the tumor cells stained positively for anti-insulin antibody. So, the neoplastic cells originated from insulin secreting beta-cells. The clinical evidence of: 1) severe neurological signs, 2) decline of blood glucose concentrations, and 3) reaction to administration of glucose also suggests that the tumor cells are functional². Later in the disease, however, animals may become unresponsive to supplemental glucose therapy.

Neurological signs in this dog were induced by prolonged hypoglycemia, and hyperinsulinism. In the cerebrum, ischemic nerve cells with eosinophilic shrunken cytoplasm and pyknotic or lytic nucleus were found in the superficial layer of the cortex. Microglial cells with elongated nuclei (rod cells), enlarged astrocytic nuclei with dark nuclear membrane, and enlarged nuclei of vascular endothelium were also observed. Ischemic changes associated with hypoglycemia of dogs is previously reported³, and

the pathological findings in those dogs were consistent with the present case. Therefore, we diagnosed this dog with an insulin-secreting islet cell carcinoma accompanied by secondary cortical necrosis of the cerebrum.

The mechanism of neuronal damage induced by hypoglycemia is not fully understood. The distribution of hypoglycemic brain damage was discussed in insulin-induced hypoglycemic model of rats⁴, and the existence of neurotoxic substance (excitotoxin) has been suspected⁵. Excitotoxic neuronal death with DNA fragmentation and activation of immediate early gene expression is thought to contribute to neuropathogenic conditions in hypoglycemia⁶.

AFIP Diagnoses:

1. Pancreas: Islet cell carcinoma, Miniature Schnauzer, canine.
2. Cerebral cortex: Neuronal necrosis, superficial, laminar, multifocal.

Conference Comment: Neuronal necrosis has been described in hypoglycemia associated with the biochemical disturbances related to other conditions, specifically pregnancy toxemia in ewes⁷, and is similar to the neuronal necrosis described in a dog with an insulinoma⁸ and in the case presented here. The pattern of neuronal necrosis related to hypoglycemia is confined to the superficial laminae of the cerebral cortex. Ischemia also causes similar lesions of neuronal necrosis but, in contrast, it affects the middle laminar cortex.⁹

The mechanisms of cell death in hypoglycemia and ischemia have many parallels. Both ischemic and hypoglycemic cell death are caused by the release of excitotoxins that bind to neuronal surface receptors and cause necrosis. One difference is that the predominant excitotoxin responsible for hypoglycemia-induced necrosis is aspartate and for ischemic-induced necrosis is glutamate. Another basic difference between these two causes of necrosis is that ischemia results in a pan-necrosis (necrosis of neurons, glial cells, and vessels) due to lack of blood supply, whereas hypoglycemia only causes selective death of neurons because blood supply is not compromised.^{9, 10}

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CASE IV - 4188 (AFIP 2852792)

Signalment: Juvenile female varying hare (*Lepus timidus*).

History: The hare was found dead.

Gross Pathology: Normal body condition. A few subcutaneous hemorrhages. Slightly enlarged spleen. Multifocal tiny grayish-white foci on the liver. Numerous intestinal *Cittotaenia* sp.

Laboratory Results: *Francisella tularensis* was isolated from the bone marrow.

Contributor's Morphologic Diagnosis: Liver: Hepatitis, necrotizing, acute, multifocal.

Contributor's Comment: This represents a typical case of tularemia in hares. The disease in this species is an acute fatal septicemia. Gross findings include moderately enlarged spleen and numerous pinpoint, pale foci in the liver, spleen, and bone marrow. Often there are also subcutaneous hemorrhages. Histologically, acute multifocal complete necrosis is seen in the liver, spleen, and bone marrow. Infrequently, a hemorrhagic necrotic enterocolitis has been detected.

Francisella tularensis is a gram-negative, pleomorphic, nonmotile coccobacillus. It is classified into three biovars: *F. tularensis tularensis* (type A), *F. tularensis palearctica* (type B), and *F. tularensis mediaasiatica*. Biovar *palearctica* is divided into three biotypes: *I*, *II*, and *japonica*. Biovar *tularensis* is the most pathogenic and occurs in North America. It has recently been isolated also from Central Europe. Other biovars are less virulent. Biovar *palearctica* (holoarctica) occurs in Eurasia and, to a lesser extent, in North America. Biovar *mediaasiatica* occurs in Central Asia and biovar

japonica in Japan. The other species, *F. philomiragia* and *F. novicida*, are of unknown pathogenicity.

Tularemia occurs all over the Northern hemisphere, in Scandinavia, Central Europe, Russia, Japan, China, the USA, and Canada. The natural hosts of the disease are wild rodents and lagomorphs. Ticks are considered to be important biological vectors of the disease and can also transmit the bacteria vertically to their ova. Other arthropods such as mosquitoes, flies, fleas, and lice can transmit the disease. The bacteria are quite resistant and can survive in cold environments, in water, soil, and cadavers for several months.

Over 250 animal species can be infected, including man. The severity of the disease varies from fatal septicemia to asymptomatic infection. Rodents and lagomorphs are the most susceptible and carnivores are usually resistant. Among domestic animals, severe infections have been described in sheep, foals, fur animals, and cats.

The disease in man can manifest in several different forms: ulceroglandular (the most common form), glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal. Also meningitis has been described. Infection is acquired through skin wounds or mucous membranes when handling sick animals, from dust or soil contaminated with rodent feces, from blood sucking insects or tick bites, or from contaminated drinking water or food.

In Finland epizootics of *F. tularensis* occur among wild hares (varying hares (*Lepus timidus*) and European brown hares (*Lepus europaeus*)). Only very few cases have been seen in muskrats (*Ondatra zibethicus*) or beavers (*Castor canadensis*, *Castor fiber*). The annual number of cases in humans as well as animals varies. The disease in man is most common in the late summer and autumn. The annual incidence in humans is 1-2/100,000 population and most of the cases are mild. Most human outbreaks have been associated with mosquito bites (ulceroglandular form) or with hay or soil dust (pneumonic form).

AFIP Diagnosis: Liver: Necrosis, random, multifocal, varying hare (*Lepus timidus*), lagomorph.

Conference Comment: The contributor gives a thorough overview of this highly infectious, zoonotic disease. The organism enters via percutaneous inoculation by arthropods, penetration of skin or mucous membranes, ingestion, or inhalation. Organisms are phagocytized by macrophages and disseminate via the lymphatics, and can invade and damage vascular endothelium causing vasculitis and thrombosis. The bacteria cause inflammation and multifocal necrosis in the liver, spleen, kidneys, lungs, and lymph nodes.⁴

Along with *F. tularensis*, the differential diagnosis for hepatic necrosis includes *Clostridium piliforme* (Tyzzer's disease), *Salmonella* sp., *Yersinia* sp., *Toxoplasma gondii*, and *Listeria monocytogenes*. The following may be used to differentiate these from tularemia: *C. piliforme* is present within the cytoplasm of hepatocytes and will stain positively with silver stains; Salmonellosis causes paratyphoid nodules in the liver, characterized by individualized necrotic hepatocytes surrounded by inflammatory cells; *Yersinia* sp. produce large, lobulated colonies of bacteria in the liver; intralésional tachyzoites are observed with toxoplasmosis; and *Listeria monocytogenes* is a gram positive coccobacillus.

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