Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 6

2 October 2019

Conference Moderator:
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Chief Scientific Officer/Pathology Manager
EPL, Inc.
Sterling, VA

CASE I: A15-62176 (JPC 4118173).

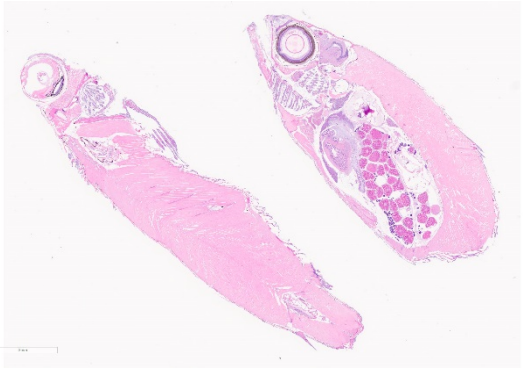
Signalment: Adult (age unknown), female, cardinal tetra (*Paracheirodon axelrodi*) fish

History: Following transport by air, high mortalities began in the group of fish approximately 24-hours after arrival in quarantine facilities at a public aquarium. There were no reported gross lesions. Skins scrapes, gill, and fin clips were reported as negative for external parasites.

Gross Pathology: Four fish were received whole fixed in formalin. Skin surfaces contained multiple, ill-defined areas of pallor and scale loss suggestive of erosion or ulceration.

Laboratory results: N/A

Microscopic Description: There is severe ulceration of the dorsal cranial region, snout, and lips, extending onto the ventral mandibular region and into the oropharynx. Lesions are characterized by extensive epithelial loss, with variable loss or necrosis of dermal connective tissues. Remaining dermal tissues are pale staining, have lost morphologic detail and are infiltrated by masses of a monomorphic population of slender, approximately 4-6 um long, bacterial rods. Necrosis and edema extend into underling muscle and adjacent soft tissues, including the olfactory rosette. Affected myofibers are similarly pale staining, with coagulated, vacuolated and fragmented



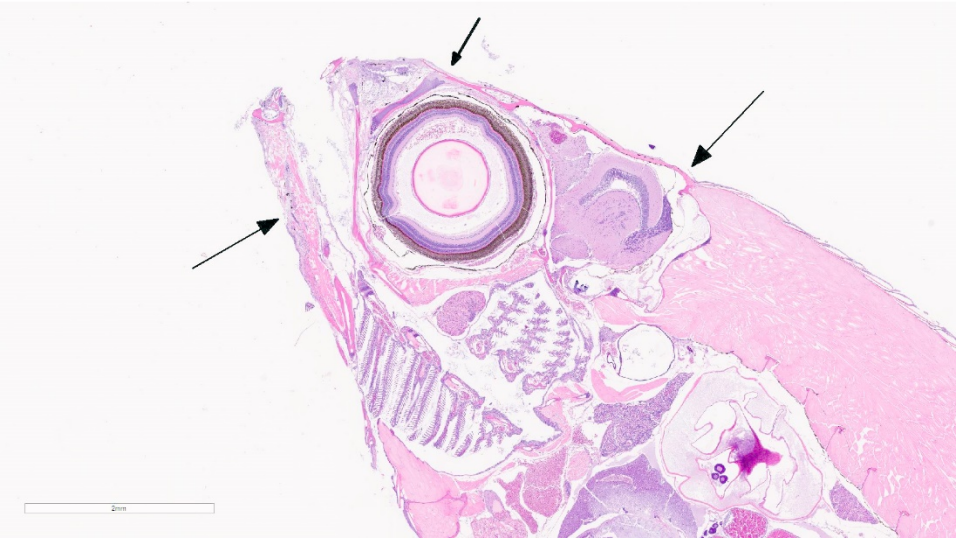
Body, cardinal tetra: Two sagittal sections of the tetra are submitted for examination. (HE, 5X)

cytoplasm, devoid of cross striations. Associated hypodermal adipose and areolar connective tissues are edematous, contain abundant cellular debris, and have lost tinctorial properties and morphologic detail. Bacteria are scattered throughout. Inflammatory responses vary from none to infiltration by small to moderate mixed populations of lymphocytes and macrophages. A focal area of hemorrhage extends from the forebrain into the cranial cavity.

Contributor's Morphologic Diagnosis: Facial skin: Ulcerative dermatitis, necrotizing, acute, focally extensive, severe, with slender bacterial rods, and subadjacent necrotizing myositis, cellulitis, and olfactory mucosal necrosis

Contributor's Comment: Microscopic examination reveals ulcerative skin lesions containing large numbers of long, slender bacterial rods consistent with *Flavobacterium columnare*, the causative agent of columnaris disease. Similar ulcerative lesions were present in all fish examined, varying only in lesion distribution and severity. Additional findings included the presence of several encapsulated larval nematodes in the coelomic cavity.

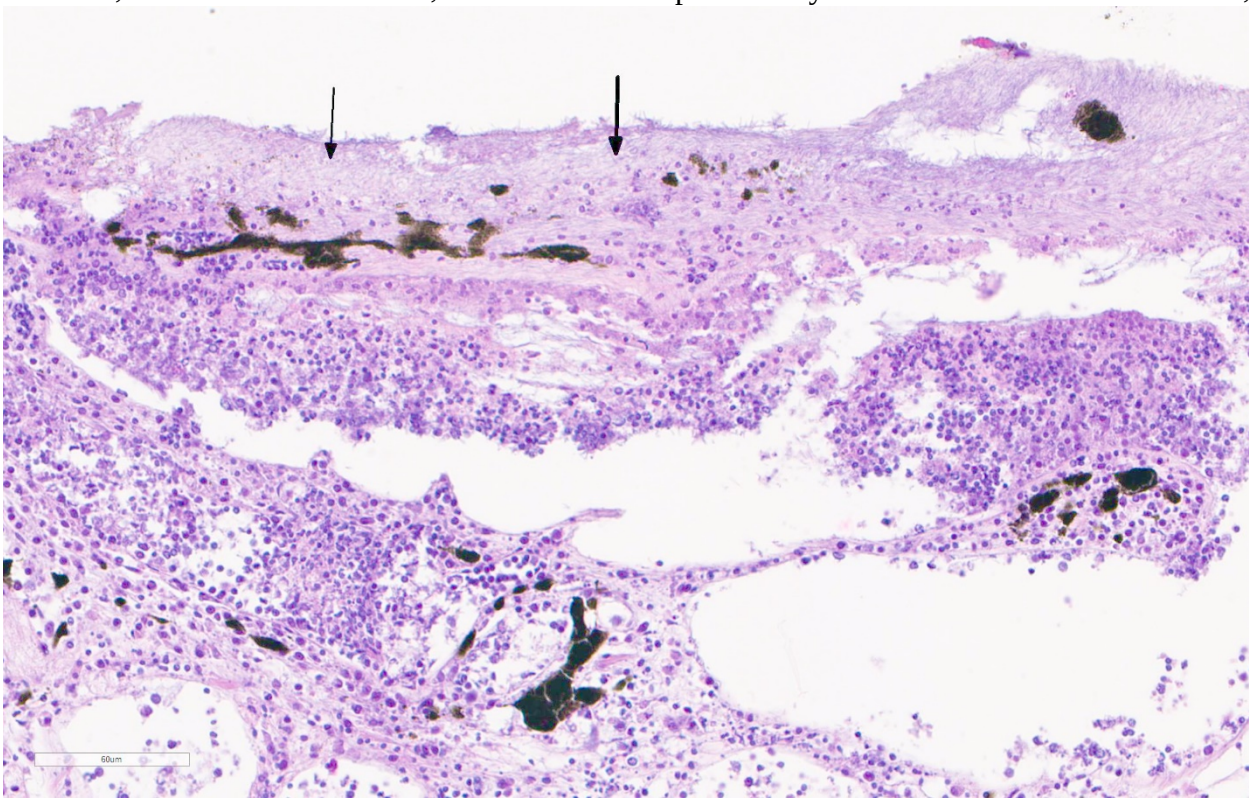
Columnaris disease occurs worldwide in many wild and cultured freshwater fish, including tropical aquarium species.[3] In the southeastern United States it is a major pathogen affecting channel catfish aquaculture.[4] The term "saddle back" is commonly used to describe symmetrical lesions over the dorsum of the back, but this is only one manifestation of the disease, which frequently involves various combinations of the gills, perioral area, fins, and caudal peduncle. The clinical course of disease, acute to chronic, and extent of lesions is dependent on the virulence of the bacterial strain involved.[3] Lesions generally begin as foci of depigmentation and erosion, but can rapidly progress to large ulcers with exposure of underlying skeletal muscle. Lesions often have a yellowish discoloration and narrow hemorrhagic border. Microscopically, lesions are primarily necrotizing, often in the presence of massive numbers of the long slender bacteria, particularly within dermal connective tissues. Bacteria are readily visualized in H&E- and Giemsa-stained sections. Despite the large numbers of bacteria and their destructive nature in tissue, inflammatory cell infiltration is often minimal. Secondary infection



Body, cardinal tetra: There is diffuse loss of the epithelium over the face and within the oral cavity between the two arrows. (HE, 19X)

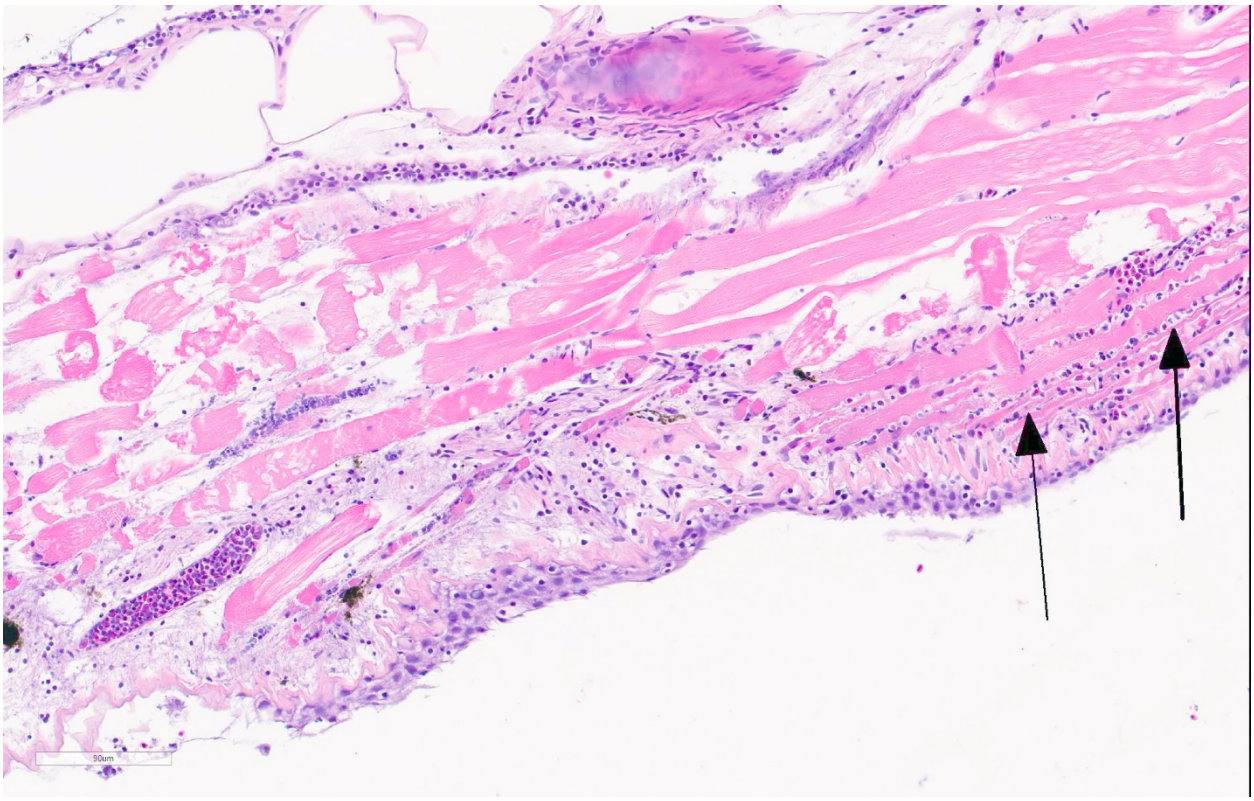
by the oomycete *Saprolegnia* sp. is common.[3,4]

Flavobacterium columnare is widespread in freshwater environments and survival is promoted in hard alkaline water with high organic loads. While highly virulent strains of *F. columnare* exist, the bacteria often presents as a typical opportunist following episodes of environmental stress, such as transport, as seen in this case. Other environmental factors conducive to infection include higher temperatures, high stocking densities, elevated nitrite levels, and slow water flow. Disease pathogenesis is poorly understood, but may initially involve factors related to the ability of the bacteria to respond to chemotaxic factors, adhere to host surfaces, and aggregate in thick mats. Three genomovars of *F. columnare* with variable pathogenicity are known to exist. Pathogenicity is enhanced by the presence of a thick capsule and the production of chondroitin AC lyase, which degrades chondroitin sulfates and hyaluronic acid in connective tissues. Extracellular proteases also contribute to tissue damage and promote invasion. Some studies indicate impairment of the host alternative complement pathway through sialic acid production and it is hypothesized that lack of inflammation may be related to the production of pro-apoptotic factors that inhibit phagocytes. Reported disturbances in blood parameters, presumably the result of water imbibition, include decreases in PCV, electrolytes and serum proteins.[3]



Snout, cardinal tetra. The denuded dermis of the snout is edematous, infiltrated by innumerous degenerate lymphocyte and histiocytes, and is covered by a mat of 1x5 um filamentous bacilli which are present at all levels of the inflamed dermis.(HE, 200X)

The name “columnaris disease” has been used consistently since the condition was first described in 1922. However, the taxonomic status of the agent has been revised numerous



Jaw, cardinal tetra. The skeletal muscle of the jaw is multifocally necrotic, as evidenced by vacuolation, fragmentation, and loss of satellite nuclei, and infiltrated by degenerate inflammatory cells. The interstitium contains numerous filamentous bacilli. (HE, 234X)

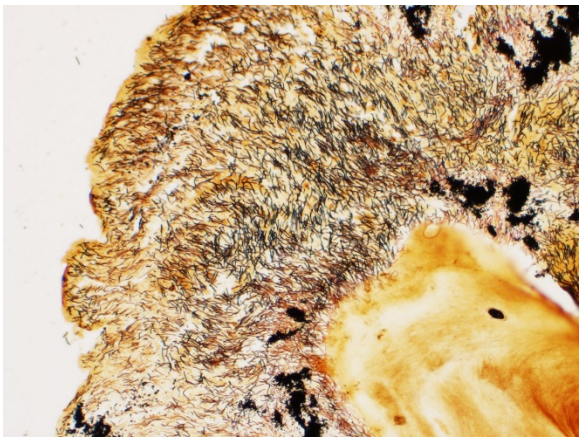
times and includes the earlier combinations *Bacillus columnaris*, *Chondrococcus columnaris*, *Cytophaga columnaris*, and *Flexibacter columnaris*.[3] The current name, *Flavobacterium columnare*, was recognized in 1996.[2] The genus *Flavobacterium* contains additional important fish pathogens that can produce lesions similar to *F. columnare*, including *Flavobacterium psychrophilum*, the cause of coldwater or peduncle disease in salmonid and other cold freshwater species,[6] and *Tenacibaculum maritimum*, in marine fish.[1] Collectively, bacteria in this gram-negative genus average 2-5 µm in length, although forms up to 40 µm can occur. Longer rods are flexible and move by gliding motility. Colonies are typically yellow, a product of non-diffusible carotenoid or flexirubin-type pigment production.[2]. Isolation of the bacteria in culture requires the use of low nutrient agar media, such as Shieh or tryptone yeast extract salts (TYES). The organism will now grow on standard bacterial media such as trypticase soy agar (TSA). Confirmatory diagnostic tests include ELISA, FA, and LAMP methods, as well as conventional and qPCR procedures.[3]

Contributing Institution:
Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, www.vet.uga.edu/VPP

JPC Diagnosis: Head: Dermatitis, necrotizing**,** and lymphocytic, focally extensive severe, with skeletal muscle degeneration and necrosis and innumerable filamentous bacilli.

JPC Comment: The contributor has given us an outstanding review of this common bacterial pathogen of a wide range of

freshwater fish. *Flavobacterium columnare* has been seen as a causative factor in a number of large-scale fish dieoffs, to include multiple species in separate reported dieoffs in the Buffalo Pound and Blackstrap Lakes, which affected yellow perch and lake whitefish respectively, and several thousand carp in the St. Lawrence river.[8] In these mortality events, *F. columnare* was considered one of a number of factors, to include environmental stress due to ambient heat and lessened oxygen availability, and other bacterial pathogens, including *Aeromonas hydrophila* which was co-cultured from the dead fish.[8]



Snout, cardinal tetra. A silver stain demonstrates the large number of Flavobacterium infiltrating the denuded dermis and underlying tissue. (Warthin-Starry 4.0, 400X)

Increased water temperature has been noted as a major contributoring factor to the increase in *F. columnare* infections on a global basis. It was identified in a study by Pulkkinen et al. as a major factor in the increase in virulence of *F. columnare* over a period of 23 years in salmon farms in Finland. In addition to higher temperatures causing stress to fish,[6] global warming also extends the periods in which *F. columnare* can grow and cause outbreaks, as well as facilitate faster tissue invasion by virulent strains as increase in chondrolysin lyase activity (facilitating dermal invasion) is seen at higher temperatures.[3] In farmed fish, increased stocking levels contribute to outbreaks of *F. columnare* by increasing the organic load in the water, as well as nitrate concentration and the possibility for co-infections with other bacterial pathogens or ectoparasites.[3]

The moderator commented on the acute nature of the lesion which was illustrated by the lack of granulomatous inflammation, and believes that many of the inflammatory cells present within the lesion are lymphocytes and few are actually tissue macrophages (as would fit with the history of fish death within 24 hours after the stress of transport, as well as the normal progress of the disease – "the fish go down quickly".) as bacteria may be lost before autopsy, the moderator recommends wet-mounted skin scrapings in animals that are still living. After approximately 15 minutes, F. columnare will assume a typical "haystack" formation for which it is famous.

A differential diagnosis for facial necrosis in ornamental fish is spaC-type *Erysipelothrix.*[5] This report describes a disease of ornamental fish resulting in facial cellulitis, necrotizing dematitis and myositis and disseminated coelomitis with numerous colonies of gram-positive organisms. *Erysipelothrix* was recovered by from numerous animals but were genetically divergent from existing species of *E. rhusiopathae* and *E. tonsillarum* known to be pathogenic in fish and marine mammals.[5]

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CASE II: AP 17-4634 (JPC 4120175).

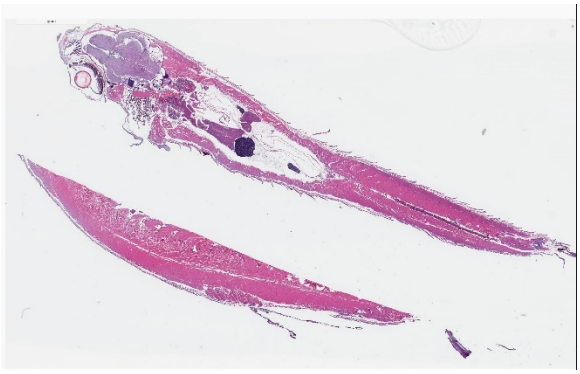
Signalment: 10-month-old male wildtype line (unspecified), zebrafish, *Danio rerio*

History: A wild-type control zebrafish for a leukemia study was swimming in circles and removed from the study for pathology evaluation.

Gross Pathology: No notable gross findings were observed.

Laboratory results: N/A

Microscopic Description:
There are randomly distributed and variably sized cross-sections of parasitic complexes containing numerous spores within the white mater of the posterior brain and brain stem. The majority of parasitic spores appear as uninucleate structures by H&E staining and are approximately 3.0 µM wide x 5.0 µM long with posterior vacuoles. Low numbers of glial cells are found at the periphery of some complexes while the neuropil appears devoid of cells at other locations were



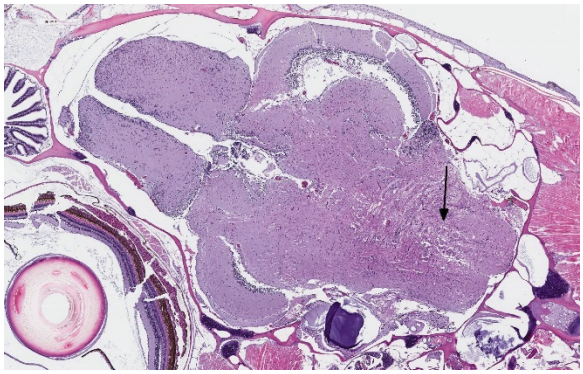
Zebrafish, sagittal sections. Two sections are presented for examination. No lesions are present on subgross examination. (HE 5X)

complexes are observed. Spores were not detected in other sampled tissues.

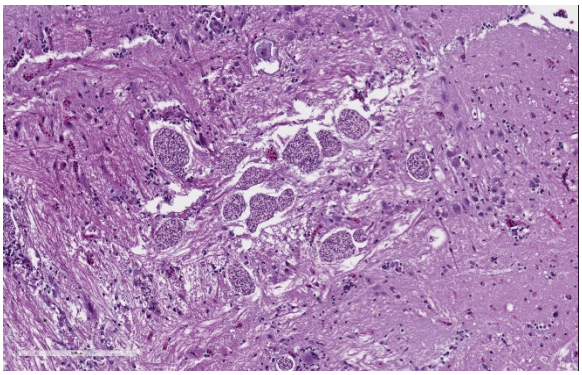
Contributor Morphologic Diagnosis:
Brain, neuropil: Parasitic complexes, multifocal, with morphology that is most consistent with *Pseudoloma neurophilia* infection.

Contributor Comment: *Pseudoloma neurophilia* is a commonly encountered and well described microsporidian parasite of zebrafish. Unlike other microsporidia, *P. neurophilia* does not appear to form xenomas. Histologic and ultrastructural studies of oral exposure to the proliferative parasitic stages show that early developmental phases interface with zebrafish host cells through a glycocalyx-like coating. Sporophorous vacuoles are then formed where the sporogonic stages remain to undergo karyokinesis, producing tetranucleate stages that then divide into uninucleate sporoblasts and spores. Spores remaining within these parasite complexes may or may not elicit an immune response.[1]

Recent studies are better clarifying the genomic basis of P. neurophila infections as well as the effects of silent infections on research.[1,4,8-10] P. neurophilia may be transmitted either through a vertical or horizontal route. It often presents in zebrafish colonies as a chronic, subclinical infection that primarily affects the brain and skeletal muscle.[5] Infected fish demonstrate a range of presentations included altered behavior, reduced growth or spinal deformities (e.g., lordosis and kyphosis). Alternatively, there may be no visible indications of infections and only baseline mortality rates for a facility. The variable clinical presentations and the potential for false negatives using either histology or molecular testing when conducting sentinel surveillance makes eradication of microsporidial infections challenging. Additionally data gathered from fish from infected colonies has the potential for inconsistent results for behavioral, immunology and hematopoiesis studies. The negative effects of microsporidial infections in colonies used for research studies is especially profound when immuno-suppressive therapies, like gamma irradiation, are used as parasitic infections often worsen. Interestingly aspects of the subclinical disease as well as understanding how these infections become clinically significant following immunosuppression in zebrafish are being advanced as one way to study aspects of microsporidial infections in humans.[4,6,8,9]



Brain, zebrafish. A cluster of microsporidial sporophorous vesicles are present within the hindbrain. (HE, 36X)



Brain, zebrafish. Sporophorous vesicles measures up to 60um in diameter and contain numerous spores. (HE 200X)

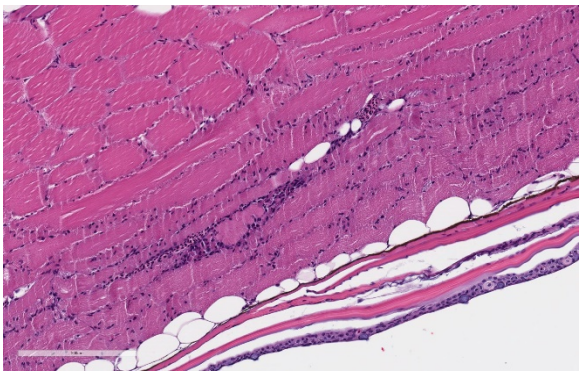
Contributing Institution:
Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, www.vet.uga.edu/VPP

JPC Diagnosis: Hindbrain: Microsporidial xenomas, multiple.
2. Skeletal muscle: Degeneration and necrosis, multifocal.

JPC Comment: *Pseudoloma neurophilium* is a common microsporidial parasite of zebrafish. Transmission occurs vertically and horizontal routes of transmission are the result of consumption of environmentally resistant spores in water contaminated by carcasses and eggs. Standard sterilization techniques, such as bleaching, do not impact spores, and spores contained within embryonated eggs are shielded from disinfectants.[9]

A recent study also demonstrated reduced survivability of *P. neurophila* spores following cryopreservation.[3] This is an important finding as cryopreservation of sperm is widely used in the maintenance and distribution of many strain type zebrafish used in research. Other zebrafish pathogens examined in this fashion were *Mycobacterium marinum* and *chelonae* (minimal impact upon freezing and thawing), and eggs of *Pseudocapillary tomentosa* (no survival).[3]

Due to their social nature, zebrafish are used in a wide variety of behavioral research studies on human diseases, such as autism, schizophrenia, depression, and PTSD, as well as to study the behavioral effects of a wide variety of pharmaceuticals.[8,11] Startle behavior, shoaling, and interfish distance of laboratory zebrafish are all interpretable responses in judging behavioral abnormalities. Fish infected with *P. neurophilia* have been shown to have



Skeletal muscle, zebrafish. Multifocally, occasional myofibers of the skeletal muscle are shrunken, granular, and low numbers of lymphocytes and histiocytes invade the perimysium. (HE, 200X)

abnorming shoaling behavior and interfish distance, which may potentially complicate behavioral studies using this animal model.[11]

A recent publication from the research group at Oregon State University at Corvallis, one of the leading groups in the area of zebrafish research, recently identified a number of species of aquarium fish that may also be infected with *P. neurophilia* in a mixed-species aquarium setting.[7] While long considered to be an infection restricted to *Danio rerio*, there are now five families and eight species which have been identified to be infectable with this parasite. The species include giant danio, medaka, fathead minnows, goldfish, platys, Siamese fighting fish, and neon tetras.[7]

Contributing Institution:
St. Jude Children's Research Hospital, Department of Pathology
https://www.stjude.org/research/departments-divisions/pathology.html

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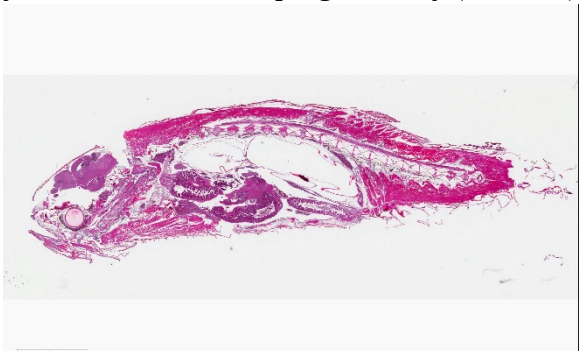
CASE III: AFRIMS Case 1 (JPC 4113195).

Signalment: Adult, male zebrafish (*Danio rerio)*

History: This animal was one of four maintained in a private aquarium and was >1 year old. The animal progressively (1 month)



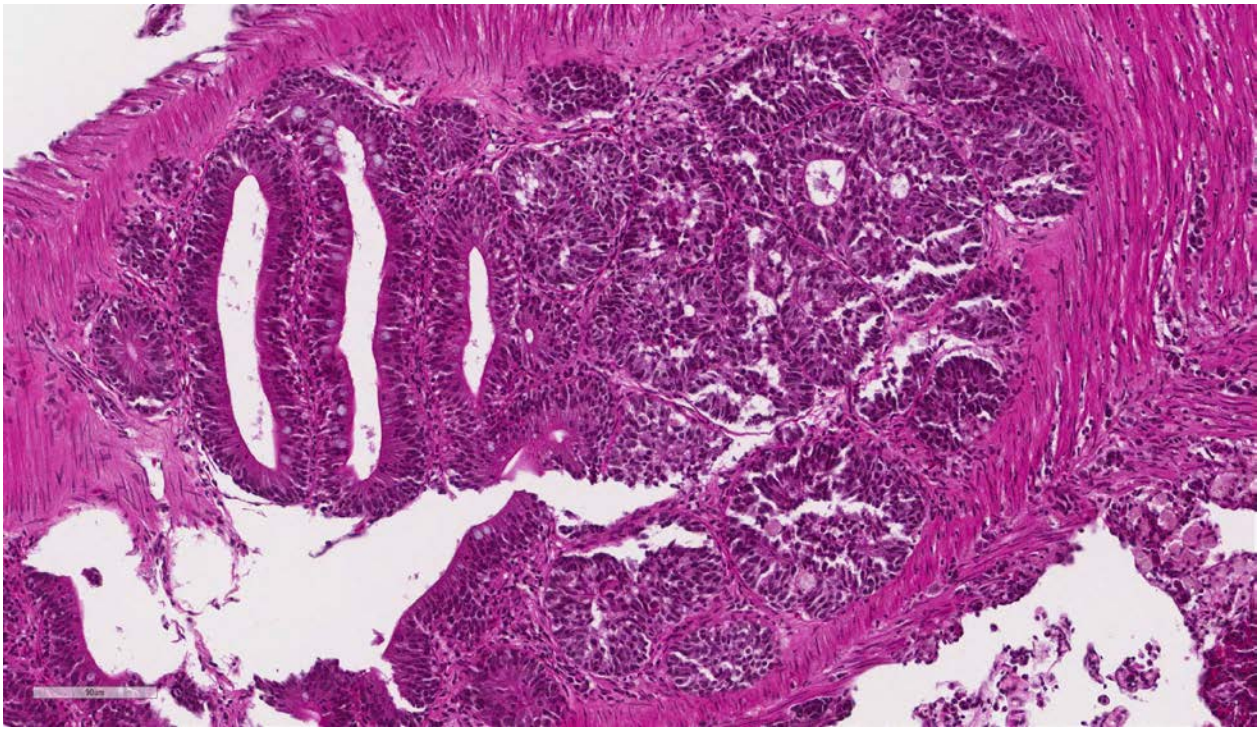
Presentation, zebrafish. A sagittal section of a zebrafish is submitted for examination. (HE 7X)

was observed to have developed a swollen abdomen and then became anorexic for 1 week. The last two days prior to euthanasia, the animal lost buoyancy control and swam irregularly within the water column. Necropsy did not reveal any abnormalities. All other animals in the tank were within normal limits.

Gross Pathology: Necropsy did not reveal any abnormalities.

Laboratory results: N/A

Microscopic Description:
Intestine: Multifocal to coalescing, markedly expanding and effacing the intestinal mucosa and submucosa and extending multifocally into the muscularis is a densely cellular, unencapsulated, poorly circumscribed neoplasm composed of polygonal cells arranged in abortive tubules in which the cells pile up and frequently have lost polarity, as well as disorganized islands and trabeculae on a fine fibrovascular stroma. Neoplastic cells have variably distinct cell borders, small to moderate amount of basophilic granular cytoplasm, oval to elongate nuclei with dense, finely stippled chromatin and occasionally 1 distinct nucleolus. Mitoses are infrequent at approximately 1 per 3 high powered fields observed. There is moderate anisocytosis and anisokaryosis, and often neoplastic cells within tubules appear to undergo maturation to mucoid cells which are characterized by large polygonal cells with abundant microvacuolated eosinophilic cytoplasm and small peripheralized nuclei with dense chromatin and no observable nucleoli. No mitoses were observed in this population. Neoplastic tubules are occasionally either occluded by the proliferating cells or are rarely ectatic and contain eosinophilic cellular and karyorrhectic debris, mucous, sloughed cells, scattered granulocytes and lymphocytes.



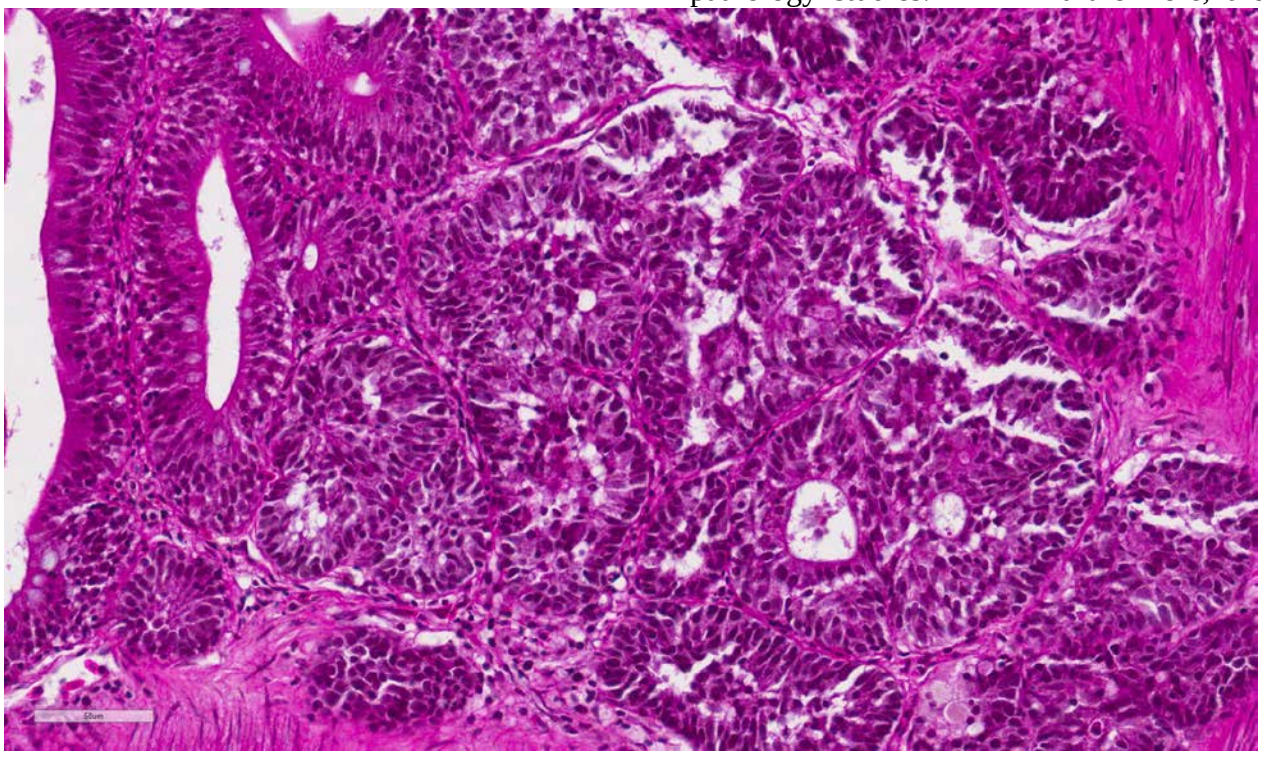
Intestine, zebrafish. The architecture of the large segment of the intestine at right is markedly altered, with a loss of typcal villar arthcitecture, and formation of prominent acini which resemble crypts. (HE, 100X)

Multifocally, extending transmurally and occasionally disrupting the serosal surface, the myocytes within the affected muscularis are separated, surrounded and replaced by small islands of neoplastic cells, eosinophilic amorphous material (edema), streams of lymphocytes and granulocytes. In these areas, myocytes are vacuolated and swollen (degenerate). Adjacent mucosa, unaffected by the neoplastic process, is frequently hyperplastic with epithelial piling and formation of papillary fronds which occasionally occlude the crypts. Focally, within a pancreatic vessel there is an organizing fibrin thrombus; however neoplastic cells are not observed within vessels or lymphatics. Occasionally rhabdomyocytes within the body wall are degenerate, necrotic or atrophic and separated by the previously described edema and inflammatory cells.

Liver; kidney; rectum; branchial arch; gill; thyroid gland; esophagus; oral cavity; brain; eye; spinal cord; vertebral column; air sac: No significant findings.

Contributor's Morphologic Diagnosis:
Intestine: Adenocarcinoma

Contributor's Comment: Fish have been and continue to be used extensively as comparative animal models for human neoplastic disease, and the zebrafish is by leaps and bounds the primary species employed. There are many reasons why zebrafish have made such headway into the fields of cancer research, and while the adult animal may appear to share little with primates, there are a wide range of evolutionarily preserved signaling pathways, translational regulation of cellular division and molecular markers of neoplasia which make fish extremely relevant to comparative pathology studies.[5,9,10,11] Furthermore, the



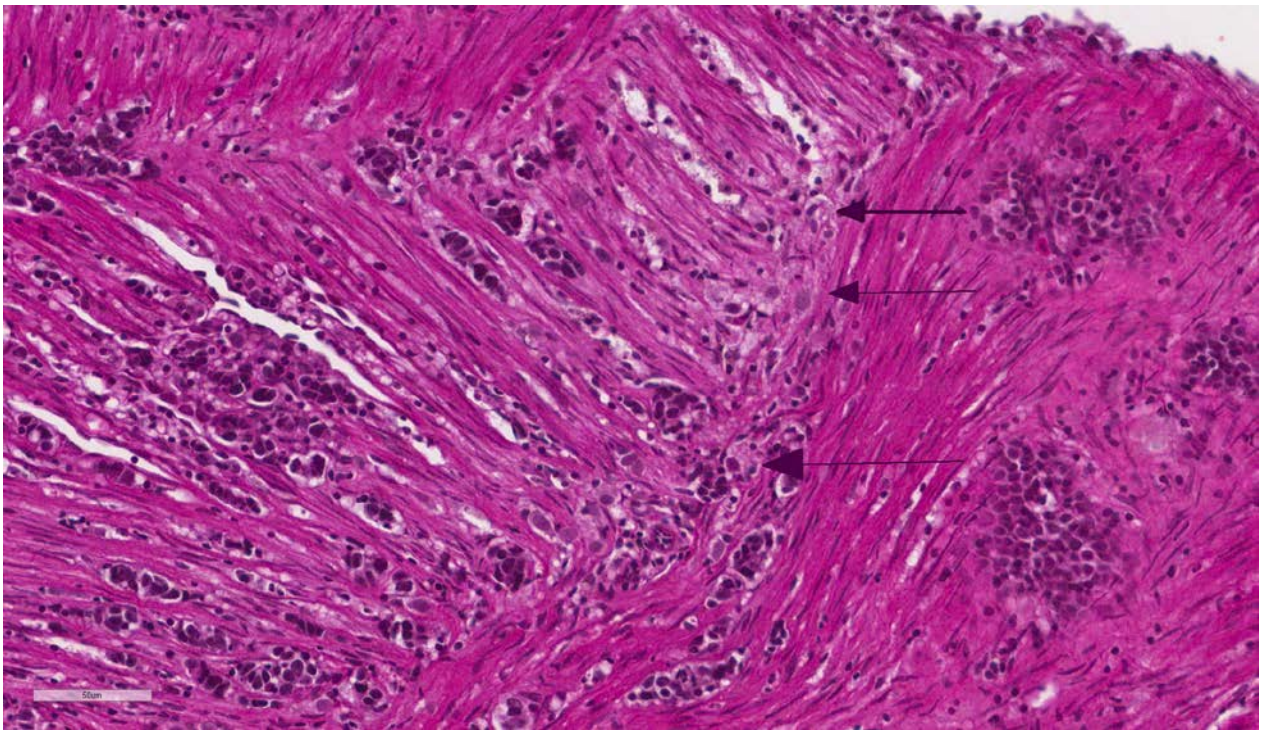
Intestine, zebrafish. Areas of dysplasia and acinar formations of neoplastic cells replace normal architecture. (HE, 234X)

embryology of fish and humans share significant commonality and as such present a valuable resource for study of the development of human disease, especially with embryonal tumors.[5,10] A recent and extremely exciting development of a transparent adult zebrafish allows for in vivo assessment of neoplastic metastatic behavior using labeled neoplastic cells.[13]

In addition to spontaneous tumors, neoplastic disease has been induced in fish and in particular in zebrafish using a variety of methods. genetic manipulation, xeno-transplantation, chemical carcinogenesis, forward and reverse genetic screens and radiation induction of neoplasia have all been described.[5,9,10,11,12] Mutant zebrafish lines have been created which are highly susceptible to the development of tumors, especially, but not limited to, those which are rare in other vertebrate species such as chordomas, pineocytomas, hepatoblastomas, ocular medulloepitheliomas and olfactory esthesioneuroepitheliomas.[12]

Zebrafish lack a stomach, and as such, the esophagus connects directly to the intestine. At a cellular level, with the exception of Paneth cells, the zebrafish intestine is composed of all of the same cell types which make up the intestinal tract in mammalian species. The intestine is a contiguous simple tube which has morphological differences at the cellular level, primarily associated with the cellular populations and changes in the epithelium along its length, but that does not have macroscopic features which allow differentiation of large and small sections. [6,10]

Intestinal adenocarcinoma in zebrafish has been reported to occur with some frequency



Intestine, zebrafish. Neoplastic cells, seen here infiltrating the intestinal wall, have two morphologies – a more mature mucous-like cells, and more numerous small cells with a small rim of eosinophilic cytoplasm surrounding a hyperchromatic nucleus. (HE,400X)

as a spontaneous neoplasm,[2,3,7,10,11] as well as occurring in the genetically modified adenomatous polyposis coli-deficient zebrafish (APC) model.[2,3,10] Additional reports of experimentally induced intestinal tumors have been associated with other genetically modified zebrafish lines, animals exposed to carcinogens and transgenic as well as in association with the nematode parasite, *Pseudocapillaria tomentosa*.[4,10]

A large study was conducted at Oregon State University examining submissions to the Zebrafish International Resource Center (ZINC) over a 10-year period. Approximately 2% of the cases submitted during that period were diagnosed with intestinal lesions, and of those, 113 tumors were diagnosed, with greater than 50% receiving diagnoses of adenocarcinomas.[7] The histopathological criteria for diagnosis of the various intestinal lesions, which were described in the evaluation, are abstracted below as Table 1 adapted from Paquette et al.'s retrospective study on intestinal neoplasia in zebrafish.[7]

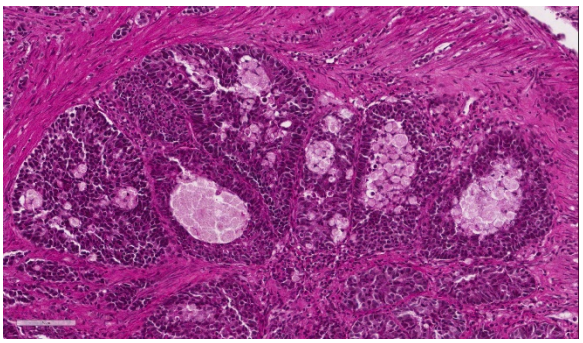
Table 1. Defining Histological Signs of Intestine Presentations as Observed within Zebrafish Submitted to the Zebrafish International Resource Center Diagnostic Service 2000-2012[7]

Intestinal Presentation	**Defining Signs**
Normal Intestine	One cell thick layer of columnar epithelial cells lining mucosal folds with basally-oriented oval nuclei; mucosal folds become progressively shorter caudally, causing "villi" (the normal undulating structure of the intestinal wall appears villous, but lacks the true anatomic characteristics of villi) to appear shorter as the intestine approaches the excretory vent (anus); lamina propria, but no submucosa; inner circular and outer longitudinal smooth muscle layers invest the intestine throughout its length. Mucosal mucus (goblet) cells can be observed and increase in number distally.
Hyperplastic Intestine	Multilayered and increased numbers of epithelial cells, especially within basilar mucosal folds; "piling-up" of mucosal epithelial cells; nuclear pseudostratification; enhanced nuclear basophilia; pseudocrypt formation resulting from increased mucosal folding; anisokaryosis frequently observed and increased mitotic figures.
Dysplastic Intestine	Features of hyperplastic intestine in addition to increased nuclear and cellular pleomorphism, and occasionally aberrant mitotic figures, the

	loss of nuclear polarity and disorganization or absence of pre-existing histoanatomic architecture
Intestinal adenocarcinoma	Features of dysplastic intestine plus formation of disorganized pseudocrypts with invasion deep into the lamina propria and frequently through the basement membrane into the underlying muscularis layers; bizarre mitotic figures; neoplastic epithelial cells are pleomorphic and may be columnar, cuboidal or attenuated; hyperchromatic nuclei; annular strictures and fibroplasia frequently accompany tumorigenesis; pseudocrypts formed by the folding of neoplastic mucosal epithelium often resembled pseudoacinar structures that contained intraluminal sloughed rafts of necrotic neoplastic cells
Intestinal small cell carcinoma	Sheets and nests of round, polygonal or fusiform cells with minimal cytoplasm; hyperchromatic nuclei with granular chromatin and inconspicuous nucleoli; extensive fibroplasia; tumor cells occasionally formed an insular or organoid pattern characteristic of neuroendocrine tumors.
Intestinal tubular/tubulovillous adenoma	Focal adenomatous polypoid structures with clusters resembling mammalian glandular colonic crypts. The pseudocrypts often are lined by hyperplastic mucosal epithelium where the cells are crowded and have hyperchromatic nuclei. Increased mitotic figures are observed. Tubulovillous adenoma is essentially similar to tubular adenoma with a combination of both villous and pseudocrypt structures

The neoplasm in this case was diagnosed as part of the histopathological evaluation of the sampled tissues, rather than as a post-mortem necropsy finding. The clinical symptomology prior to euthanasia is consistent with the final diagnosis, however is relatively non-specific for any abdominal neoplasm as well as a wide range of other pathological processes. The histo-pathological features of this tumor are consistent with a diagnosis of intestinal adenocarcinoma.

Contributing Institution:
Armed Forces Research Institute of Medical Sciences (AFRIMS). http://afrims.amedd.army.mil/usamd-afrims.html

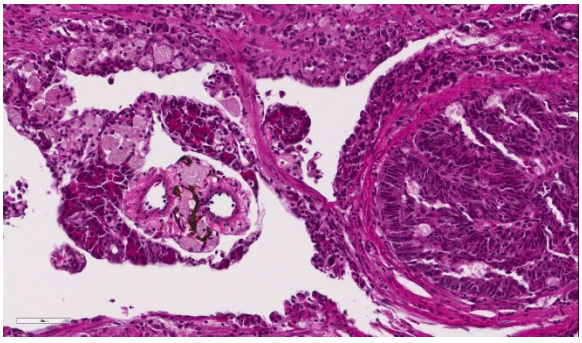


Intestine, zebrafish: Dilated acinar lumina contain sheets of necrotic mucus-producing cells. (HE, t34X)

JPC Diagnosis: 1. Intestine: Adenocarcinoma.
2. Pancreas: Intestinal adenocarcinoma, metastatic.

JPC Comment: The contributor has provided an excellent and comprehensive review of this neoplasm and its importance in a common laboratory species. Realizing the complexity of diagnosis of these tumors based solely on their morphologic appearance,[7] Paquette et al. in 2015[8] published a study detailing the immunohistochemical profile of these tumors, identifying them as neoplasms of epithelial origin (rather than their primary differential of tumors of neuroendocrine origin.) These neoplasms stained positive for AE1/AE3 (cytokeratin), while no tumors exhibited any immunopositivity for neuroendocrine markers (chromagranin A or S-100.)[8]

An interesting study by the Oregon State group in 2018 strongly suggests that this particular entity is transmissible.[1] The condition has been identified across zebrafish of multiple genetic backgrounds, suggesting genetics did not play a strong role. Feeding diets of fish with a high prevalence to fish with a low prevalence did not increase the frequency of the finding, suggesting diet was not a factor. Connecting tanks of fish with high prevalence to those of low prevalence in the same recirculating system did not result in a spike in tumors. Following a cohabitation protocol with fish with a known high incidence of the tumor, sampling of the biome of these fish with high-throughput 16S rRNA sequencing was performed, which indicated a high rate of infection in animals with tumors with a yet unidentified species of *Mycoplasma*, presumably spread to naive fish via a fecal-oral route. The authors do admit that a) other agents, potentially oncogenic viruses, may still be causative or play a role in causation, and b) the *Mycoplasma* infection may be a result of the pathological change rather than its cause. They recommend managing fish with the condition as having a potentially transmissible condition.[1]



Intestine, zebrafish: Neoplastic cells infiltrate the pancreas (left) and line the intestinal serosa (right). (HE, 400X)

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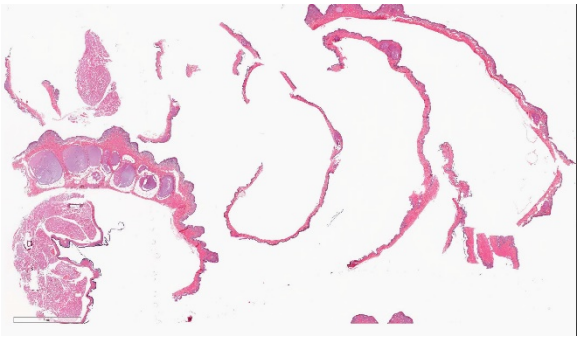
CASE IV: WSC 18-19 #2 (JPC 4117006).

Signalment: 1-year-old, male, Puerto Rican crested toad *(Peltophryne lemur)*

History: The toad was found dead in its enclosure after an approximately several weeks long treatment for skin issues.

Gross Pathology: At necropsy, the toad was moderately desiccated and the skin of the ventrum and legs was diffusely brown and was easily peeled away from the body.

Laboratory results: N/A

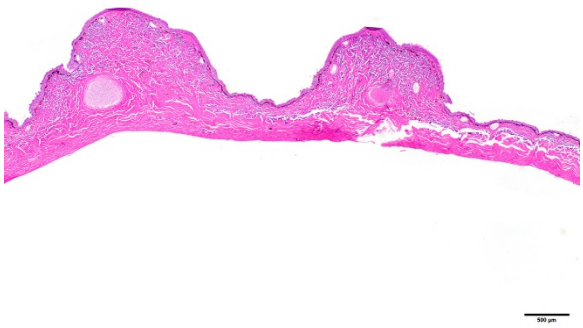


Skin, Puerto Rican crested toad. Multiple sections of skin are submitted for examination. (HE, 5X)

Microscopic Description:
There is multifocal to diffuse hyperplasia of the epidermis which is often covered by orthokeratotic hyperkeratosis. This keratin layer is often deep brown. Within the hyperplastic epidermis there are rare apoptotic keratinocytes and intracellular edema (spongiosis).There is a nearly diffuse layer of prominent melanocytes within the superficial dermis immediately adjacent to the basal layer of the epidermis. There is frequent thickening of the dermal Eberth-Katschenko (calcium) layer.

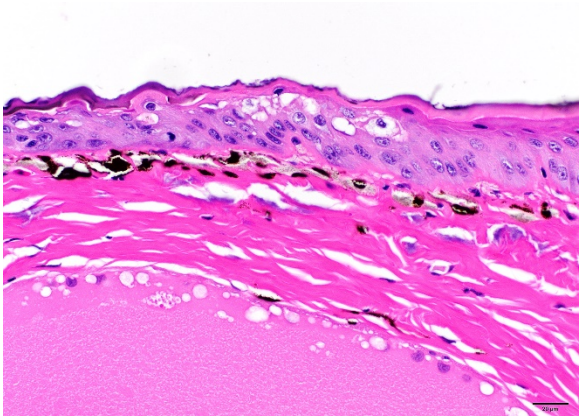
Contributor Morphologic Diagnosis:
Skin, Epidermal hyperplasia, orthokeratotic hyperkeratosis, spongiosis, rare keratinocyte apoptosis, and dermal melanocytosis, moderate, diffuse with thickened Eberth-Katschenko layer

Contributor Comment: The changes seen in the sections of skin from the submitted toad are consistent with the entity described as Brown Skin Disease of Puerto Rican Crested Toads (PRCT). Brown skin disease (BSD) is an idiopathic disease that has only been described in this species of toad.



Skin, Puerto Rican crested toad. Epidermal hyperplasia with markedly thickened Eberth-Katschenko layer. (HE, 20X) (Photo courtesy of: Kansas State Veterinary Diagnostic Laboratory, Department of Diagnostic Medicine/Pathobiology)

Grossly the disease is characterized by leathery, brown discoloration of the skin that can occur anywhere on the body with abnormal shedding of the skin (dysecdysis) particularly on the ventrum.[1] Microscopically BSD is typically characterized by epithelial hyperplasia, spongiosis, orthokeratotic or parakeratotic hyperkeratosis with “nuclear ghosts” in keratinized epithelial cells, and occasional basal layer disorganization.[1] Other features include single cell necrosis throughout the epithelium, erosion or ulceration, and thickening of the Eberth-Katschenko layer (a normal layer of mineralization present in the skin).[1] See Crawshaw et al for excellent comparisons of normal and affected PRCT skin histology images.

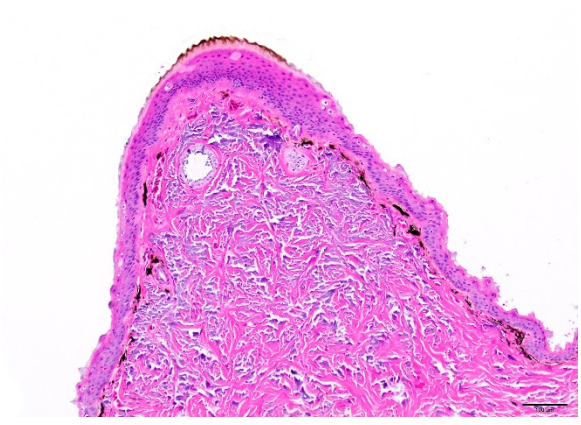


Skin, Puerto Rican crested toad. Epidermal hyperplasia with spongiosis and increased dermal melanocytes (HE, 400X) (Photo courtesy of: Kansas State Veterinary Diagnostic Laboratory, Department of Diagnostic Medicine/Pathobiology)

Examination of many cases has failed to reveal a cause for the disease. Attempts at bacterial cultures have revealed growth of a number of different bacterial genuses including *Aeromonas, Acinetobacter*, and many others but these have been considered normal flora or contaminants and attempts to identify common fungi of toads including chytrid fungi have all been negative.[1] In one study, supplementation of Vitamin A in 48 captive born PRCT failed to reduce the

severity of disease when it developed.[2] Further testing for bacterial or fungal organisms was not pursued in this case. Another PRCT was submitted from the same zoological collection and was similarly diagnosed with BSD. In that case bacterial culture of the skin revealed abundant growth of *Providencia rettgeri, Acinetobacter sp., Pseudomonas putida, Citrobacter freundii, Enterococcus faecalis, Microbacterium sp., and Staphylococcus sp. (non-hemolytic).* Fungal cultures of this second toad revealed unidentified yeast and further testing was not pursued.

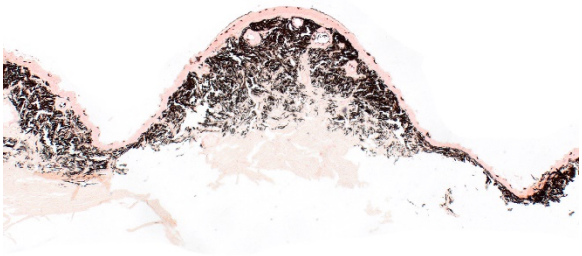
Puerto Rican Crested Toads are a threatened species and have been listed as endangered by the International Union for Conservation of Nature (IUCN) since 1987.[1] Since then a number of the species have been housed in a number of zoos in an attempt to save the species. Given the lack of a convincing infectious cause for this disease is it possible that a genetic component contributes to this disease. Evidence for this includes the initial case of BSD occurring in one male of a group of four toads that represented the last four of the "Northern" race of PRCT, six offspring from a pairing of this same group, and multiple animals from succeeding generations from this initial pairing.[1] Despite this, cases of BSD have occurred in unrelated individuals from the "Southern" race housed at different zoos in close proximity to affected animals and on similar diets thus the true etiology remains elusive.[1]



Skin, Puerto Rican crested toad. Epidermal hyperplasia with brown orthokeratotic hyperkeratosis, increased dermal melanocytes, and thickened Eberth-Katshenko layer (HE, 100X) (Photo courtesy of: Kansas State Veterinary Diagnostic Laboratory, Department of Diagnostic Medicine/Pathobiology)

Contributing Institution:
Kansas State Veterinary Diagnostic Laboratory, Department of Diagnostic Medicine/Pathobiology, www.ksvdl.org

JPC Diagnosis: Skin: Epidermal hyperplasia and hyperkeratosis, diffuse, marked, with dermal melanosis and mineralization.



Skin, Puerto Rican crested toad. Thickened Eberth-Katshenko layer highlighted with Von Kossa staining (Von Kossa, 40X) (Photo courtesy of: Kansas State Veterinary Diagnostic Laboratory, Department of Diagnostic Medicine/Pathobiology)

JPC Comment: The contributor has provided a concise review of this species-specific disease which unfortunately is a problem in a threatened species. The contributor describes the common finding of brown leathery skin in affected toads, which does not apparently contain an increase in dermal melanocytes to explain the coloration. Attempts to soak and remove affected skin from individuals have been successful, but the disease recurs again soon after.[1]

A wide range of environmental factors (to include husbandry issues) and infectious

agents have been incriminated as causing skin lesions in anurans. Once considering the problem of dysecdysis (abnormal skin shedding), low humidity, low temperature, and poor nutrition have all been implicated in this dysecdysis.[2]

Hypovitaminosis A has been particularly incriminated in amphibians with dysecdysis. Like all other species, retinoids must be obtained via the det in the form of provitamin A carotenoids or preformed Vitamin A. When absorbed, enterocytes hydrolyze dietary retinol esters or carotenoids to retinol where they are combined with dietary fat and cholesterol to form retinyl ested-laden chylomicons and resecreted. Dissolution of chylomicra in circulation allows the delivery of retinoids to peripheral tissues; remaining retinoids are taken up by the liver. In the skin, retinoids perform an important function in the regulation of gene transcription and affect processes of cellular proliferation, differentiation, and apoptosis.[2]

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