

**The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2002-2003**

**CONFERENCE 23
23 April 2003**

Conference Moderator: Dr. Donald Nichols, Diplomate ACVP
Department of Pathology
National Zoological Park
Smithsonian Institution
Washington, DC 20008

CASE I - No.1 NIAH (AFIP 2840314)

Signalment: One-week-old, male, specific-pathogen-free White Leghorn chickens.

History: There are no significant clinical signs in the chickens inoculated with virus. The SPF chickens were inoculated orally with chicken kidney cell culture of 10^7 plaque-forming unit (PFU) of serotype 1 group I avian adenovirus (GIAAV). The chickens were killed and necropsied 5 days after inoculation

Gross Pathology: Macroscopically, the gizzard was dilated with liquefied contents. The koilin layer (keratinoid layer) of the gizzard contained multifocal white lesions with occasional erosions.

Laboratory Results: Histologically there was liquefactive degeneration of the koilin layer with heterophilic infiltration, degeneration and depletion of the gizzard glandular epithelium with basophilic intranuclear inclusion bodies, and infiltration with macrophages and some heterophils in the lamina propria. The intranuclear inclusions of the gizzard glandular epithelial cells showed positive reactions against GIAAV antigens by immunoperoxidase staining. The intranuclear inclusion bodies of the degenerative glandular cells consisted of numerous viral particles (averaging 72 nm in diameter, from 63 to 88 nm). Large aggregates of hexagonal virus particles were observed, varying from immature (particles with electron-lucent cores) to mature types (particles with electron-dense cores). Electron-dense amorphous substance was noted concurrently with the viral particles. There was a crystalline arrangement of virus particles in the nuclei.

Contributor's Morphologic Diagnosis: Gizzard: Ventriculitis, inclusion body, multifocal, severe, with liquefaction of koilin layer, chicken, etiology: adenovirus.

Contributor's Comment: Avian adenoviruses are divided into three groups, which are often referred to as subgroups in the literature. Group I includes the viruses of inclusion body hepatitis, hydropericardium syndrome, and gizzard erosion as well as necrotizing pancreatitis in layer chicks. Group II includes the viruses of turkey hemorrhagic enteritis and marble spleen disease in pheasants. The only member of group III is egg drop syndrome virus, occurring in chickens, and first described in 1976.

The group 1 avian adenovirus isolate from layers was serotype 8. Adenoviral gizzard erosions have recently been seen in broilers at processing plants in Japan. Gizzard erosions with hemorrhages were detected in the growing broilers on the farm. The virus isolates from gizzard erosion in broilers were serotyped to serotype 1. Gizzard erosion due to fishmeal was prevalent in broiler chicks prior to 20 years ago. We have not encountered gizzard erosion in broiler chickens, since diets are no longer supplemented with fishmeal.

AFIP Diagnoses: 1. Ventriculus: Ventriculitis, necrotizing, heterophilic and lymphohistiocytic, multifocal, moderate, with koilin degeneration, and intraepithelial intranuclear inclusion bodies, etiology consistent with adenovirus, White Leghorn chicken, avian.

2. Proventriculus and duodenum: No significant lesions.

Conference Comment: The family Adenoviridae includes two genera, Aviadenovirus and Mastadenovirus (important mammalian pathogens - canine adenovirus type 1 and 2, equine adenovirus, adenovirus of deer). Aviadenoviruses may be either primary or opportunistic pathogens. Two new adenovirus genera (Siadenovirus, Atadenovirus) have been proposed, which correspond to the current groups II and III of avian adenoviruses. Adenovirus virions are nonenveloped, icosahedral, 70-90 um in diameter; the genome is double stranded DNA. Ultrastructurally, adenoviral infection is characterized by crystalline arrays of virions within host cell nuclei.

Koilin is a carbohydrate-protein complex that is secreted by the glandular epithelium of the ventriculus and hardens on the luminal surface into a dense layer when contacted by the hydrochloric acid secreted by the proventriculus. In this case, the proposed pathogenesis involves aviadenovirus infection of the glandular epithelium causing degeneration and loss, which may result in abnormal secretion of koilin, subsequently causing erosion of the ventriculus.

Contributor: National Institute of Animal Health, 3-1-5 Kannondai, Tsukuba, Ibaraki, 305-0856, Japan

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CASE II - X (AFIP 2843446)

Signalment: Adult, male raccoon (*Procyon lotor*)

History: The animal was found in an animal enclosure at the Conservation and Research Center acting depressed and reluctant to move. Due to the potential for rabies infection, the animal was euthanized and submitted to the Department of Pathology for full necropsy.

Gross Pathology: The raccoon was in poor nutritional condition. All four limbs were thickened and firm. The periosteum of the long bones and phalanges were expanded by radiating bony trabeculae and the surface was rough. The thickening of the periosteum did not extend into the joint spaces, but proliferative periarticular bone caused limited range of motion in the tarsal-metatarsal joints. Skeletal muscles of the limbs were viable, but edematous. Within the caudal portion of the middle lung lobe was a spherical, 2 cm diameter, pink, firm nodule. Several similar, smaller nodules that were up to 0.5 cm in diameter were present throughout the pancreas.

Laboratory Results: None.

Contributor's Morphologic Diagnoses: 1. Radius and ulna: Periosteal exostoses, diffuse, severe, raccoon (*Procyon lotor*).
2. Skeletal muscle: Parasitism, aphasmid, *Trichinella* sp., multiple
3. Skeletal muscle: Parasitism, sarcocyst, multiple

Contributor's Comment: Hypertrophic osteopathy is a rare phenomenon that has been reported in dogs, cats, sheep, deer, horses, gibbons, and lions. It is most often associated with intrathoracic disease such as heartworm infection, primary or metastatic lung neoplasia, or granulomatous pneumonia, but has also been reported with canine

urinary bladder rhabdomyosarcoma and ovarian tumors in the horse. There is one report of a condition resembling hypertrophic osteopathy that was associated with infection by *Hepatozoon americanum* in dogs.

The diaphyseal regions of the long bones of the limbs are most often affected, but phalanges, ribs and vertebrae may also be involved. There is an ascending widening of the bone and intense pain on palpation. Histologically, there is periosteal proliferation of irregular and anastomosing bony trabeculae causing compression of the surrounding musculature. Lymphocytes and plasma cells may be present in the surrounding connective tissue. The proliferative bone does not involve the joint surfaces, and restriction of movement, as seen in this case, can be attributed to interference between adjacent long bones.

Pathogenesis of this condition involves prolonged hypoxia, which triggers increased blood flow to the periosteum via a vasomotor reflex, with subsequent osteogenesis. Cutting the vagus nerve in affected animals decreases the blood flow to the limbs and causes regression of the lesion. Removal of the inciting pulmonary lesion has also been shown to be curative of hypertrophic osteopathy.

In this raccoon, a 2 cm mass lesion diagnosed histologically as a bronchoalveolar adenoma in the caudal portion of the middle lung lobe was likely the cause of the hypertrophic osteopathy lesions.

Trichinella species are common parasites of wild pigs and carnivores in the United States. Several genotypes of *Trichinella* are maintained in a sylvatic cycle, but only one, *Trichinella* T5, has been recognized in raccoons. The larval stage encysts in the host muscle and is transmitted to other carnivores through scavenging and cannibalistic behavior. These parasites are commonly resistant to freezing, especially in species adapted to arctic environments such as polar bears.

Similarly, *Sarcocystis* species may encyst in the muscle of an intermediate host during the asexual stage of the life cycle, and are frequently an incidental microscopic finding. Raccoons generally serve as a definitive host, with sexual reproduction occurring in the intestine. However, as in this case, raccoons may play both roles in the life cycle of sarcocysts.

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- AFIP Diagnoses:**
1. Radius and ulna: Periosteal exostoses, circumferential, severe, with muscular atrophy and fibrosis, raccoon (*Procyon lotor*), carnivore.
 2. Skeletal muscle: Nematode larvae, intrasarcoplasmic, few, etiology consistent with *Trichinella* sp.
 3. Skeletal muscle: Protozoal cysts, intrasarcoplasmic, few, etiology consistent with *Sarcocystis* sp.

Conference Comment: The contributor has concisely summarized hypertrophic osteopathy in veterinary species. In humans, this bone disorder is termed hypertrophic osteoarthropathy, has an uncertain pathogenesis, and is considered a paraneoplastic syndrome that is often associated with primary thoracic, and rarely, extrathoracic lesions. Unlike animals, in chronic human adult cases, there is joint involvement adjacent to the affected bones, resulting in arthritis, and there is clubbing of the phalanges. In humans, periosteal new bone forms at these sites: metacarpals, metatarsals, proximal phalanges, and the distal ends of long bones.

Contributor: Department of Pathology, Smithsonian National Zoological Park,
Washington, DC 20007

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CASE III - L99-3227 (AFIP 2739184)

Signalment: About 20 adult (1-1.5 cm long) Dwarf African clawed frogs (*Hymenochirus curtipes*) were submitted to the Utah Veterinary Diagnostic Laboratory.

History: The frogs were from several tanks from a tropical fish wholesaler that had increased numbers of animals returned sick or dead. The frogs were purchased from several different breeders. They were housed in large tanks equipped with sand, gravel and UV filters.

Gross Pathology: About half the animals were dead. Some of the live frogs had pieces of skin hanging from their backs and legs. All the animals were thin.

Laboratory Results: Trace mineral and vitamin E levels were similar to those measured in "normal" Dwarf African clawed frogs obtained from several other sources. No significant microorganisms were isolated from the tegument and internal tissues.

Contributor's Morphologic Diagnoses: Skin, proliferative dermatitis with intraepidermal fungal organisms (phylum Chytridiomycota). Other lesions include skeletal myodegeneration and multifocal necrotizing hepatitis.

Contributor's Comment: Chytridiomycosis is a fungal disease described several years ago in frogs in Australia and Panama. Several long-term ecologic studies suggest that it contributed to large population declines in these areas (Daszak et al, 1999; Berger and Speare, 1998). Infections are also reported in captive amphibians in the United States (Pessier et al, 1999). Diagnosis can be made by identifying developing and mature sporangia in fresh skin scraping or within the epidermis of histologic sections. Infected frogs are reported to have abnormal posture, lethargy, and loss of righting reflex. Animals may have abnormal skin sloughing and epidermal ulceration. The epidermal response is hyperplastic with minimal inflammation. It is speculated that fungal toxins are responsible for the clinical signs and animal deaths. Most animals become infected and sick shortly after metamorphosis, as tadpoles may spread the organism but are resistant to disease. Captive amphibians usually develop disease secondary to other diseases, improper husbandry, or other conditions that compromise host immune systems. Changes in tanks, nutrition and water temperature probably contributed to disease development in these frogs.

AFIP Diagnosis: Skin, epidermis: Hyperplasia, diffuse, moderate, with hyperkeratosis and numerous fungi, etiology consistent with *Batrachochytrium dendrobatidis*, Dwarf African Clawed Frog (*Hymenochirus curtipes*), amphibian.

Conference Comment: This emerging aquatic fungal pathogen of both captive and wild frogs and toads primarily infects the ventral parts of the body that are in contact with moisture, including the abdomen, pelvis, medial thighs, and feet. There are three forms of *Batrachochytrium dendrobatidis* thalli recognized in tissue section: uninucleate, multinucleate, and zoosporangium. Characteristically, there is a lack of inflammation associated with the fungi. The exact pathogenesis and cause of mortality is unknown, but three theories have been proposed:

1) epidermal hyperplasia and orthokeratotic hyperkeratosis incited by chytridiomycosis infection may interfere with important functions of amphibian skin (water absorption, osmoregulation, and respiration) by creating an impervious barrier, 2) systemic absorption of a fungal toxin, and 3) a combination of these two factors. Tadpoles can carry *B. dendrobatidis* in their keratinized mouthparts, without affect, but when they undergo metamorphosis, their skin becomes keratinized and the infection spreads, which may result in death.

Contributor: Utah Veterinary Diagnostic Lab, 950 E 1400 North, Logan, UT, 84341

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CASE IV - 02:5662 (AFIP 2840467)

Signalment: Adult, male, Wolf Eel (*Anarrhichthys ocellatus*), 5.2 kilograms

History: This fish developed a large unilateral swelling on the right side of its jaw. It was treated with chloramphenicol *ip* for 4 days before it died. This presentation had been seen in other fish in this exhibit and was always fatal.

Gross Pathology: Multiple formalin-fixed tissue sections including gill, liver, stomach, pancreas, intestine, kidney, spleen and the oral mass were submitted for histopathological examination. There were no significant gross lesions identified in the gill or coelomic viscera. The oral mass was soft, tan, friable muscle.

Laboratory Results: No clinical laboratory pathology was reported.

Contributor's Morphologic Diagnosis: Skeletal muscle (buccal wall): Myositis, histiocytic, interstitial, diffuse, severe, with necrosis and degeneration of myofibers, fibrinoid vasculitis, and myriad ciliated protozoa histomorphologically consistent with *Uronema marinum*.

Contributor's Comment: The exact anatomical location of this section of skeletal muscle is difficult to discern from the slide alone. The specimen is taken from beneath the buccal mucosa. Myofibers throughout this section are swollen and contain vacuolated to granular eosinophilic cytoplasm lacking nuclei or containing scattered pyknotic debris. The interstitium is markedly expanded by an infiltrate of large numbers of viable and necrotic macrophages admixed with fibrin, edema, erythrocytes, cellular debris, and innumerable ciliated protozoa. The ciliated protozoa are round to pyriform, approximately 15-25 X 20-50 um in diameters, with solitary round basophilic heterochromatic nuclei and varying numbers of brightly eosinophilic homogenous intracytoplasmic droplets (phagocytized erythrocytes). There are fibrinoid changes in the walls of some blood vessels, along with rare medial and intimal invasion by ciliates.

The histomorphology of this protozoan is consistent with that of *Uronema marinum*. *Uronema* is a free-living, facultatively parasitic, hymenostomatid protozoan that is capable of causing fatal infections in marine fish. *Uronema* is often referred to as the saltwater variant of the freshwater species known as *Tetrahymena*. *Uronema* can be introduced to aquariums and can cause severe losses if unchecked. Unlike other protozoal ectoparasites of marine fishes, e.g. *Cryptocaryon irritans*, *Uronema* more

frequently is associated with deep tissue invasion such that most infections manifest as ulcerative dermatitis, myositis and invasion of coelomic viscera, including kidney and liver.

Mortalities with *Uronema* have been reported previously in marine fish. High organic loads in aquariums are thought to favor the growth of this protozoan. A possible route of infection in this case could have been trauma to the buccal mucosa with subsequent deep infection by *Uronema* from the environment.

AFIP Diagnosis: Skeletal muscle: Myositis, histiocytic, diffuse, moderate, with hemorrhage and numerous ciliated protozoa, Wolf Eel (*Anarrhichthys ocellatus*), piscine.

Conference Comment: *Uronema* is an uncommon, free-living, opportunistic, ciliated, protozoal pathogen of marine fish that causes acute myodegeneration and myositis, ulcerative dermatitis, and occasionally, meningitis and encephalitis. Uronemosis may affect numerous species of saltwater fish over a wide temperature and salinity range. As the contributor stated, *Tetrahymena* causes similar lesions in freshwater fish. *Anarrhichthys ocellatus* is not a true eel; it is classified in the family Anarhichadidae with wolffishes. It can grow to 2.5 meters in length, and has an ocean range from Alaska to California.

Contributor: Department of Pathobiology and Veterinary Science, University of Connecticut, 61 North Eagleville Road, Storrs, CT, 06269

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Kathleen A. Szabo, DVM
Major, Veterinary Corps, U.S. Army

Wednesday Slide Conference Coordinator
Department of Veterinary Pathology
Armed Forces Institute of Pathology
Registry of Veterinary Pathology*

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