Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2011-2012

Conference 16

29 February 2012

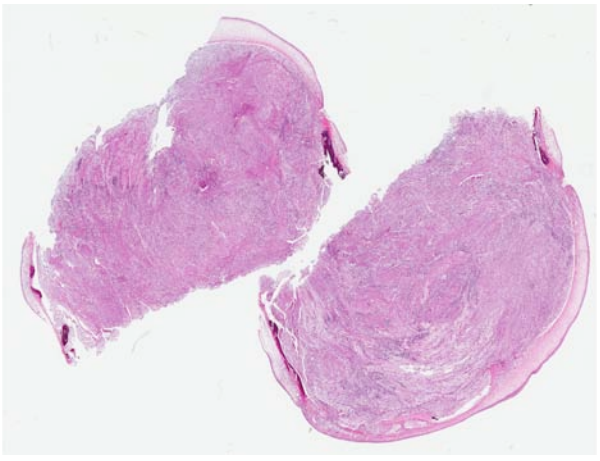
CASE I: 09.6781/09.1998 (JPC 3135245).

Signalment: Age unknown (adult) male silver carp (*Hypophthalmichthys molitrix*).

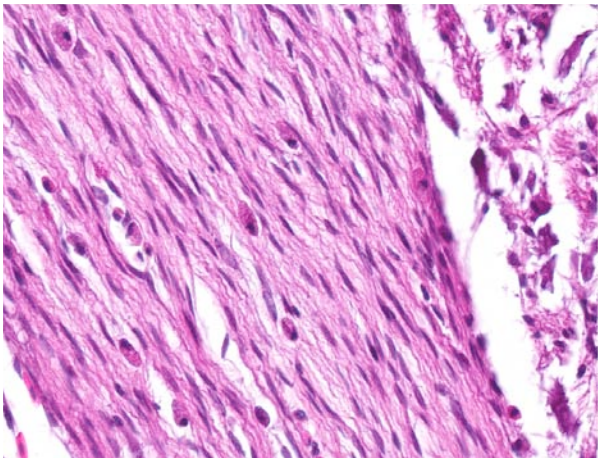
History: Abnormal growth noted in both eyes over a period of several months; left eye enucleated 2 months prior to enucleation of the right eye.

Gross Pathology: A 1cm diameter homogenous, soft white-tan mass occupies the vitreous, posterior and part of the anterior chamber in both globes, obscuring the lenticular and iridial structures.

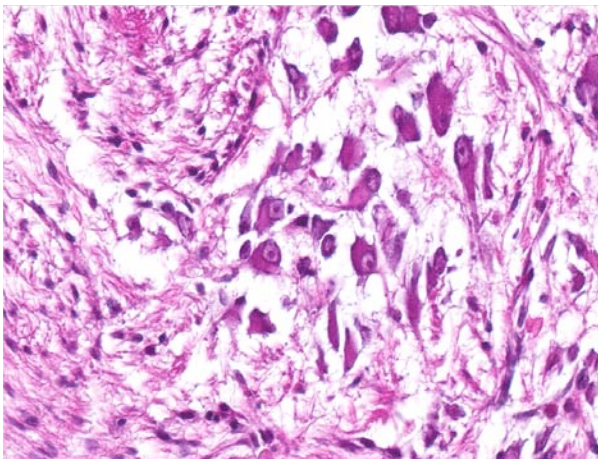
Contributor's Histopathologic Description: 109.001998 Left eye: Six sections of the globe are examined (slides A-C). There is a densely cellular, poorly circumscribed population of neoplastic cells filling the anterior and posterior chambers and vitreous. Neoplastic cells are dimorphic: the first population is spindloid with a small amount of fibrillar eosinophilic cytoplasm, a large oval nucleus with finely stippled chromatin and infrequent visible nucleoli. Mitotic figures average six per ten high power fields. The second neoplastic cell population is composed of well differentiated neurons, and this population is scattered throughout the fibroblastic



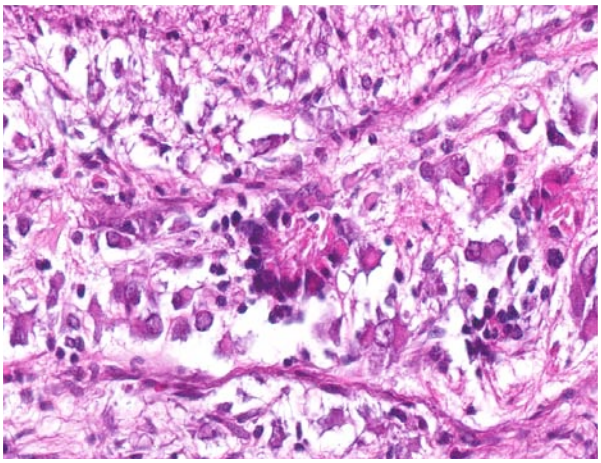
1-1. Globe, silver carp: The anterior chamber and vitreous, sclera, uvea, choroid, and even the cornea is infiltrated by a densely cellular neoplasm composed of broad streams and bundles. (HE, 40X)



1-2. Globe, silver carp: Bland spindle cells are the primary cell population; mitoses are rare. (HE, 400X)



1-3. Globe, silver carp: A second neoplastic cell type are large polygonal cells resembling neurons. (HE, 400X)



1-4. Globe, silver carp: Small triangular cells with apical nuclei form prominent rosettes (neuroblasts). (HE 400X)

neoplastic cell population. Neoplastic cells are arranged in thick interlacing fascicles and are infiltrated by a diffuse population of heterophils and macrophages. Neoplastic cells dissect through the stroma of the cornea and sclera.

109.006781 Right eye: One section of completely excised tissue is examined. The architecture of the iris, ciliary body and lens is diffusely effaced by a poorly circumscribed population of neoplastic cells that fills the vitreous body and protrudes into the anterior chamber. Neoplastic cells are dimorphic: the first population is spindloid with tapered cell borders, a moderate amount of fibrillar eosinophilic cytoplasm and a large elongate nucleus with stippled chromatin. These neoplastic cells are arranged in thick interlacing fascicles. Interspersed within this population of neoplastic spindle cells, there is a subpopulation of polyhedral to stellate neoplastic cells with abundant eosinophilic cytoplasm, a large eccentric nucleus with a fine chromatin pattern and prominent eosinophilic nucleolus. Mitotic figures average three per ten high power fields for all neoplastic cell types. Neoplastic cells invade the lens capsule and disrupt the lens, and there is moderate histiocytic and lymphoplasmacytic inflammation surrounding the lens fragments.

Contributor's Morphologic Diagnosis: Left and right globes: Ganglioneuroma.

Contributor's Comment: A diagnosis of ganglioneuroma is made based on the presence of the well differentiated neuronal and spindle neoplastic cell populations within the mass. Reported tumors of nerve cell origin within the peripheral nervous system of fish are rare and include schwannomas, neurilemmomas, retinoblastomas and ganglioneuromas. Ganglioneuromas are associated with spinal ganglia in both marine and freshwater species, and are typically reported within body cavities[6] and in association with gill filaments and the root of the tail fin.[1] These tumors are soft, pale and whitish and sometimes lobulated. The histologic features of this tumor include a classic single or grouped neoplastic ganglion cell with prominent vesicular chromatin and eosinophilic nucleoli, separated by interlacing fascicles of spindle neoplastic cells. Unlike retinoblastomas, ganglioneuromas do not exhibit rosette formation, and unlike schwannomas, they contain neuronally differentiated cells. Neoplasms of the retina in fish have been reported in association with exposure to methylazoxymethanol experimentally.[2,5] The species in which this tumor more frequently occurs include cats, pigs, cattle, dogs and horses, as well as reports of cockatiels and rats. The bilateral origin of the tumor in this case suggests an underlying genetic predisposition, which may be analogous to that of retinoblastomas.

Immunohistochemical staining with GFAP and vimentin was performed. Few foci of cellular processes within the spindloid cell population in the neoplasm were positive for GFAP, and there was positive internal control staining within the optic nerve. Vimentin staining, however, was negative for neuronal and spindle cells and there was no positive internal control staining within the optic nerve or elsewhere in the tissue.

JPC Diagnosis: Eye, globe: Ganglioneuroblastoma.

Conference Comment: The differential diagnoses proposed by conference participants included neuroblastoma, ganglioneuroma, and ganglioneuroblastoma. Neuroblastomas are derived from neural crest cells, may metastasize, and common sites include the adrenal medulla and sympathetic ganglia. Ganglioneuromas may arise from primitive neuroepithelial cells which exhibit neuronal differentiation and usually exhibit a benign behavior.

Common sites include the neuraxis, autonomic and cranial nerve ganglia, and adrenal medulla.

Conference participants felt that there were three distinct cell populations in this particular neoplasm, including the spindle cells, ganglion cells, and neuroblast cells with carrot-shaped nuclei that formed scattered rosettes. For this reason, we favor a diagnosis of ganglioneuroblastoma. Ganglioneuroblastomas exhibit histologic features of Conference participants also felt this neoplasm was malignant due to its locally invasive nature.

Additional differential diagnoses considered for this neoplasm include medulloepithelioma, melanoma, and retinoblastoma. Retinoblastomas present with numerous neuroblasts arranged in rosettes and can be easily confused with medulloepitheliomas in fish, except they are generally not as invasive and do not form heteroplastic elements such as undifferentiated mesenchymal cells, bone, or cartilage.[7]

There was some variation among sections due to the submission of both eyes, and some conference participants had evidence of retinal detachment and atrophy, as well as a lenticular cataract with Morgagnian globules in the lens. There were also areas of woven bone surrounding some sections of the cornea, and conference participants hypothesized that this could represent osseous metaplasia from the neoplasm or from exposed lens fibers.

S-100, neurofilament protein (NFP), synaptophysin, chromagranin A, and glial fibrillary acidic protein (GFAP) immunohistochemical stains were performed on this case, but all were non-contributory, which is likely due to the fact that the immunohistochemical stains at the Joint Pathology Center laboratory are not optimized for piscine tissues.

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CASE II: A10-42431 (JPC 3167832).

Signalment: Sex undetermined juvenile Moorish idol (*Zanclus cornutus*).

History: The body of an approximately 6cm juvenile Moorish idol was received in formalin. The fish was one of a group of recently shipped animals under observation and treatment in quarantine prior to display in a public aquarium. Constant low level mortalities had occurred from the time of arrival.

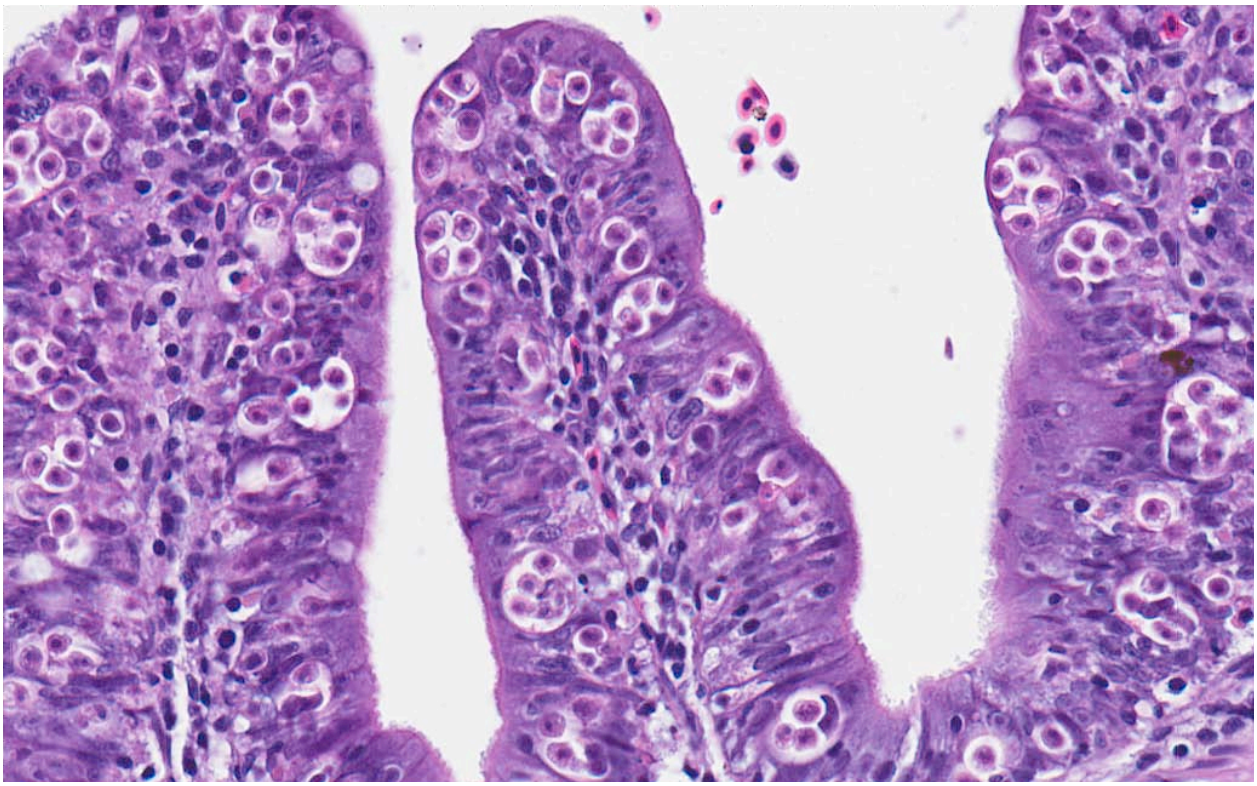
Gross Pathology: Reported gross findings varied among individuals and included lethargy, emaciation, abdominal distension, and intestines severely distended by thick yellow contents.

Laboratory Results: Intestinal mucosal scrapings were reported to contain numerous signet ring-like structures with multiple internal spheres.

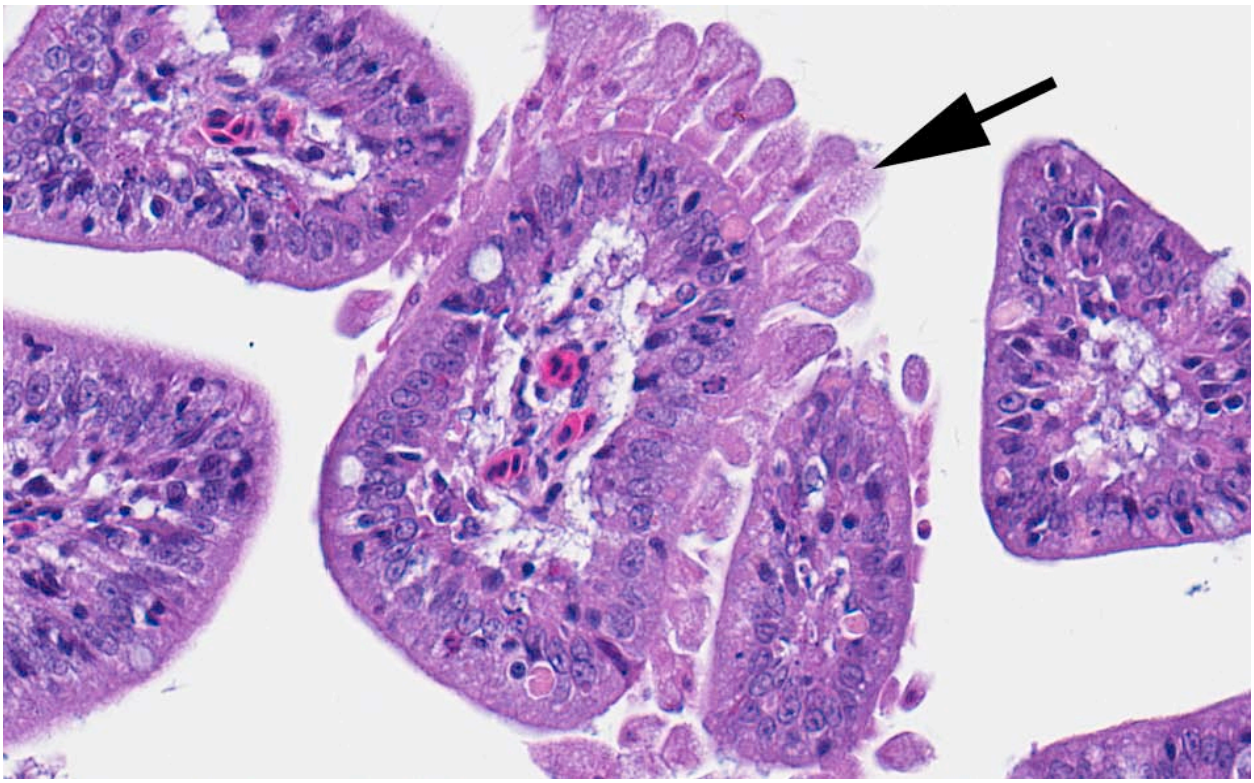
Contributor's Histopathologic Description: Intestine: Multiple intestinal segments are characterized by plicae irregularly expanded by variable, mild to moderate, mixed populations of lymphocytes, plasma cells, and macrophages containing granular to refractile debris. Within affected areas, the normal mucosal architecture is altered by a combination of mild epithelial hypertrophy, hyperplasia, and pseudostratification, with compression and distortion of individual cells resulting from the presence of large numbers of approximately 20 µm, ovoid, intraepithelial parasitic bodies. Individual bodies (pansporoblasts) are defined by a thin pale eosinophilic limiting membrane and compressed peripheral nucleus, surrounding a vacuolated space typically containing 4-8 ovoid, 5 x 8 µm structures (sporoblasts and accessory cells). The round to ovoid internal structures have moderate amounts of slightly vacuolated pale eosinophilic cytoplasm and possess 1 or 2 central, condensed, basophilic nuclei, often with a prominent nucleolus. Similar structures are present within luminal debris. Occasional lifting of the epithelium from the basement membrane is evident in some areas and pockets of refractile debris are scattered throughout the epithelium.

Contributor's Morphologic Diagnosis: Intestine: Enteritis, segmental, subacute to chronic, moderate, with numerous intralesional myxozoal pansporoblasts.

Contributor's Comment: Microscopic findings are consistent with widespread intestinal infection by the myxozoan parasite *Enteromyxm* (*Myxidium*) *leei*. *Enteromyxm leei* causes severe enteritis and losses, particularly in sea bream and sea bass mariculture in the Mediterranean area.[6,8] The parasite exhibits an unusually low degree of host specificity and infections have been transmitted to over 25 marine fish species in



2-1. Intestine, Moorish idol: Approximately half of the intestinal epithelial cells contain myxosporidian pansporoblasts. (HE, 400X)



2-2. Intestine, Moorish idol: In an adjacent section of intestine, a florid carpet of ciliates multifocally cover the intestinal villi. (HE, 400X)

a public aquarium.[8] An alternate host or actinosporean form of the parasite has not been identified and direct transmission has been demonstrated by cohabitation, exposure to contaminated effluent, and by the feeding of infected tissue.[4] Gross signs may include emaciation, darkening, lethargy, abdominal distension, pale soft liver, enlarged gallbladder, and a thickened and hemorrhagic intestinal wall.[6,8] Lesions occur throughout the intestine from the pyloric ceca to rectum, and occasionally in the gallbladder and intrahepatic bile ducts.[1,6,8] Development occurs between epithelial cells near the basement membrane. Pansporoblasts, up to 40 µm, consist of an enveloping pericyte or primary cell surrounding a clear space housing two developing sporoblasts and two or more accompanying accessory cells.[1] Although Giemsa, Gram, and acid-fast stains were performed, mature spores were not identified. Mature spores, when present, are arcuate, average 6.9 x 14.7 µm, and have two elongate polar capsules tapering to their distal ends.[3]

Myxozoans are important metazoan parasites of freshwater and marine fish closely related to the Cnidaria. This large group of obligate parasites includes over 1350 species in 52 genera, involving intracellular and intercellular histozoic representatives, as well as many coelozoic forms. Life cycles are complex and not fully understood for many species, although at least 25 are known to involve an invertebrate (oligochaete, polychaete, or bryozoan) alternative host. Taxonomy is largely based on the morphology of myxospores, characterized by multiple cells arranged in 1-7 spore valves, 1-2 amoeboid sporoplasms, and 2-7 nematocyst-like polar capsules with coiled extrudable polar filaments.[2,7]

Additional findings present in some sections include plasmodia of a coelozoic myxozoan associated with mild bile duct ectasia, epithelial attenuation, and periductular fibrosis. Some intestinal sections also include a focal area with large numbers of elongate structures with abundant finely granular to vacuolated cytoplasm, one to rarely two small nuclei, and a broad base of attachment to the mucosal epithelium. The nature of this organism is unknown, but possibly represents a developmental stage of another myxozoan.

JPC Diagnosis: Intestine: Enteritis, histiocytic and lymphocytic, segmental, moderate to marked, with mucosal hypertrophy and hyperplasia and numerous intracytoplasmic myxosporidian pansporoblasts.

Conference Comment: Conference participants discussed the usual mode of transmission for this myxozoan. Direct fish-to-fish transmission can occur through the ingestion of exfoliated infected intestinal epithelium.[5] The intestinal epithelium contains

autophagic vacuoles, a common finding in fish with gastrointestinal disturbances. Conference participants also discussed the presence of exocrine pancreatic atrophy, lack of coelomic adipose tissue, and atrophy and loss of the dorsal skeletal muscle, all of which are consistent with the reported gross findings and indicate cachexia. Participants attributed areas of myodegeneration in the dorsal skeletal muscle to possible capture myopathy.

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CASE III: 63048 (JPC 4002871).

Signalment: Approximately 9-year-old male mudpuppy (*Necturus maculosus*).

History: Keepers reported this mudpuppy, a collection animal at the Maryland Zoo in Baltimore, to the veterinary staff for acute coelomic distention and floating upside-down. On physical exam, the animal was dull but responsive, and had gained approximately 250g since its most recent weight check. Ultrasound exam revealed a large amount of free fluid within the coelomic cavity, while the heart, liver, and intestines all appeared to be within expected limits. The coelomic fluid had low specific gravity, 1.009, and cytologic examination of a concentrated sample showed many lymphocytes and presumptive granulocytes, as well as a single, septate, branching fungal hypha. Treatment was initiated, including twice daily coelomocentesis, ceftazidime (20mg/kg q72h), and once daily baths in intraconazole and Wright-Whitaker solution. On the fourth day of treatment, the animal was found moribund and floating upside-down. Shortly thereafter, the heartbeat could not be detected by Doppler.

Gross Pathology: On necropsy, there were multiple variably-sized areas of light red discoloration on the skin of the ventrum and around the cloaca (livor mortis). The musculature of the body wall was diffusely gelatinous on palpation (edema). Approximately 10mL of colorless, slightly turbid fluid was present within the coelomic cavity. The liver was predominantly brownish black with subtle, multifocal, light tan mottling of the capsular surface. The testes and kidneys appeared mottled light tan to dark brown. All other major organs were grossly unremarkable.

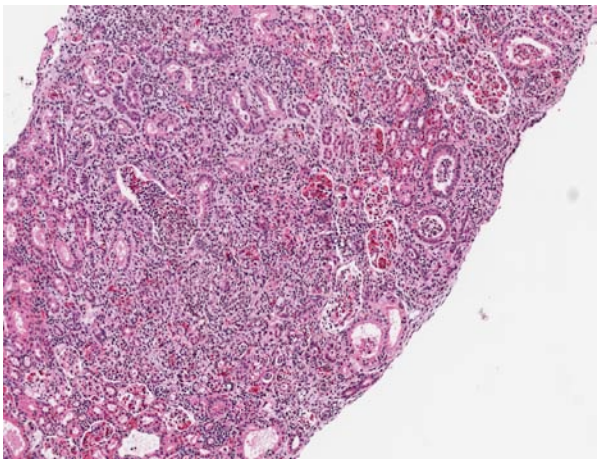
Laboratory Results: Fungal culture of the coelomic fluid yielded 2+ *Exophiala* species. Further speciation was not provided. There was no growth on aerobic bacterial culture.

Contributor's Histopathologic Description: Kidney: Multifocally infiltrating the interstitium and tubules is an inflammatory cell population consisting primarily of cells with large, multilobulated nuclei (neutrophils) and fewer cells with large, round to reniform nuclei (macrophages). Admixed with the inflammatory cells are variable amounts of fibrin, necrotic cellular debris, extravasated red blood cells (hemorrhage), and numerous slender, 5-7 µm diameter, septate fungal hyphae characterized by light brown pigment and acute angle dichotomous branching. Tubules are occasionally dilated and contain low numbers of red blood cells and viable and degenerating leukocytes.

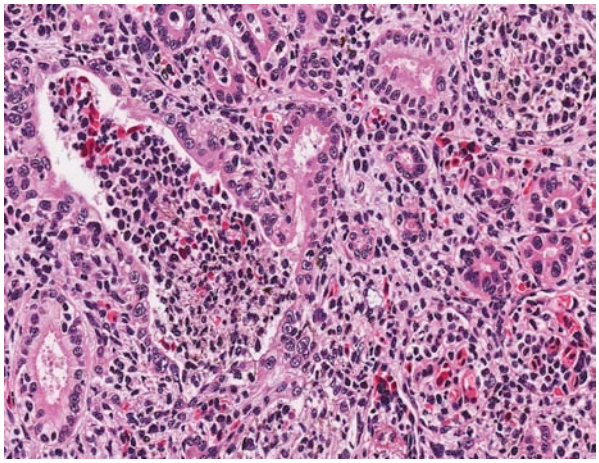
Liver: The periportal areas are infiltrated by moderate numbers of macrophages and scattered neutrophils, admixed with occasional fragments of pigmented fungal hyphae. Portal areas consistently contain four or more bile ductules (biliary hyperplasia). There are moderate numbers of clustered and individual melanomacrophages scattered throughout the hepatic parenchyma.

** Some slides also contain sections of testes, which display inflammation and necrosis similar to that observed in the kidney sections.

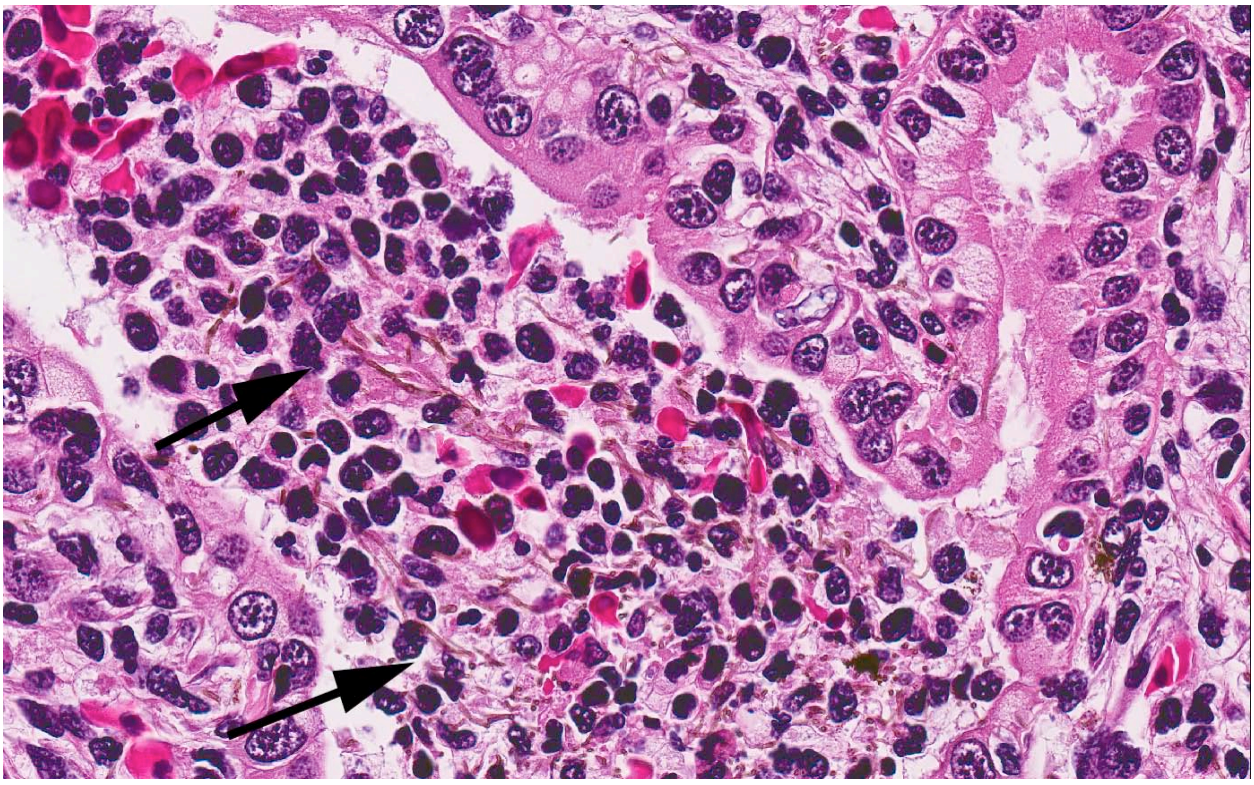
Contributor's Morphologic Diagnosis: 1. Kidney: Tubulointerstitial nephritis, neutrophilic and histiocytic, multifocal, subacute, severe, with necrosis, and intralesional pigmented fungal hyphae.
2. Liver: Hepatitis, periportal, histiocytic and neutrophilic, multifocal, subacute, moderate, with



3-1. Kidney, mudpuppy: The renal interstitium is markedly expanded by large numbers of neutrophils and histiocytes, effacing normal renal tubular structure. (HE, 120X)



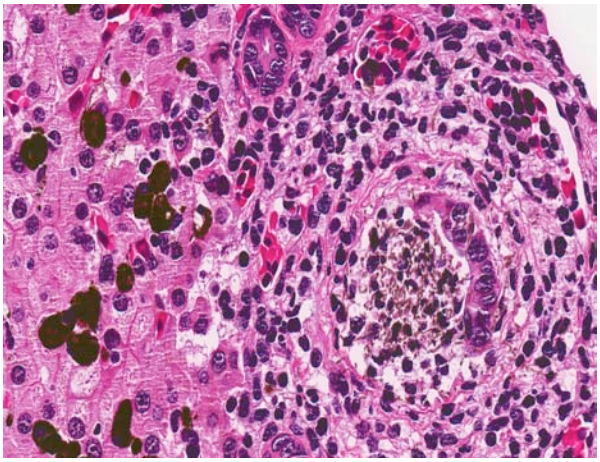
3-2. Kidney, mudpuppy: Numerous neutrophils, sloughed epithelial cells, and fewer histiocytes markedly distend renal tubules. (HE, 240X)



3-3. Kidney, mudpuppy: A higher magnification of this tubule reveals numerous pigmented fungal hyphae (arrows). Rare fungal hyphae are present within these areas. (HE 400X)

biliary hyperplasia, and intralesional pigmented fungal hyphae.

Contributor's Comment: Death of this mudpuppy was attributed to phaeohyphomycosis, a condition characterized by the presence in tissues of pigmented, or dematiaceous, hyphae, pseudohyphae, yeasts, or any combination of these forms.[12] Phaeohyphomycosis, which can be caused by over one hundred different types of pigmented fungi, encompasses a wide spectrum of opportunistic mycoses in humans and domestic animals, reports of which range from superficial cutaneous infection to fatal encephalitis.[1,2,5,6,12] Similarly, in amphibians, the disease may be limited to ulcerative or granulomatous lesions of the skin, or be disseminated systemically.[4] The term "phaeohyphomycosis" should not be confused with "chromoblastomycosis", which refers specifically to a localized cutaneous or subcutaneous pigmented fungal infection characterized by the presence of rounded sclerotic bodies.[12]



3-4. Liver, mudpuppy: Multifocally, pyogranulomas (right) are scattered thoughout the section. Fungal hyphae are present within pyogranulomas; in this case they are present within a degenerating biliary ductule. At left, numerous melanomacrophages are scattered throughout the hepatic parenchyma. (HE 400X)

Fungal culture of the mudpuppy's coelomic fluid yielded growth of a black mold morphologically consistent with an *Exophiala* species. As with other dematiaceous fungi, *Exophiala* is widely distributed in soil, decaying vegetation, and water. Species of *Exophiala* are notoriously difficult to classify and identify due to their complicated life cycles and morphologic plasticity, and recently molecular diagnostic tools have become important in defining the taxonomy of the group.[13,14] It has been reported as an emerging opportunistic pathogen in both immunocompetent and immune suppressed people,[14] including solid organ transplant recipients.[3,11] *Exophiala* species are significant pathogens in a

variety of fresh and saltwater fish including salmon,[8] flounder,[10] and sea dragons.[13]

Infection with dematiaceous fungi is typically initiated by introduction of the agent via an abrasive or penetrating wound, or by inhalation of fungal elements from the environment.[12] In this case, the presence a large mat of interwoven fungal hyphae within the central air space of one lung, and the lack of visible external wounds, were compatible with inhalation being the route of entry.

JPC Diagnosis: 1. Kidney: Nephritis, tubulointerstitial, pyogranulomatous, diffuse, moderate, with numerous pigmented fungal hyphae.
2. Liver: Hepatitis, pyogranulomatous, multifocal, mild to moderate, with rare pigmented fungal hyphae.

Conference Comment: Dematiaceous fungal hyphae are septate, 2-8 µm wide, and have non-parallel pigmented walls and random dilated, round, thick walled segments up to 25 µm in diameter that resemble chlamydospores or chlamydoconidium. Fungi grow in tissue as yeast, pseudohyphae and hyphae and may be extracellular and intracellular. Organisms that cause chromoblastomycosis, a condition mentioned by the contributor, are pigmented, but do not form hyphae in tissue, and their division by septation is distinctive. Additionally, they form large 4-15 µm, round, thick and dark-walled yeast-like cells, known as sclerotic bodies or muriform cells.[9]

There was some slide variation, and some sections had large hyphal mats within the ureter. This feature, along with the unusual and striking amount of inflammation within Bowman's space, interpreted as reflux tubulitis, led conference participants to consider an ascending infection.

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CASE IV: 07-2329-3 (JPC 4003032).

Signalment: Young fry (age not specified: estimated at 2 months), male and female (several fry submitted) brook trout (*Salvelinus fontinalis*).

History: Sudden and rapid increase in mortality in brook trout fry. The water temperature had risen from 10°C to 13-14°C in the past few weeks. No clinical signs were reported by the owner. Fingerlings were not affected. Over 90% of the fry died within a few days.

Gross Pathology: None (fry were sent opened and formalin-fixed).

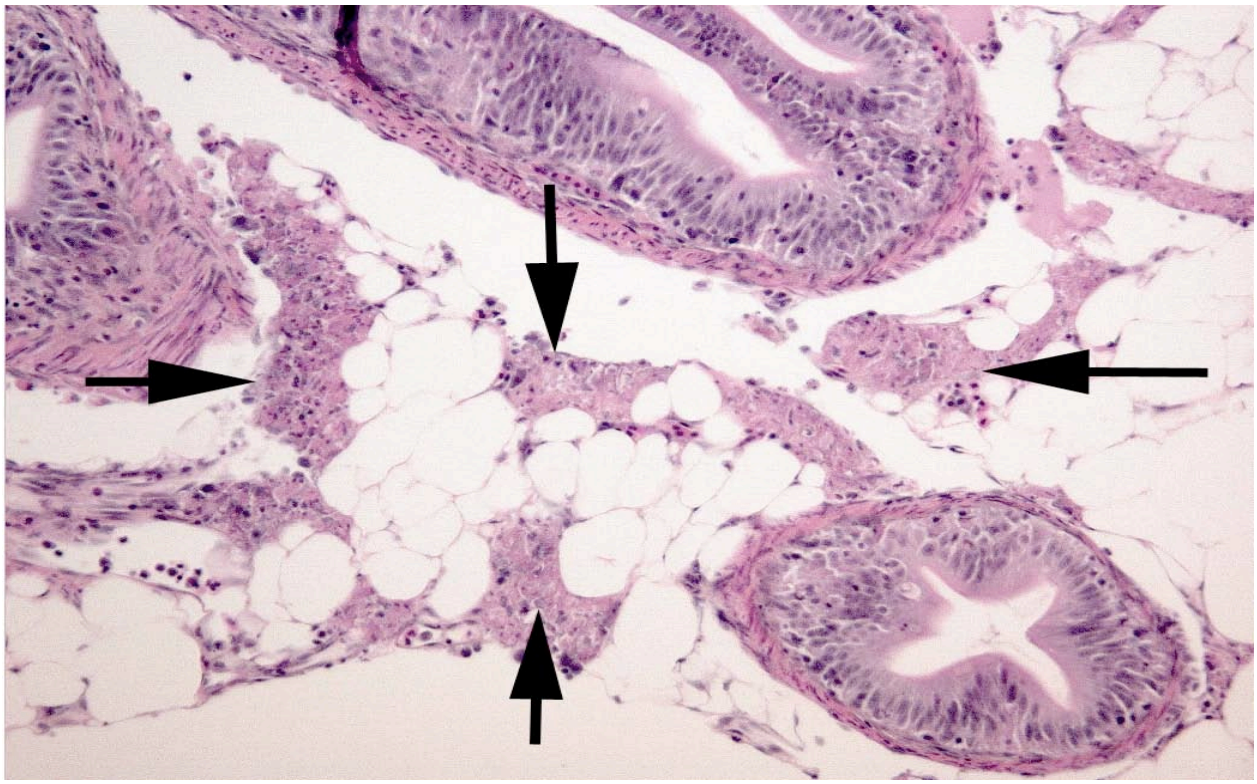
Laboratory Results: After the initial presumptive diagnosis was given, fresh fry from the same tanks were sent to a fish virology laboratory. Infectious Pancreatic Necrosis Virus (IPNV) was isolated in high titers.

Contributor's Histopathologic Description: Submitted slides are parasagittal sections from 4 fry (which were needed to show the lesions), so there is significant variation among slides. Depending on the sections, lesions vary from necrosis of single or small clusters of cells to massive coagulative necrosis of exocrine pancreatic epithelium. There are mild to moderate associated degenerative changes in the adjacent adipose tissue. Inflammation is essentially absent. In some sections, there is also occasional single cell necrosis of hepatocytes and/or pyloric cecal epithelium ("McKnight cells"). In a few sections, there is rare degeneration and necrosis of myocytes.

Contributor's Morphologic Diagnosis: Acute, multifocal exocrine pancreatic necrosis.

Contributor's Comment: Based on the case history, microscopic lesions and isolation of IPNV in high titers, Infectious Pancreatic Necrosis (IPN) was diagnosed. IPN is caused by an *Aquabirnavirus*, family *Birnaviridae*, a double-stranded RNA virus.[6] IPVN has a worldwide distribution; it is a major cause of mortality and economic loss in young freshwater-farmed salmonids, and in juvenile salmon transferred from fresh to sea water.[4,6] IPVN is a pathogen of salmonids, but has also been isolated in healthy individuals from many other fish species and aquatic invertebrates.[4]

IPN is a highly contagious disease of usually acute onset that only affects young fish (<6 months), especially fry; older fish can be infected but are chronic carriers.[4] It can present, as in our case, as a



4-1. Exocrine pancreas, brook trout. There is diffuse necrosis of exocrine pancreatic epithelium with no inflammation and mild degenerative changes in the adjacent adipose tissue. Cross sections of normal pyloric ceca are also present (HE 100X). Image courtesy of the University of Montreal, Faculty of Veterinary Medicine, Montreal, Canada (http://www.medvet.umontreal.ca)

sudden and rapid increase in mortality with minimal clinical signs. When present, clinical signs include dorsal darkening, trailing white feces, abdominal distension, exophtalmos, hemorrhages on the ventrum, pale gills and corkscrew spiral swimming.[4] Mortality is variable, but it is most rapid and severe at temperatures between 10-14°C[4]; it can approach 100%. There is no treatment, but fasting and lowering water temperature can diminish the mortality. IPVN infection is often limited to a carrier state; infection is lifelong in carriers and in fish that survive the disease. Many of these fish chronically shed the virus. IPVN can be transmitted vertically and horizontally. It has marked tropism for the exocrine pancreatic epithelium, but IPVN has been demonstrated in kidney, liver and occasionally in other organs in post-smolt Atlantic salmon (*Salmo salar* L.).[1] Findings of an *in vitro* study showed that IPNV induced apoptosis followed by postapoptotic necrosis in fish embryonic cells.[2] *In vivo*, IPNV has been shown to induce apoptosis in hepatocytes of post-smolt Atlantic salmon[6]; in the exocrine pancreatic epithelium, apoptosis has not been demonstrated.

Gross lesions in fry, when present, include pale viscera with a few petechiae, and a mucoid plug in the GI tract.[4] The most characteristic microscopic lesion of IPN is, as its name implies, exocrine pancreatic necrosis[3,5,6], with or without secondary degenerative changes in the adjacent adipose tissue. Other lesions that can be observed are apoptosis of pyloric cecal epithelium ("McKnight cells"), hepatocyte single cell necrosis/apoptosis, focal myocyte degeneration/ necrosis and, in severe cases, necrosis of renal tubular and interstitial cells.[4,5] Hepatic lesions have been shown to play a major role in IPN in post-smolt Atlantic salmon.[5] A presumptive diagnosis can be established by histopathology but must be confirmed by virological methods (e.g. virus isolation; RT-PCR). Virus isolation in itself is not confirmatory as IPNV can be demonstrated in clinically healthy/carrier fish; thus, a high titer should be present to establish a definitive diagnosis.[4] Immunohistochemistry can also be used, and has the advantage of antigen localization. Other viruses can cause exocrine pancreatic necrosis in salmonids; the main differential diagnoses for the lesions in our case are sleeping disease in rainbow trout (*Oncorhynchus mykiss*) and pancreas disease in salmon, both caused by closely related alphaviruses (*Togaviridae*).[8] However, in both cases the skeletal and cardiac muscles are also main targets, with significant necrosis, and pancreatic involvement is minimal in sleeping disease.[8]

JPC Diagnosis: 1. Exocrine pancreas: Necrosis, multifocal to coalescing.
2. Intestine: Enteritis, necrotizing, multifocal, mild.

Conference Comment: IPN is mainly a disease of first-feeding salmonid fry, although it has been found in many other fish species, as noted by the contributor. The reason for this selective species susceptibility is unknown. The main target tissues of IPN virus in salmonids are the mucosal epithelial cells lining the gastrointestinal tract and the interstitial cells and macrophages of the kidney. Some species, such as the brook trout, harbor the virus in leukocytes and kidney stem cells; whereas others such as halibut and turbot have affected cells in the intestine, liver, and kidney. Apoptosis is an early and primary mechanism of cell death associated with IPN, as seen in the McKnight cells of the intestinal epithelium, occurring within two hours of infection and well before more extensive necrosis occurs later in the disease.[7]

Conference participants considered the pyknosis and loss of cellular detail in some sections of liver to be autolysis. Conference participants also discussed the epithelial lifting in the secondary lamellae of the gills, which is likely an artifact of formalin fixation rather than edema.

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