CASE I – 03F034 (AFIP 2890942)

Signalment: Channel catfish (Ictalurus punctatus) from recently constructed and filled farm pond.

History: Outbreak occurred in spring of 2003 and mortality was approximately 35%. Affected fish were observed swimming close to surface of pond.

Gross Pathology: Gill lamellae were hyperemic, blunted and thickened. All other internal organs and tissues were grossly normal.

Laboratory Results: Water quality data was not available

Contributor’s Morphologic Diagnosis: Gill Lamellae – inflammation, granulomatous, chronic, diffuse, severe with epithelial hyperplasia, cartilage necrosis and intralamellar myxozoan-like parasites

Contributor’s Comment: The lesions are those of proliferative gill disease (PGD) of channel catfish. This is an important disease of cultured channel catfish causing high mortality and significant economic loss. The disease is caused by a myxozoan parasite that has life stages in different hosts. A worm (Dero digitata) commonly found in catfish ponds can become infected with the myxozoan parasite Aurantiactinomyxon sp. (actinosporean stage). Another stage of this myxozoan parasite named Henneguya ichtaluri (myxosporean stage) also infects catfish gills. Catfish can be experimentally infected 5 to 6 days after exposure to worms infected with Aurantiactinomyxon sp (1). Experimental infection is characterized by inflammation, epithelial cell hyperplasia, gill necrosis and the presence of cysts containing spores. The sequences of the SSU rRNA genes of the actinospore and myxospore life stages have been recently demonstrated to be identical (2).
In tissue section, *H. ictaluri* cysts are located within central regions of gill lamellae and are surrounded by heavy infiltrates of macrophages. Individual cysts appear to lack a capsule and consist of aggregates of small basophilic spores. Cartilage is either conspicuously absent where inflammation and parasites are located or is undergoing degenerative changes.

Please note: some sections of gill lamellae contain low numbers of monogenetic trematodes, *Chilodonella* sp protozoa and *Henneguya exilis* cysts. These are producing minimal tissue reaction and are not part of PGD.

**AFIP Diagnosis:** Gill: Branchitis, histiocytic and proliferative, diffuse, moderate to severe, chronic, with lamellar fusion, blunting, and loss and protozoa, etiology consistent with myxozoan parasites, channel catfish (*Ictalurus punctatus*), piscine.

**Conference Comment:** There is some variability among slides concerning the number and types of parasites present as well as the presence of cartilage necrosis. Proliferative gill disease (PGD) (a.k.a. hamburger gill disease) of channel catfish occurs throughout commercial catfish-producing regions of the United States and has caused severe economic losses to individual producers since 1981. The disease is characterized by a rapid onset with 10% to 95% mortality. Respiratory efficiency is reduced and fish may gasp for air at dissolved oxygen concentrations that are normally adequate. Fish present in severe respiratory distress with swollen, friable gills that may bleed when touched. Microscopically, there is intense granulomatous inflammation, swelling and epithelial hyperplasia of the gills with necrosis of the primary lamellar cartilage and distortion of lamellae. Cysts have also been observed in the brain, spleen, liver, and kidney. Water temperatures between 16 and 20°C favor optimal growth of the organism.

PGD must be differentiated from disease caused by microsporidian parasites such as *Pleistophora* sp., *Glugea* sp., *Loma* sp. and *Noserag* sp. which are capable of infecting skeletal muscle, gills, epithelial and connective tissue resulting in high mortality. Microsporidian infection results in the formation of large cysts (up to several millimeters) called xenomas that are filled with numerous refractile spores. Xenomas induce proliferative inflammation leading to granuloma formation. Additional differentials for gill lesions include the gram-negative bacterium *Flavobacterium columnare* (formerly *Flexibacter columnaris*) causing “columnaris disease or saddleback disease”; and non-inflammatory hyperplasia of gill epithelium associated with poor water quality and overcrowding.

Other myxosporidian parasites of fish include:
- *Myxosoma cerebralis*: "Whirling disease" in trout; cartilage necrosis of head and spine
- *Henneguya* sp.: “Blister disease” in freshwater fish and catfish; gill epithelial and epidermal hyperplasia with pseudocysts

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• **Hofferellus carassii**: “Kidney bloater disease” in goldfish in North America and Asia; marked dilation of some, but not all, renal tubules

• **Ceratomyxa shasta**: Ceratomyxosis; granulomatous peritonitis in salmonids

• **Sphaerospora** sp.: Proposed cause of Proliferative Kidney Disease (PKD, PKX, X Disease); myxozoan parasite; granulomatous interstitial nephritis in salmonids

Readers are encouraged to review Wednesday Slide Conference case 2, conference 22, 1996 for another case of PGD.

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**References:**

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**CASE II – L03 1060 (AFIP 2893497)**

**Signalment:** Grouped samples of hepatopancreas from numerous freshwater crayfish (*Cherax quadricarinatus*) are submitted for histopathology.

**History:** Submitted for health evaluation during state inspection

**Histopathologic Description:** Hepatopancreas: Few to moderate numbers of epithelial cells lining hepatopancreatic tubules have enlarged nuclei with margined chromatin and contain homogeneous, eosinophilic to basophilic, round to oblong inclusion bodies. In some areas, supporting interstitium has multifocal infiltrates of mononuclear and granular leukocytes. Leukocytes center about squamous epithelial lined ducts, which contain large numbers of rod shaped bacteria.

**Contributor’s Morphologic Diagnoses:** 1. Intranuclear inclusion bodies in hepatopancreatic epithelial cells
2. Hepatopancreatitis, mononuclear and granulated leukocytes

**Contributor’s Comment:** The presence of baculovirus virions were demonstrated in tissue sections by electron microscopy.

Rod shaped nuclear viruses are the most common viral agents detected in cultured crustaceans and all infect the epithelial cells of the digestive tubules and hepatopancreas (13). Baculoviruses of penaeid shrimps have been demonstrated to be serious pathogens, however the virulence and clinical significance of others (crayfish) remains unclear (13).

Baculovirus was discovered in crayfish in 1996 as the result of an inspection of an aquaculture facility in Northern Utah (14). Samples of crayfish were taken from the facility and tested at the Ross Smart Veterinary Diagnostic Laboratory at Utah State University. This virus was confirmed using electron microscopy. The baculovirus is known to infect crayfish in Australia and California, but had not been seen in Utah until its presence was confirmed in 1997 (14). The baculovirus is often found in the crayfish's internal organ called the hepatopancreas. The virus is not known to cause extensive mortality, but is suspect of stunting their growth and affecting their immuno-competency (14). The disease is not a threat to humans.

**AFIP Diagnoses:** 1. Hepatopancreas, tubular epithelial cells: Amphophilic intranuclear inclusion bodies, multifocal, moderate numbers, with karyomegaly, freshwater crayfish (*Cherax quadricarinatus*), crustacean.
2. Hepatopancreas: Granulomas, multifocal, few, with mixed bacteria.

**Conference Comment:** There is some variability among slides. Some sections contain granulomas with a central core of mixed bacteria. As mentioned by the contributor, baculoviruses are known to cause disease in shrimp and prawns and have recently been recognized as pathogens of several species of crayfish.

Baculoviruses are a family of large, rod-shaped viruses which contain a circular, double-stranded genome ranging from 80-180 kbp. Baculoviruses have very species-specific tropisms among invertebrates with over 600 host species having been described. They are not known to replicate in mammalian or other vertebrate animal cells. (16)

Diseases of crayfish caused by baculoviruses include White Spot Virus (WSV) infection and *Cherax quadricarinatus* bacilliform virus (CqBV) infection. WSV has been associated with high mortality (90%) in crayfish fed frozen imported prawns. CqBV infections in *Cherax quadricarinatus* can result in lethargy, weakness, a failed-tail-flick response and an inability to right themselves when placed on their back. CqBV infections are associated with hepatopancreatic tubule epithelial cell necrosis and atrophy. Nuclear changes include hypertrophy, margination of chromatin and the formation of a single, large, granular eosinophilic inclusion body. (17)
The following is an excerpt of supplemental information on penaeid (shrimp) baculovirus provided by the Office International des Epizooties (OIE manual):

Infections of the hepatopancreas by the nuclear polyhedrosis viruses *Baculovirus penaei* (BP) and *Penaeus monodon*-type baculovirus (MBV) are among the most readily diagnosable diseases of the penaeid shrimps and prawns. The inclusion bodies formed by the viruses are very conspicuous and easily demonstrated by direct light microscopy with fresh specimens or by routine histological methods with fixed specimens. Direct microscopic methods are most suitable for the post-larvae stages, which are commonly moved in regional and international commerce (1, 2, 7, 8). Highly sensitive molecular methods for BP and MBV are also available and provide alternative methods for surveillance applications, especially for nonlethal testing of broodstock (8, 9).

BP and MBV are considered to be potentially serious pathogens in the larval, postlarval, and early juvenile stages of host shrimps. These viruses possess a wide geographical distribution and diverse host range, and multiple strains of both viruses have been documented. Infections by both viruses are characterized by the presence of prominent, intranuclear occlusion bodies, which are referred to as polyhedral occlusion bodies or polyhedral inclusion bodies, in affected epithelial cells of the hepatopancreas and midgut, or free within lysed cell debris in the faeces (8, 9). Crowding, chemical stress, or environmental stress may enhance the pathogenicity and increase the prevalence of MBV or BP in their hosts. Infection by BP and MBV are exclusively by the oral route in which cannibalism and faecal-oral contamination are the principal mechanisms of transmission (6, 8, 11, 12).

The geographical distribution of BP is limited to the Western Hemisphere. However, within the Americas and Hawaii, multiple geographical strains of BP exist, and some of these may be distinguished by the size of the virion or by molecular methods. BP has a widespread distribution in cultured and wild penaeid shrimps in North and South America (3, 4, 5, 8, 9, 10).

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**References:**
2. Brock JA, Main K: A Guide to the Common Problems and Diseases of Cultured Penaeus vannamei. Published by the Oceanic Institute, Makapuu Point, P.O. Box 25280, Honolulu, Hawaii, USA, 1994
14. AQUACULTURE IN UTAH, State of Utah department of agriculture and food, Dec 2000 issue
CASE III – ZH S05-0926 (AFIP 2987670)

Signalment: 2-year-old, female, Bearded Dragon (*Pogona vitticeps*).

History: In a group of 4 animals, one died unexpected without any symptoms. The other remained asymptomatic and are still alive.

Gross Pathology: At necropsy, no gross abnormalities were observed.

Laboratory Results: *Salmonella* spp. were isolated from the intestinal tract after enrichment.

Histopathologic Description: The normal lobular architecture of the liver section is disturbed by multifocal to confluent necrosis. Many hepatocytes are karyomegalic as the result of large smudgy basophilic adenoviral inclusions. Anisokaryosis of hepatocytes and a marked increase of sinusoidal inflammatory cells, which are predominantly lymphocytes, are present. In the necrotic areas there are some fibrin clots. Intranuclear inclusion bodies were also found in the intestinal epithelium but pancreas and lungs were not affected. Electron microscopy showed intranuclear inclusions consisting of crystalline aggregates of non-enveloped viral particles, some of them with electrodense centers.

Contributor’s Morphologic Diagnosis: Acute severe multifocal to coalescing necrotising hepatitis, with basophilic intranuclear inclusions in hepatocytes and Kupffer cells.

Contributor’s Comment: Adenoviruses (AdVs) are categorized into four genera: *Mastadenovirus* (from mammals), *Aviadenovirus* (from birds), and two recently accepted genera, *Atadenovirus* and *Siadenovirus*. Phylogenetic analysis indicated that Adenoviruses from reptiles belong to the genus *Atadenovirus* (1). AdV-like particles have been identified in many reptile species, including 10 snake species, 4 lizard species, and 1 crocodilian species. Lesions in reptiles associated with AdV-like agents include hepatitis, enteritis, esophagitis, splenitis, and encephalopathy (1).

The virus appears to be established in certain breeding groups of the lizard genus *Pogona* in the United States, and although transmission studies have not been conducted, vertical transmission through the egg *in utero* or at the time of oviposition seems a likely route. Adults may survive as inapparent carriers (2).
AFIP Diagnoses: 1. Liver: Hepatocellular degeneration, necrosis, and loss, multifocally-extensive, marked to severe, with basophilic intranuclear inclusion bodies, bearded dragon (Pogona vitticeps), reptile.
2. Liver: Hepatocellular lipidosis, diffuse, moderate.

Conference Comment: Adenoviruses are double-stranded DNA virus that have been reported in numerous reptiles including Nile crocodiles, boa constrictors, rosy boas, rat snakes, Gaboon vipers, Savannah monitor lizards, Jackson’s chameleon, and Rankin’s dragon. All of these reptiles had intranuclear viral inclusion bodies in the gastrointestinal tract and/or liver; most also had lesions of hepatic necrosis. Adenovirus is established in some breeding groups of bearded dragons in the United States.

Adenoviral inclusions are intranuclear and typically appear basophilic to amphophilic with hematoxylin and eosin. Adenoviral inclusions are often referred to as large and “smudgy” in appearance.

Ultrastructural characteristics of reptilian adenoviruses include the formation of paracrystalline arrays in the nucleus of infected cells. The viral particles are 60-66 nm in diameter, non-enveloped, and have electron-dense or electron-lucent cores.

As mentioned by the contributor, adenoviruses are currently classified into four genera. Below is a comparative list of adenoviruses and some of the diseases they cause (3):

Genus Aviadenovirus (Group 1 avian adenoviruses):
• Fowl, goose, duck, pigeon, turkey adenovirus: Inclusion Body Hepatitis (IBH); hydropericardium syndrome; respiratory disease; necrotizing pancreatitis and gizzard erosions
• Quail Bronchitis (avian adenovirus Type 1): Infects Colinus virginianus

Genus Siadenovirus:
• Marble spleen disease (MSD) (Adenovirus Type 2): Pheasants; splenic necrosis, respiratory edema, congestion and asphyxia
• Hemorrhagic enteritis (HE) (Adenovirus Type 2): Young turkeys; bloody droppings, death
• Avian adenovirus splenomegaly virus (AASV)
• Frog Adenovirus

Genus Atadenovirus:
• Egg drop syndrome (subgroup 3 avian adenovirus): Laying hens, viral replication in pouch shell gland epithelium; intranuclear inclusion bodies
• Ovine, bovine, duck, possum adenoviruses
• Reptilian Adenoviruses
  • Bearded dragon, snake, chameleon, gecko

Genus Mastadenovirus:

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• Human adenovirus: respiratory disease, enteritis; keratoconjunctivitis
• Simian adenovirus (27 different viruses): mostly subclinical; some secondary to immunosuppression, mild to moderately severe respiratory and enteric disease, keratitis/conjunctivitis
• Canine adenovirus 1: Infectious canine hepatitis
• Canine adenovirus 2: most cases secondary to immunosuppression; produces necrotizing bronchiolitis and alveolar epithelialization
• Equine adenovirus: mild respiratory disease except in CID Arabian foals where adenoviral infection leads to severe bronchiolitis and atelectasis
• Bovine adenovirus: respiratory tract disease, pyrexia, KCS, colic, associated with respiratory and enteric disease in calves but not considered the primary pathogen in either syndrome
• Ovine adenovirus: respiratory tract disease, conjunctivitis, enteritis
• Porcine adenovirus: widespread, mostly subclinical, pneumonia, enteritis associated with encephalitis and diarrhea
• Murine adenovirus: oncogenic in newborns, experimentally induce CNS lesions
• Guinea Pig Adenovirus: pneumonitis
• Adenovirus can experimentally cause tumors in hamsters
• Wildlife: Adenovirus Hemorrhagic Disease in California mule deer produces similar lesions to Bluetongue virus and Epizootic Hemorrhagic Disease (EHD) ( orbiviruses). Black-tailed deer adenovirus: hemorrhagic vasculitis, pulmonary edema. Brown bear, coyotes, wolves and raccoons also susceptible to CAV-1

Note, several viral isolates share the same name with isolates from other genera and are only differentiated by letter designators. For a complete table of the adenoviruses, readers are encouraged to visit the ICTV website listed in reference 3.

Readers are encouraged to review Wednesday Slide Conference case 2, conference 29, 1995 for another case of adenovirus in the intestine of a reptile.

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References:
CASE IV – 366724 (AFIP 2888664)

Signalment: Adult female green tree frog (Rana sp.)

History: Owner presented the frog to Angell Memorial Animal Hospital for a one-week history of decreased appetite, lethargy, and progressive erythema along the ventral abdomen and hind legs. The frog shared a peat moss enclosure with another green tree frog, which showed similar clinical signs. Both affected frogs exhibited accelerated skin shedding.

Gross Pathology: On gross examination, the body exhibited dark red, pinpoint mottling of the skin of the abdomen and pelvic extremities. The dorsal skin showed fewer pinpoint lesions but exhibited irregular shedding with numerous flakes of keratin that were easily removed with minimal digital pressure.

Laboratory Results: Routine fungal cultures were negative. Aerobic/anaerobic bacterial cultures exhibited mild to moderate growth of Sphingobacterium multivorum (incidental environmental flora).

Contributor’s Morphologic Diagnosis: Skin (ventral abdomen): Moderate to severe diffuse orthokeratotic hyperkeratosis and acanthosis with intralesional chytrid organisms and mild superficial perivascular dermatitis

Etiologic diagnosis: Chytridiomycosis

Etiology: Batrachochytrium dendrobatidis

Contributor’s Comment: Chytridiomycosis, recently identified as an emerging infectious disease of post-metamorphic amphibians, has been implicated in episodes of mass mortality with subsequent population declines in Australia, the Americas, Africa, Europe, Asia and India [1,2,3,4]. The etiologic agent, Batrachochytrium dendrobatidis, was first identified from carcasses collected at sites of mass mortalities in Australia, Costa Rica and Panama [1,4]. The gross lesions vary greatly in severity. They consist of epidermal hyperkeratosis with petechia, sloughing, ulceration and hemorrhage and usually involve the toes, pelvic extremities and ventral abdominal skin [1,2,3]. The organisms were identified as a non-hyphal chytrid fungus based on histologic examination of hematoxylin and eosin-stained tissue sections, transmission electron microscopy and 18s rDNA sequence data [1,4].

The organism has a wide host range that includes urodeles, aquatic amphibians, terrestrial and semi-terrestrial frogs and toads. It has a predilection for lower
environmental temperatures (montane forests) [2]. Although it has been seen mainly in adult amphibians, tadpoles, which have normally non-keratinized skin, can serve as carriers and will on occasion exhibit lesions around the keratinized mouth parts [1,3].

The organisms infect only the stratum corneum and stratum granulosum, and develop into a 6-15 micron diameter spherical thallus (zoosporangia) with a discharge papilla that projects to the surface. The zoosporangia appear to be intracellular and display a relatively uniform, 1-2 micron thick, smooth wall. Within the zoosporangia there are variable numbers of any of four developmental stages of the chytrid (zoospores). On routine hematoxylin and eosin-stained tissue sections, zoospores appear as round to oval basophilic bodies with poorly defined margins. The zoosporangia can retain their shape for some time after the zoospores have been released [1,3].

AFIP Diagnosis: Skin: Epidermal hyperplasia and hyperkeratosis, multifocal, moderate, with minimal subacute dermatitis, and numerous intracorneal fungi, etiology consistent with Batrachochytrium dendrobatidis, green tree frog, amphibian.

Conference Comment: The contributor provides an excellent review of an emerging aquatic fungal pathogen of captive and wild frogs and toads.

Chytridiomycosis is now believed to have originated in Africa with the earliest case found in an African Clawed Frog (Xenopus laevis) in 1938. In the wild, X. laevis does not show clinical signs and the species has not experienced any sudden die-offs, suggesting it is the natural host. Interestingly, X. laevis was also geographically disseminated around the world in massive numbers for over 3 decades following the discovery of the pregnancy assay for humans in 1934 (the assay is based on the principle that ovulation in X. laevis is induced by injection with urine from pregnant women because of high levels of gonadotropic hormones in the urine). Other factors contributing to the spread of the disease include the establishment of feral populations of X. laevis in importing countries (e.g. U.K., U.S.A., and Chile) and transmission to other amphibian species (e.g. the American bullfrog) which subsequently served as vectors through international trade as food items.(5) Recent in vitro studies have shown that B. dendrobatidis can survive in sterile river soil and sand for up to three months, can attach and grow on feathers and survive drying for up to three hours, suggesting that birds may be disseminating the disease between frog habitats.(6)

The 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 (such as water absorption, osmoregulation and respiration) by creating an impervious barrier; 2) systemic absorption of a fungal toxin; and 3) a combination of the two.

Recent work shows that although there is little genetic variation among isolates of B. dendrobatidis, different strains exist with significantly different virulence and behavior.
As mentioned by the contributor, changes such as hyperkeratosis and congestion occur more frequently on the ventrum. Additionally, the stratum corneum sloughs following a high intensity build-up of sporangia rather than being shed continuously.

Readers are encouraged to review Wednesday Slide Conference case 3, conference 23, 2002 and case 4, conference 29, 1999 for additional cases of chytridiomycosis in frogs.

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