Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 13

5 January 2022

CASE I: A21-106 (JPC 4167686)

Signalment:
9-month-old White's tree frogs (*Ranoidea caerulea,* formerly *Litoria caerulea*).
Multiple individuals (three females and one male) submitted.

History:
Sudden deaths of multiple cohabitants over the course of a few weeks.

Gross Pathology:
No relevant gross findings.

Laboratory Results:
None provided.

Microscopic Description:
The epidermis is diffusely hyperplastic with multifocal to coalescing areas of orthokeratotic hyperkeratosis. The hyperkeratotic stratum corneum is diffusely expanded by 1-5 layers of retained keratinocytes. Retained keratinocytes contain moderate to high numbers of intracellular, round, fungal structures measuring 5-10 µm in diameter (thalli). Thalli variably contain roughly round, basophilic zoospores measuring 2-3 µm in diameter. Infrequently, spike-like projections extend from thalli toward the skin surface (discharge tubules). Minimal lymphocyte exocytosis is observed in the affected epidermis. Few lymphocytes, histiocytes, and granulocytes are scattered in the superficial dermis.

Contributor's Morphologic Diagnoses:
Skin: Epidermal hyperplasia and hyperkeratosis, diffuse, moderate to marked, chronic, active, with high numbers of intracorneal thalli, morphology consistent with *Batrachochytrium dendrobatidis.*

Contributor's Comment:
Batrachochytrium spp., the causative agents behind the infectious disease chytridiomycosis, are fungal pathogens responsible

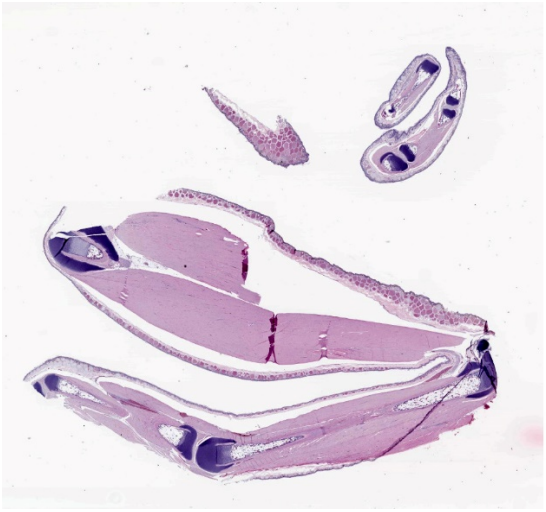

Figure 1-1. Legs, frog. Cross section of legs are submitted for examination; there are no apparent lesions at subgross magnification. (HE, 5X)

for mass morbidity and mortality in amphibian populations worldwide.[11] Two species have thus far been implicated in global amphibian population declines; *Batrachochytrium dendrobatidis (Bd),* which has been identified in over 50 countries and over 500 amphibian species,[1] and *Batrachochytrium salamandrivorans (Bsal),* which primarily affects salamanders and newts.[8] *Bd* infects the keratinized layer of the epidermis. The disease is therefore primarily seen in post-metamorphic adults, but *Bd* can infect the keratinized oral discs of tadpoles.[7]

Amphibian skin is actively involved in maintenance of homeostasis, gas exchange, and electrolyte balance. Through infection of the superficial epidermis, *Bd* impairs the function of amphibians' epidermal ion transport channels, disrupting osmoregulation and resulting in systemic electrolyte imbalance.[15] Mechanisms by which *Bd* causes systemic electrolyte imbalance include direct disruption of epidermal intercellular junctions via production of virulence-associated proteins;[3] influencing the expression of genes involved in collagen, fibrinogen, elastin, keratin, and ion channel production;[15] and possibly direct inhibition of sodium transport channels.[4, 17] Disease in affected animals typically does not elicit a strong immune response, and it has been hypothesized that *Bd* may also impair host immune responses.[6] Overall, disease course and outcome are influenced by a variety of pathogen, host, and environmental factors, but the resulting hyponatremia, hypokalemia, and hypochloremia eventually lead to arrhythmia, cardiac arrest, and death.[4, 11, 15]

Affected amphibians typically first present with nonspecific signs of disease such as anorexia and lethargy. Some affected animals exhibit abnormal or excessive shedding patterns.[10] Sudden death without any premonitory signs is also possible.[11]

Gross lesions can include rough skin texture, greyish discoloration of the skin, erythema,

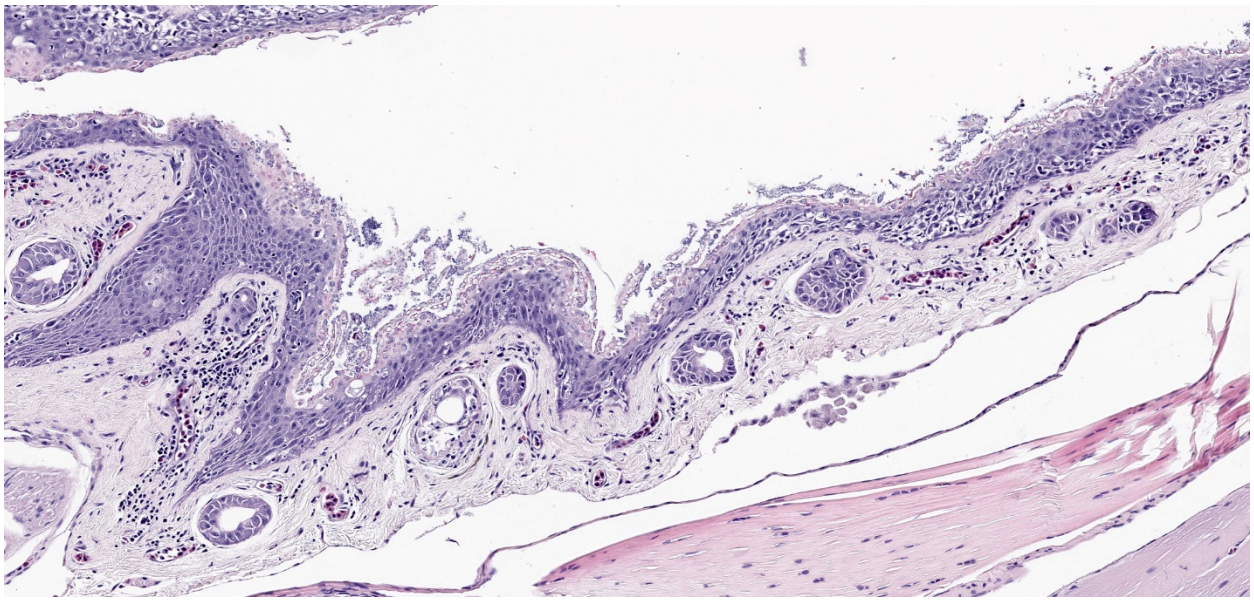

Figure 1-2. Skin, frog. There is marked diffuse epidermal hyperplasia and hyperkeratosis. There are numerous empty thalli and fungal hyphae within the hyperkeratotic debris, and colonies of opportunistic bacteria. There is intraepidermal edema and multifocal keratinocyte necrosis. (HE, 400X)

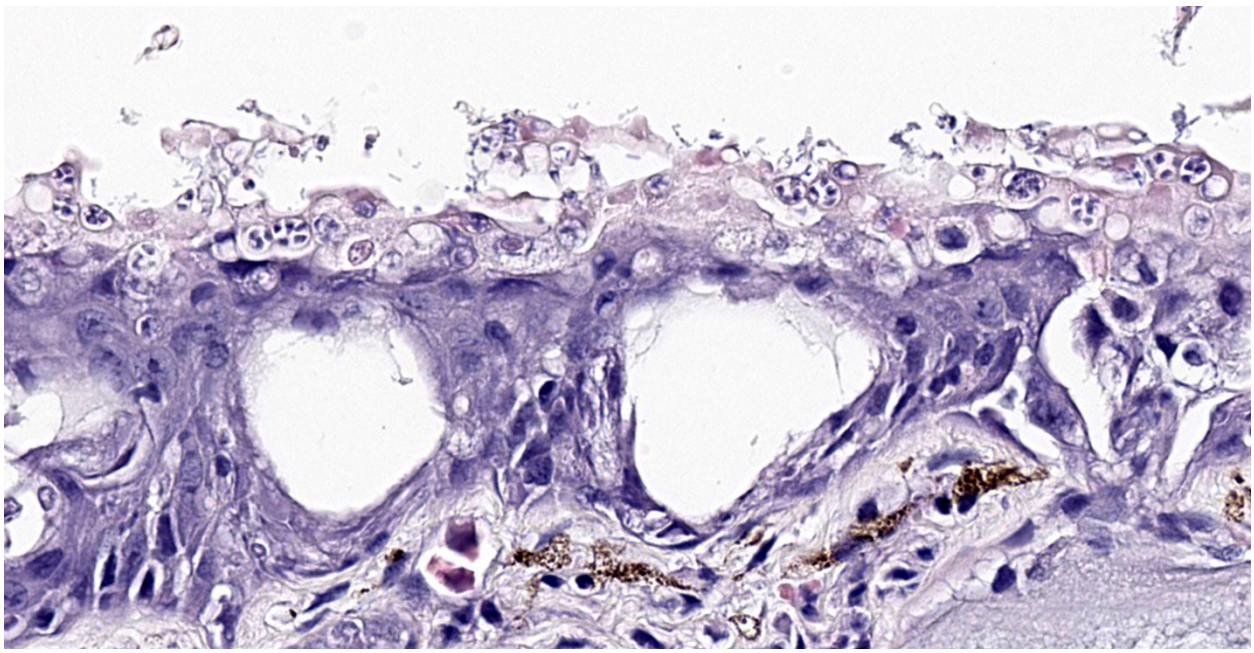

Figure 1-3. Skin, frog. The thickened keratin layer (contains abundant zoosporangia filled with 1-2 micron basophilic zoospores. Many empty thalli with fine internal septations (diamonds) are also present. (HE, 381X)

ulceration, abnormal shedding, and thinning and sloughing of the skin.[4, 11] However, in cases of sudden death, no gross lesions may be appreciated. Histologically, fungal thalli are identified in the stratum corneum and stratum granulosum with associated hyperkeratosis and epidermal hyperplasia. Lesions are typically most pronounced on the feet and ventral body.[2] Thalli are round and 5-15 µm in diameter with a variably present projection extending toward the skin surface known as a discharge tubule. Depending on their stage of development, thalli may be empty and clear-staining with only thin internal septa visible or filled with few, round, basophilic zoospores that are 2-3 µm in diameter.[11] Inflammatory cell infiltrates are not commonly observed in association with thalli.[11]

Histochemical stains such as Gomori's methenamine silver stain (GMS) and Periodic-acid Schiff for fungus stain (PAS-F) can be used to highlight intracorneal thalli. Wet mounts and skin scrapings may also aid in diagnosis of *Bd.*

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JPC Diagnosis:
Skin, epidermis: Hyperplasia and hyperkeratosis, multifocal to coalescing, moderate to severe, with numerous fungal thalli and zoospores, etiology consistent with *Batrachochytrium dendrobatidis.*

JPC Comment:
All fungi, including those of division Chytridiomycota (commonly known as 'chytrids'), share a common aquatic ancestor from which metazoans and fungi both originated approximately 1.2 billion years ago. Given their early divergence from other

fungi approximately 750 million years ago, chytrids retain many features from their common ancestor and are suited for survival in aquatic environments, such as the production of mobile ciliated zoospores. Chytrids also feature several features shared by all fungi, such as a hyphal-like structure known as a "thallus", which is associated with a feeding structure known as a rhizoid that absorbs nutrients while also anchoring the fungus to the substrate.[9]

Chytrids are found in both aquatic and terrestrial environments. Many, such as *Batrachochytrium dendrobatidis*, parasitize animals while others target algae and plants or are saprophytic. These organisms play a major role in aquatic food chains by parasitizing large inedible algae while also producing zoospores that are a food source for zooplankton. Anaerobic chytrids are also components of the rumen microbiome. With the help of specialized organelles known as hydrosomes, these organisms facilitate the breakdown of resilient carbohydrates in plant tissues, which in turn aids volatile fatty acid and microbial proteins production by additional constituents of the rumen microbiome.[9]

Most chytrid species exhibit a similar life cycle beginning with a zoospore that subsequently progresses to a thallus and then a sporangium. Zoospores range from 2-10 µm in diameter and typically have a single posterior cilium used for propulsion in aquatic environments. Zoospores are non-mitotic, have limited energy stores derived from lipids and carbohydrates allocated during zooporogenesis, which are often visible microscopically as lipid droplets. Upon finding itself in a suitable environment, the motile zoospore encysts by retracting its cilia and forms a fungal cell wall. Previously inhibited by cilia, the now free centrioles are utilized for cell division. As the cyst germinates, a primitive germ tube forms, which subsequently arborizes into a hyphal-

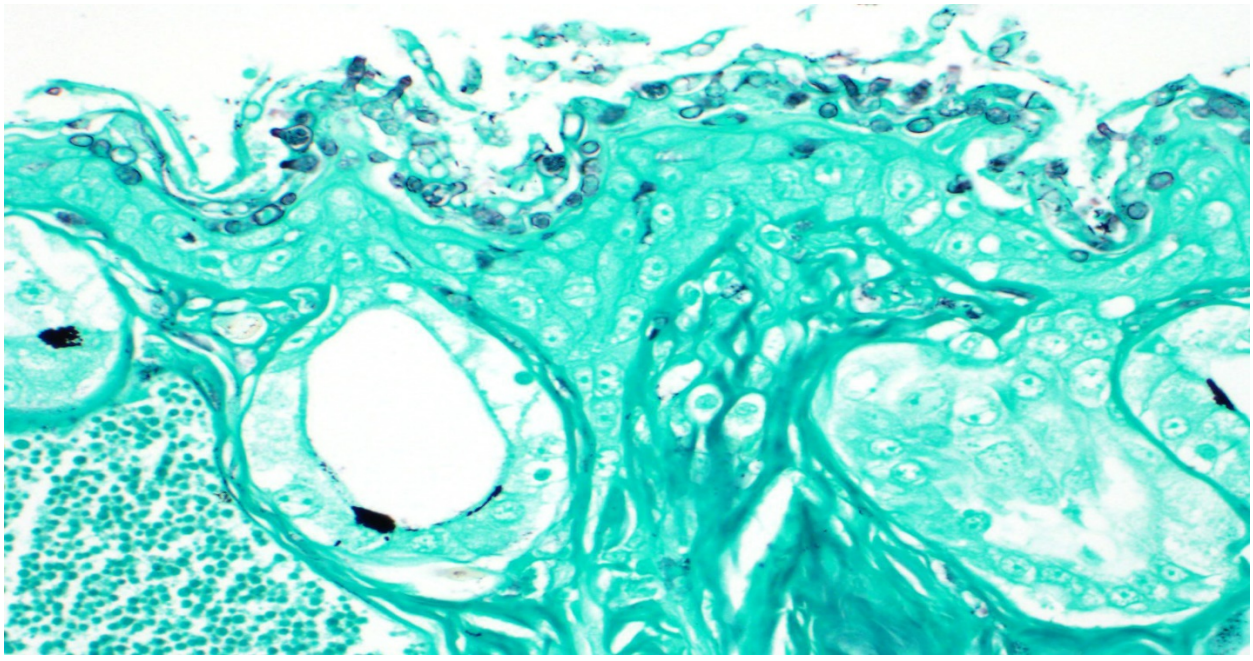

Figure 1-4. Skin, frog. A fungal stain demonstrates multiple life forms (zoosporangia, thalli and fungal hyphae) of B. dendrobatidis within the hyperkeratotic debris.

like rhizoidal system. Depending on the species, a sporangium forms at the site of the nucleus, which is either located within the cyst or from a separate location within a rhizoid as the result of nuclear migration. Within the developing sporangium, the nucleus undergoes multiple divisions without cytoplasmic division, forming a coenocyte. This is followed by ciliogenesis, by which centrioles are converted into basal bodies and the cilia are formed. Nuclei are then individually encapsulated by membrane invaginations. Following the completion of zoosporogenesis, mature zoospores are released via one or more pores (discharge papillae) within the cell wall.[9]

Batrachochytrium dendrobatidis (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal)* are the etiologic agents of chytridiomycosis, an emerging infectious amphibian disease responsible for mass die-offs worldwide. As of a 2019 report[14], chytridiomycosis has contributed to the decline of 501 amphibian species (6.5% of described amphibian species), of which 90 are extinct in the wild and 124 have experienced a >90% decline. Of the 501 species affected, all but one species decline were attributed to *Bd.* These figures constitute the greatest known loss of biodiversity attributed to a pathogen. In comparison, other well-known pathogens such as white-nose syndrome in bats (*Pseudogymoascus destructans*) and West Nile virus are responsible for the declines of six species of bats and 23 species of birds, respectively. Anurans, which comprise 89% of amphibian species, are most commonly affected and represent 93% of the species in severe decline, with 45% of severe declines and extinctions occurring in the neotropical genera *Atelopus, Craugastor,* and *Telmatobius.*[14]

A study[15] evaluating a chronically *Bd*-infected population compared to a *Bd*-free population of the endangered Australian frog *Litoria verreauxii alpina* found a higher proportion of males and females in infected populations reached sexual maturity at a younger age than non-infected populations. In males, this resulted in reduced size at maturity and overall may demonstrate an adaptive evolutionary shift in response to selection pressure induced by high *Bd*-induced mortality.[15]

In contrast to *Bd* which is predominantly associated with anuran hyperkeratosis, *Bsal* is a pathogen of salamanders and newts and is characterized by multifocal superficial erosions and deep ulcerations in the skin. Although anurans are seemingly unaffected, they can act as carriers and likely facilitate the spread of the disease as the result of inter/intraspecies contact associated with anthropogenic trade. This entity recently spread from Asia into Europe, decimating the fire salamander (*Salamandra salamandra)* population in The Netherlands in 2013.[5] Hosting one of the most diverse salamander populations in the world, The United States also imported 750,000 salamanders from 2010-2014, creating a high risk of *Bsal* introduction.[12] As a result, the US Fish and Wildlife Commission banned the import of 201 species of salamanders into the United States in 2016. Although *Bd* is reported worldwide, *Bsal* has not yet been reported in North America.[5]

Conference participants noted the dermis and underlying musculature are separated by abundant clear space. A possible explanation for this clear space is lymph sac edema, however, the moderator suggested the lack of associated flocculent material is more consistent with processing artifact.

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CASE II: R18-1576 (JPC 4137580)

Signalment:
Approximately 1-2 month old, female, Nile tilapia, *Tilapia niloticus*, fingerling, fish

History:
Mortalities of 10-30% occurred over a four-month period in a tilapia hatchery receiving untreated surface water. Reported clinical signs were limited to lethargy, erratic swimming, and exophthalmia.

Gross Pathology:
Exophthalmia was accompanied by severe renomegaly and splenomegaly, with pinpoint white nodules widely distributed throughout.

Laboratory Results:
Pure growth of *Edwardsiella anguillarum* was cultured from the brains of multiple fish and its identity confirmed by specific end-point PCR. Ancillary testing for tilapia lake virus (TiLV) and *Francisella noatunensis* subsp. *orientalis* was negative.

Microscopic Description:
Multifocal to occasionally coalescing, expansile granulomas replace hematopoietic tissue and displace adjacent tubules within the anterior region of the kidney. Central regions of granulomas are formed by extensive areas of hypereosinophilic, pyknotic, and karyorrhectic cellular debris, mixed with degenerate macrophages containing large numbers of intracytoplasmic bacterial rods. Broad mantles formed by concentric layers of epithelioid macrophages limit granulomas. Large numbers of free bacterial rods mixed with necrotic epithelial cell debris fill scattered tubular lumens. A single, similar granuloma is present in the liver.

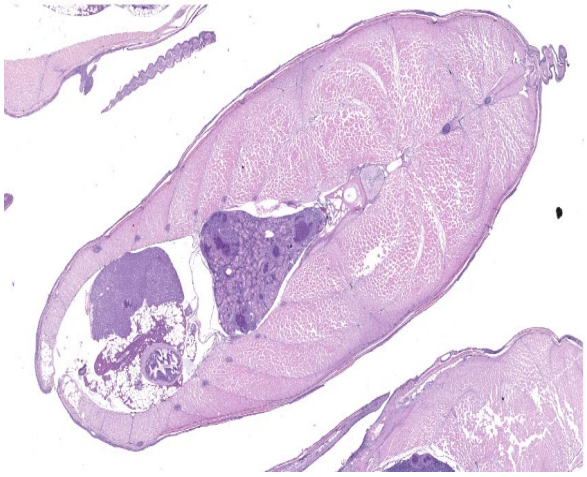

Figure 2-1. Transverse section, tilapia. A transverse section of a tilapia at the level of the anterior kidney is presented for examination. There are multifocal to coalescing areas of hypercellularity scattered throughout the kidney, and focally within the liver. (HE, 6X)

Contributor's Morphologic Diagnoses:
Kidney: Epithelioid granulomas, multifocal, subacute to chronic, severe, and scattered tubular necrosis, with free and intracytoplasmic gram-negative bacterial rods.

Contributor's Comment:
This kidney section from a tilapia was from one of 20 fish received from a group of hatchery-reared fingerlings experiencing chronic mortalities. Microscopic findings were consistent with systemic *Edwardsiella anguillarum* infection, an emerging fish pathogen with an expanding host range.[5] *Edwardsiella anguillarum* is a facultative intracellular, gram-negative, bacterial rod originally described from diseased eel (*Anguilla* spp.) in China.[11] In the United States, identification dates back to samples of striped bass (*Morone saxatilis*) archived in 1994.[10] Multi-organ necrosis and granuloma formation, with intracellular bacterial rods, characterizes lesions.[2] Although variable in distribution, severity, and chronicity among individuals, similar lesions affected the meninges, intracranial adipose, ventricular

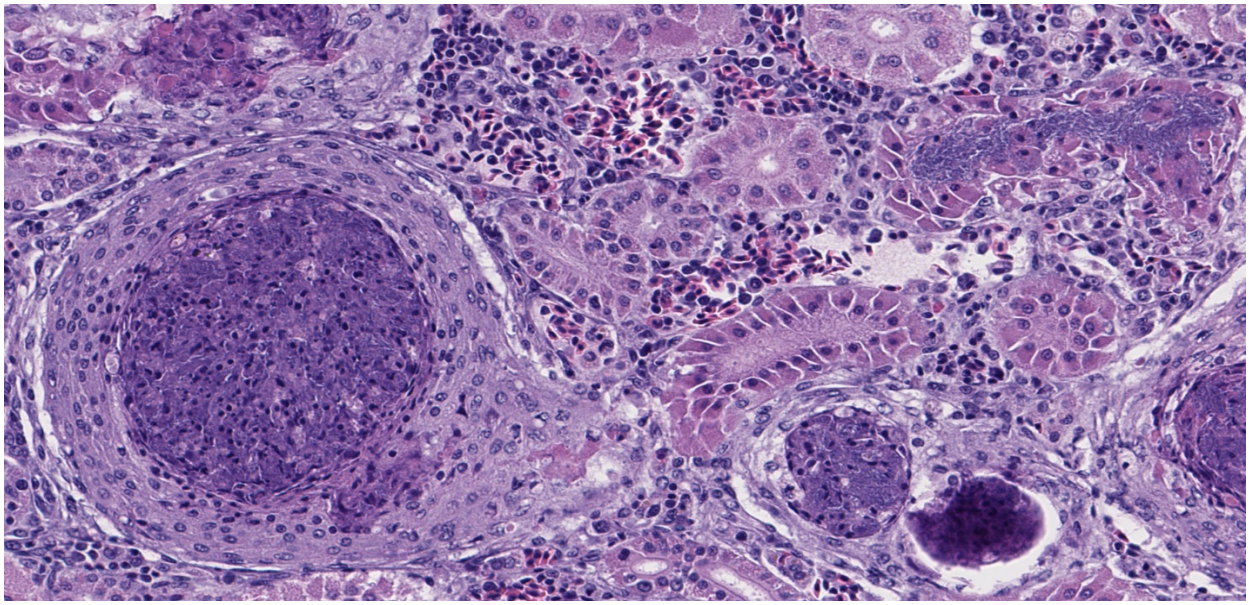

Figure 2-2. Kidney, tilapia. There are well-formed (left) and developing (lower center) granulomas scattered throughout the kidney. At right, there is granulomatous interstitial inflammation with infiltration and partial replacement of a necrotic renal tubule. (HE, 396X)

system of the brain, olfactory nerves, ocular choroid rete, heart, gills, pseudobranch, spleen, kidney, liver, gastrointestinal wall, swim bladder rete, skeletal muscle, and various connective tissue sites among the various tilapia.

Lesions are reported to be most severe in highly vascular organs, particularly spleen and kidney, but can be widely disseminated and affect almost any tissue site. Early lesions are necrotizing, containing abundant cellular debris, variable numbers of macrophages, and large numbers of 3-5µm, free and phagocytized, gram-negative, bacterial rods. Over time, thin margins of attenuated and epithelioid macrophages envelop foci of necrosis, ultimately forming granulomas with large central areas of hypereosinophilic cellular debris bordered by wide zones of epithelioid macrophages. Bacteria can be observed in H&E sections, but are best visualized with Giemsa stain. They occur primarily within macrophage cytoplasm, consistent with the facultative intracellular nature of *Edwardsiella* spp. bacteria. With chronicity, bacterial numbers decline sharply and may not be discernable in mature granulomas.[2]

The genus *Edwardsiella* contains the well-known bacterial fish pathogens, *E. ictaluri* and *E. tarda*, as well as the two recently recognized species, *E. piscicida* and *E. anguillarum*, that were split from *E. tarda*.[1,11] *Edwardsiella ictaluri* is best known as the agent of enteric septicemia of catfish (ESC), an important disease of catfish aquaculture in the southeastern United States.[5] *Edwardsiella tarda* affects both terrestrial and aquatic hosts, and has been implicated in disease outbreaks in numerous economically important fish species worldwide.[8] However, the validity of many E. tarda identifications from fish is now questionable.[5] Similar to *E. anguillarum*, *E. piscicida* appears to exhibit little host specificity and is increasing in importance as a global pathogen.[3,5] Due to shared phenotypic characteristics and high 16S rRNA sequence homology, differentiation of *E. tarda, E. piscicida,* and *E. anguillarum* require species-specific PCR

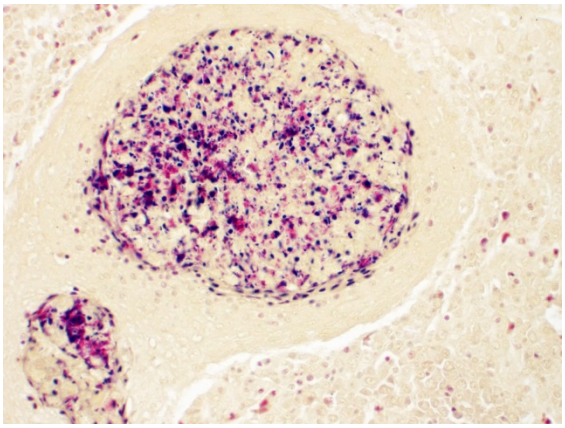

Figure 2-3. Kidney, tilapia. Granulomas contain large numbers of gram-variable rods within the necrotic center. (Brown and Hopps, 600X)

methods or evaluation of additional gene sequences (e.g. gyrB) for correct identification.[5,10] Current MALDI-TOF databases may not recognize the latter two species.

In addition to *E. anguillarum*, disease outbreaks in tilapia have been attributed to *E. tarda*, *E. ictaluri*, and *E. piscicida*, although not all *E. tarda* identifications may be reliable.[4,5,10,12] Lesions described particularly for *E. piscicida* and *E. ictaluri* resemble those seen here, suggesting the respective diseases may not be reliably differentiated by histopathology, which should be supported by appropriate bacterial culture and molecular testing.[2,3] Additional differential diagnoses include mycobacteriosis and francisellosis caused by *Francisella noatunensis* subsp. *orientalis*.

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JPC Diagnosis:
1. Kidney: Granulomas, multiple, with multifocal renal tubular degeneration, necrosis, and loss, and colonies of bacilli.
2. Liver: Granuloma, focal.
3. Skin: Epidermitis and dermatitis, erosive to ulcerative, moderate, multifocal, with *Trichodina* sp.

JPC Comment:
Family Enterobacteriaceae is composed of facultative anaerobic, non-spore forming, gram-negative rods which ferment glucose and a variety of other sugars, are oxidase negative, and catalase positive. Over 28 genera have been identified and include major pathogens such as *Escherichia, Salmonella,* and *Yersinia*, as well as opportunists such as *Proteus, Klebsiella* and *Serratia.*[10]

In the early 1960s, a unique group of isolates that did not fit into known genera were identified from both a human patient with enteric fever and from snakes in Japan with acute gastroenteritis. The isolates were subsequently identified as a new genus and species, *Edwardsiella tarda*. Additional species have since been identified and include *E. ictaluri*, *E. hoshinae, E. piscicida,* and *E. anguillarum*, infecting a wide range of hosts including freshwater and marine fish, reptiles, avians, and mammals. As noted by the contributor, multiple studies over the first half of the previous decade found the species previously identified as *E. tarda* was composed of three genetically distinct but phenotypicically identical species: *E. tarda, E. piscicida,* and *E. anguillarum*.[11]

Historically, *E. tarda* has been identified as a zoonotic pathogen that can be acquired from both fish (including ornamental pet fish) and shellfish. A range of clinical manifestations of *E. tarda* have been reported in humans and include necrotic dermal lesions, gastro-

enteritis, and septicemia in severe cases that results in osteomyelitis, meningitis, or cholecystitis. Given the recent discoveries of *E. piscicida* and *E. anguillarum,* it is unclear if these previous reports are attributable to *E. tarda* or a newly recognized species.[11]

Studies conducted prior to the discovery of *E. piscicida* and *E. anguillarum* found multiple virulence factors affiliated with *E. tarda*'s pathogenesis. EypP is an effector protein always present in virulent strains whereas avirulent strains lack the gene encoding for EypP. Additional important virulance factors include AIDA, EthA, EthB, Fur, QseB, and QseC. AIDA is an autotransporter adhesin that facilitates attachment and penetration into host cells. Both EthA and EthB are hemolysins.[6] Ferric Uptake Regulator (Fur) is a transcription factor responsible for the regulation of the pathogen's intracellular iron concentration, which is inherently protective against the formation of reactive oxygen species generated by the Fenton reaction.[6,14] Both QseB and QseC are responsible for phenomenon known as quorum sensing, which plays an important role in the pathogen's cellular invasion and intracellular survival, particularly within macrophages, by both regulating virulence factors as well as surface structure modifications (e.g. flagella and fimbriae) which may facilitate adaptation to the intracellular environment.[6]

Although *Edwardsiella ictaluri* is commonly associated with catfish and tilapia, it has also been reported in aquarium species including the green knifefish (*Eigenmannia virescens*), rosy barb (*Puntius conchonius*) and zebrafish (*Danio* spp.). Zebrafish are particularly susceptible to a genetically distinct strain from those infecting catfish and tilapia, which has destroyed between 20,000-40,000 fish. Given zebrafish are commonly utilized in the research setting, this pathogen has the capacity to significant impact studies. However, infection is typically detected during quarantine prior to research facility entry. Although in vitro studies have shown *E. ictaluri* to be susceptive to multiple antibiotics, neither bath nor oral antibiotic administration have been shown to be effective in vivo.[7]

A secondary finding in this case are multifocal regions of erosive to ulcerative dermatitis associated with superficial parasitic ciliates consistent with *Trichodina* sp. Speciation based on histologic examination alone is difficult due to high intraspecific variation, high interspecific similarity, and low host specificity.[15] All clinically important species affect the skin and/or gills and both marine and freshwater fish are commonly infested.[8]

Trichodinosis is typically a mild disease resulting in chronic morbidity or mortality, but significant losses may occur, particularly with young fish and those with secondary bacterial infections. Trichodinids exhibit a characteristic scooting motion on the tissue surface and can be diagnosed using a wet mount of skin or gill or histologically, however, these organism are easily lost during the fixation process.[8]

When viewed from the dorsal aspect, trichodinids typically measure 40-60µm (up to 120µm) in diameter and are characterized by a round shape, a central ring of hook-like denticles, and circumferential peripheral cilia.[8]

Trichodinids most commonly infest debilitated fish, such as in this case. Therefore, particularly in cases of low numbers (e.g. one per 100X field of view), a

primary underlying disease process should be ruled out in cases of trichodinosis.[8]

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CASE III: A15-23071 (JPC 4168468)

Signalment:
Juvenile, sex unknown, discus, *Symphysodon* sp., fish

History:
Multiple fixed tissues were received from a discus was that was euthanized to investigate chronic low level mortalities within a group of recently acquired juvenile fish. Clinical signs within the group included poor growth and physical condition, lethargy, anorexia, and external darkening.

Gross Pathology:
No gross findings reported.

Laboratory Results:
No laboratory findings reported.

Microscopic Description:
An extensive segment of intestine is characterized by widespread, variable expansion, blunting, and fusion of mucosal folds with patchy loss of the normal plicated architecture and associated epithelia. Affected mucosal surfaces are frequently disorganized with variably sized foci of epithelial attenuation, hypertrophy, and hyperplasia accompanied by scattered small foci of necrosis containing cellular debris, and widespread migration of lymphocytes across the epithelial border. The lamina propria and submucosa are markedly expanded by widespread infiltration with moderate to large numbers of lymphocytes and macrophages accompanied by scattered eosinophilic granular cells and plasma cells. Inflammatory infiltrates are accompanied by patchy edema, rare minimal foci of hemorrhage, scattered fibroblasts, and islands of entrapped epithelial cells. Vascular lumens are prominent and lined by mildly hypertrophic endothelial cells. Free within the intestinal lumen and embedded in the mucosa and submucosa are multiple longitudinal and transverse fragments of slender nematodes with a smooth cuticle, pseudocoelom, and inconspicuous coelomyarian musculature. Additional features include a prominent stichosome composed of a series of granular, amphophilic to basophilic stichocysts, and in some sections a stichosome nucleus. Multiple stages of germ cell development are evident within the same sections of ovarian and testicular tissue. Rare transverse sections of unembryonated eggs with hyaline eosinophilic shells are present in the mucosa and gut lumen. The submucosa contains a single granuloma with fragments of a degenerate worm.

Contributor's Morphologic Diagnoses:
Intestine: Enteritis, granulomatous, diffuse, chronic, severe, with intraluminal and intramural aphasmid nematodes, presumptive *Capillaria pterophylli.*

Contributor's Comment:
Microscopic findings in this discus fish *Symphysodon* spp., chronic enteritis in the presence of embedded aphasmid nematodes, are consistent with pathologic changes and morphologic features of intestinal capillariasis, presumptively due to *Capillaria pterophylli*. Although often identified only to

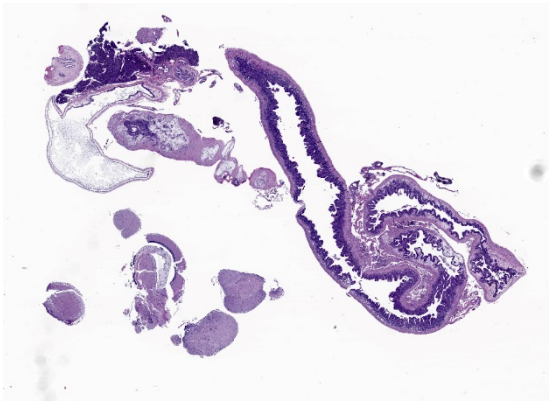

Figure 3-1. Viscera, discus. Sections of intestine, stomach, mesentery, liver, and brain are submitted for examination. (HE, 5X)

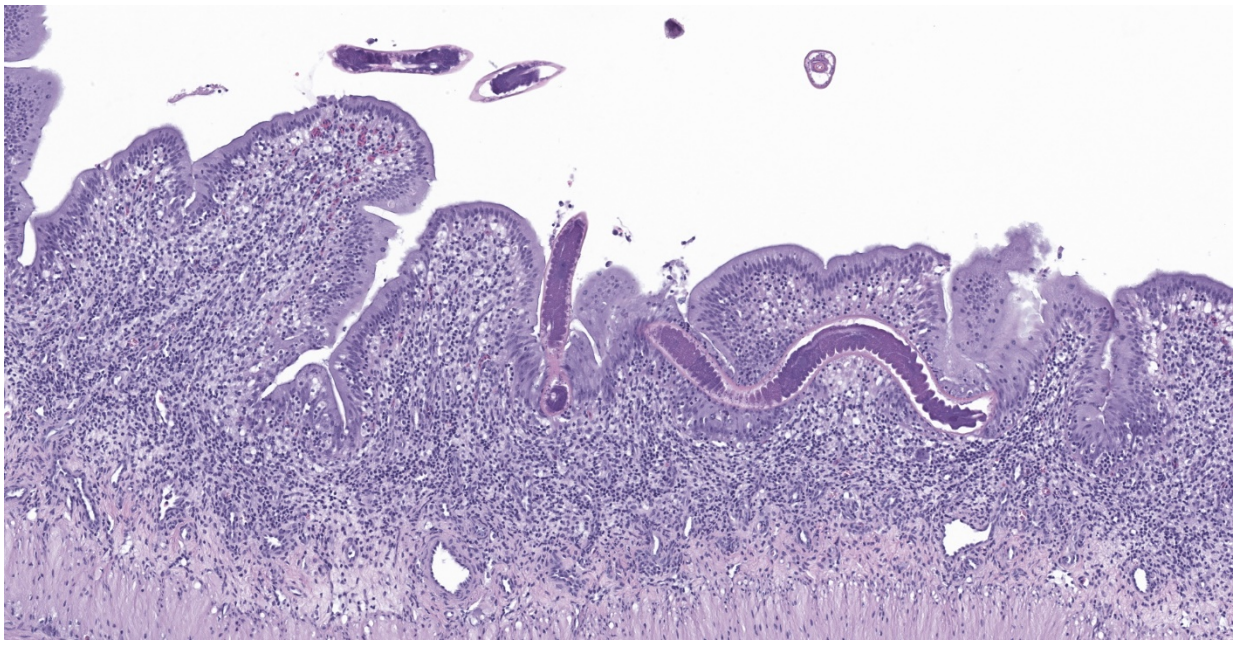

Figure 3-2. Intestine, discus. There is blunting and fusion of mucosal folds with expansions of the lamina propria/submucosa by large numbers of histiocytes and lymphocytes. Embedded within the mucosa and free within the lumen are cross and tangential sections of adult aphasmid nematodes with a prominent basophilic stichosome. (HE, 142X)

the genus level, examples of capillariasis causing losses in aquaculture operations producing ornamental fish has been reported from multiple countries and fish species. Records of *C. pterophylli* suggest primary hosts are South American fishes of the family Cichlidae, including angelfish *Pterophyllum* spp. and discus *Symphysodon* spp.[5] However, taxonomy of the Capillaridae is disputed and some classification schemes may contain as many as 22 genera. The distinctive shape, social behavior, and array of color patterns found in discus have established them as a popular aquarium species among collectors and in competitions. Their production in several Asian countries is part of a major fish culture industry. Primary differentials for chronic ill-thrift and mortalities in discus include mycobacteriosis, spironucleosis, and cryptobiosis.

While *C. pterophylli* is reported to induce similar clinical signs and pathologic changes, impacts of capillariasis in small freshwater fish are best described in research colonies of zebrafish *Danio rerio*, where parasitism by *Pseudocapillaria tomentosa* is a significant cause of chronic wasting and mortality.[3,5,6] Histologic changes include irregularities in the intestinal plicae, fusion of plicae, epithelial hyperplasia and dysplasia, and edema in the lamina propria. Inflammatory infiltrates composed of mixed lymphocytes, eosinophilic granular cells (mast cells), neutrophils, and fewer histiocytes further expand the lamina and submucosa.[2,3,6] In zebrafish colonies, *P. tomentosa*, is also associated with a high incidence of intestinal carcinomas, where *Mycoplasma* spp. are believed to initiate tumor development that is promoted by proliferative changes induced by the nematode.[2,8]

The trichurid nematodes, of which the family Capillaridae is a member, are all parasitic as adults in vertebrates. Most possess an anterior end that is narrower than the posterior end with a slender esophagus

embedded in large, basophilic, glandular cells, the stichocysts, which collectively form a stichosome. Distinct lateral chords are absent. However, hypodermal bacillary bands with associated nuclei are present but may not be visualized in all sections. Musculature is polymarian coelomyarian. In any section, ovarian and testicular tissue will contain all stages of germ cells (hologonic development). The eggs of most species have bipolar or biopercular plugs. Portions of worms are frequently found embedded within the intestinal wall.[1]

The life cycles of both *C. pterophylli* and *P. tomentosa* are direct by feco-oral transmission between tankmates.[4,7] Movements of fish in the ornamental pet trade and the sharing of zebrafish lines, lack of quarantine, and potential for eggs to survive sanitization may contribute to spread. Due to their direct life cycles, infections cannot be contained through the elimination of intermediate hosts. However, fenbendazole, levamisole, emam-ectin benzoate, and ivermectin have been described as potential treatments.[6] A real-time PCR assay has been developed and validated as a means of non-lethal sampling in zebrafish colonies.[7]

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JPC Diagnosis:

1. Intestine: Enteritis, lymphohistiocytic, diffuse, moderate, with intramucosal aphasmid adults and luminal eggs.
2. Stomach, submucosa: Granulomas, few, with edema.

JPC Comment:
Capillaria pterophylli was first described by Heinze (1933) in two species of angelfish

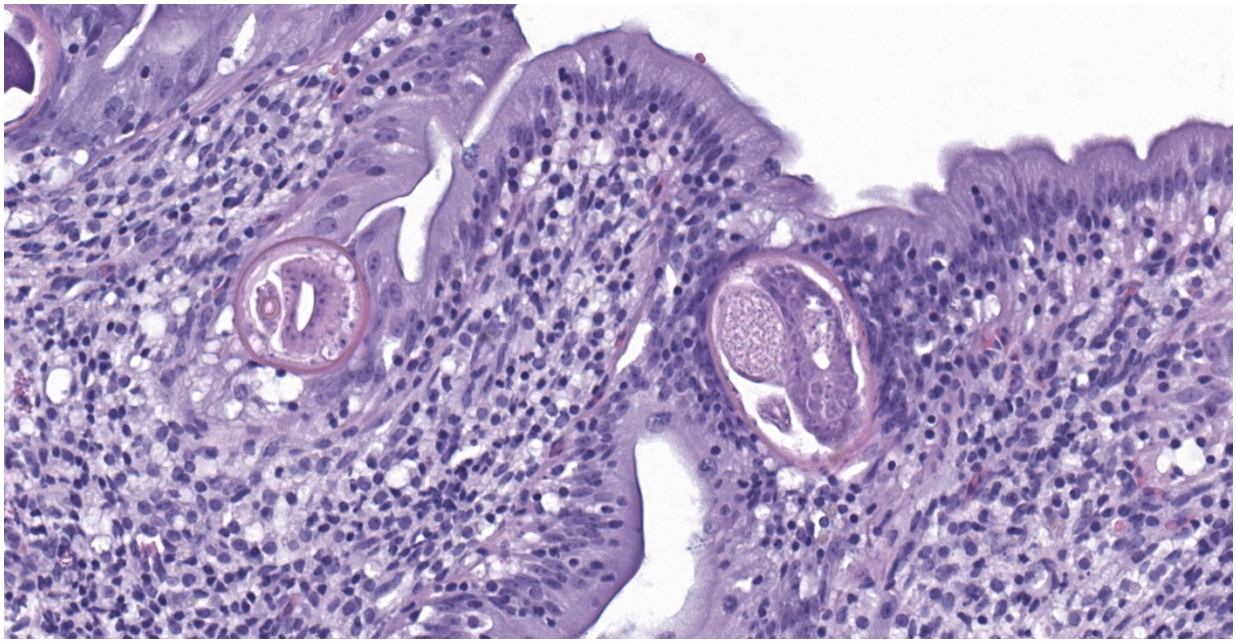

Figure 3-3. Intestine, discus. Additional nematode cross sections demonstrate an intestine lined by large uninucleate cells (section at left), and testis (section at right). (HE, 483X)

(*Pterophyllum scalare* and *P. eimekei*) native to South America, which are commonly found in aquariums. Fish of the family Cichlidae (*Pterophyllum* and *Symphysodon*) originating from South America are thought to be the primary hosts although other species of aquarium fish from different families and geographic regions have also been infected, including *Puntius tetrazona* (Cyprinidae) and *Colisa lalia* (Belontiidae). Notably, the majority of reported infected fish are not directly imported, suggesting the life-cycle can be completed in aquarium conditions.[4]

As noted by the contributor, *Pseudo-capillaria tomentosa* is a common cause of capillariasis in zebrafish (*Danio rerio*). This species serves as an important animal model used in various research studies including genetics, development, infectious disease, and toxicology. Compared to other laboratory animals, such as murine models, utilization of zebrafish in research is a relatively recent endeavor. Consequentially, infectious diseases are a commonly faced issue in the research setting with an impact on not only population management but the value of research involving their use can also be diminished as a result. Modern research facilities frequently utilize multiple biosecurity measures such as quarantine rooms, routine visual assessment for diseased fish, and screening sentinels and culled older fish for pathogens using histology and PCR/qPCR.[7]

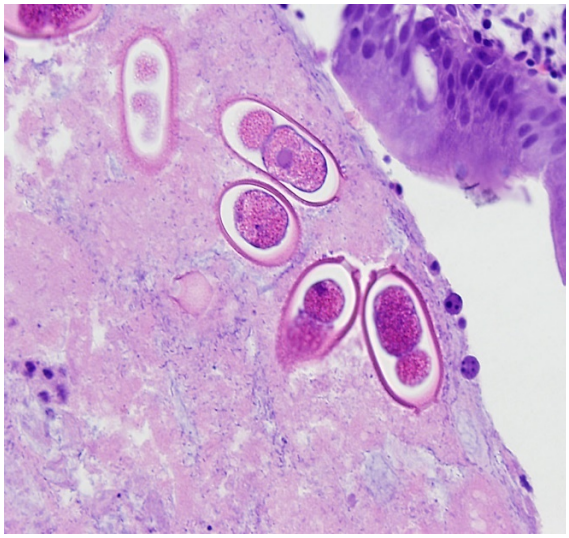

Figure 3-4. Intestine, discus. Characteristic bioperculated and embryonated Capillaria eggs are present within the intestinal lumen. (HE, 483X) (Photo courtesy of: Department of Pathology, University of Georgia, College of Veterinary Medicine, 501 DW Brooks Drive, Athens, GA 30602. https://vet.uga.edu/education/academic-departments/pathology/)

Development of non-lethal screening assays that assess tank water for pathogens have generated considerable interest concerning the infection status of individual tanks. The detection of primary pathogens in tank water such as *Pseudoloma neurophilia, Myco-bacterium marinum*, *M. chelonae* and *M. haemophilium* is a direct indication of infected (although often asymptomatic) fish given these pathogens are not found in the environment unless the fish are infected.[7]

Development of a qPCR assay capable of detecting *Pseudocapillaria tomentosa* using tank water as well as pooled fish samples was recently described with a limit of detection less than one individual nematode (0.01 nematode) in the pooled samples.[7] However, sensitivity was degraded in water samples due to contaminants such as fish DNA, algae, food residue, fish waste, other free-living organisms, and other organic and inorganic compounds such as humic acid. Given infection with this nematode is associated with chronic inflammation, emaciation, and predisposition to intestinal neoplasia, use of this assay may be an effective tool toward reducing confounding lesions and development of pathogen free piscine models in research over the long-term.[7]

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CASE IV: AK895 (JPC 4168135)

Signalment:
Approximately 7-year-old, female, rainbow trout (*Oncorhynchus mykiss*, Walbaum)

History:
The trout was reared in a concrete tank in a trout farming facility, provided with 10-12°C well water and fed *bis in die* with a commercial diet. A 9 cm x 7 cm mass was noted, bulging from the dorsal fin and altering its normal profile. Behavioral alterations were not present. The trout was euthanized with tricaine methanesulphonate overdose and underwent complete necropsy and histopathological examinations.

Gross Pathology:
The mass was characterized by multinodular appearance, black to dark red discoloration with multifocal areas of ulceration, and soft consistence, with lardaceous appearance of the cut surface. When the coelomic cavity was opened, no alterations of the main internal organs were noted.

Laboratory Results:
None provided.

Microscopic Description:
A multinodular neoplastic mass severely expands the dermis and the adjacent skin and extends to the cut borders. The mass is highly cellular, well-defined, non-encapsulated, and multifocally infiltrating the adjacent skeletal muscles. Neoplastic cells are haphazardly arranged in streams and fascicles, with occasional whorl formation. Cells are spindle-shaped and characterized by a scant fibrillary eosinophilic cytoplasm, a fusiform to wavy nucleus with basophilic finely stippled to vesicular chromatin, and up to 2 visible nucleoli. Cells are supported by scant fibrovascular stroma, associated to multifocal deposition of a loosely arranged clear matrix,

Figure 4-1. Presentation, trout. Upon gross inspection, arising in the area of the dorsal fin, there is a soft, multinodular red-black neoplasm. (Photo courtesy of Dept. Comparative Biomedicine and Food Science – University of Padova, AGRIPOLIS – Viale dell'Università 16, 35020 Legnaro (PD) - Italy)

with interspersed myriads of intensely basophilic granules (presumed proteoglycans). Neoplastic cells with multiple nuclei are rarely encountered. Anisocytosis and anisokaryosis are moderate, and mitotic figures are less than 1 per hpf. Numerous small and newly formed capillaries engorged by erythrocytes (neovascularization and congestion) are also visible, with occasional intravascular deposition of polymerized fibrillary eosinophilic material (fibrin thrombi) and erythrocyte extravasation (microhemorrhages). Small foci of inflammatory cells mainly composed of lymphocytes, plasma cells and, to a lesser extent, macrophages are also visible. At the periphery of the mass, small areas characterized by cellular hypereosinophilia and swelling, retention of cell borders, loss of cellular detail and nuclear pyknosis, loss of nuclei, and occasional faint eosinophilic nuclei or nuclear debris (karyorrhexis) (coagulative necrosis) are observed. Scattered melanophores are also noted throughout the mass.

Occasional filamentous bacteria elements are noticed and associated with the pocket scales and with small areas of epidermal ulceration. The neoplasia focally infiltrates the skeletal muscles causing sarcolemmal disruption, cytoplasmic flocculation, fragmentation, and hyalinization (muscle necrosis). This change is associated with infiltration of the myofibers by macrophages with foamy cytoplasm and occasional satellite cells (muscle regeneration) are evident. Intact small peripheral nerves are also noted at the edge of the mass.

The mass was stained with Alcian blue, Fontana-Masson, and immunohistochemically tested for S-100 expression. The Alcian-blue staining confirmed the mucinous origin of the extracellular matrix, while the Fontana-Masson staining yielded negative results, reasonably ruling out a melanocytic origin for this neoplasm. S-100 IHC specifically labeled neoplastic cells cytoplasm, reinforcing the hypothesis of a nervous origin.

Figure 4-2. Presentation, trout. The coelomic cavity and contents are free of tumor. (Photo courtesy of Dept. Comparative Biomedicine and Food Science – University of Padova, AGRIPOLIS – Viale dell'Università 16, 35020 Legnaro (PD) - Italy)

Contributor's Morphologic Diagnoses:
Rainbow trout (*Oncorhynchus mykiss)*, dorsal fin: myxoid nerve sheath tumor (NST).

Contributor's Comment:
Nerve sheath tumors (NSTs) were previously classified as peripheral nerve sheath tumors (PNSTs), but the term NST is now preferred given the complete lacking of an involvement of central nerves. In veterinary medicine, NSTs are classified as schwannoma, neurofibroma, perineurioma, and malignant nerve sheath tumor (MNST).

NSTs may arise in different body sites, predominantly in skin, spinal and cranial nerves, and autonomic ganglia of the heart, mediastinum, and thorax. In cats and dogs, NSTs are frequently characterized by the deposition of myxoid extracellular matrix.[9] Similar to mammals, numerous piscine species can be affected by neoplasia, and some species, in particular, are predisposed to NST development. Among them are Goldfish (*Carassius auratus*)[10], snappers (Lutjanidae)[2,13], coho salmon (*Oncorhynchus kisutch*)[6], damselfish (*Pomacentrus partitus*)[11], and rainbow smelt (*Osmerus mordax*)[7]. In particular, schwannoma is the most common neoplasia in goldfish[3,10,12], and are reported also in seabream (*Sparus aurata*)[4], and zebrafish (*Danio rerio*)[5].

In humans, domestic animals, and goldfish[12], schwannomas show some characteristic patterns that can be of aid in the histopathological diagnosis. They frequently form cellular foci composed of in interlacing bundles of spindle cells (Antoni A areas) and hypocellular and less compact areas associated with loose myxoid material (Antoni B pattern).

When these two distinct histological patterns are missing several differentials need to be taken into consideration, including neuro-fibroma, perivascular wall tumors, sarcoid, myxoma/myxosarcoma, fibroma, melanoma, leiomyoma/leiomyosarcoma, low-grade MNST, ganglioneuroma and, in general, other soft tissue sarcomas.

IHC generally helps in such cases, and panels including S-100, NGFR, Olig-2, PRX, and

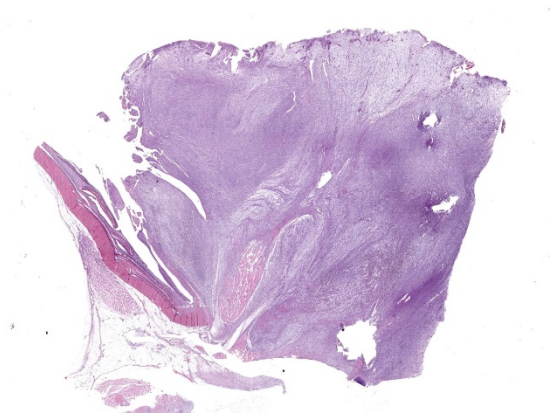

Figure 4-3. Scaled skin, trout. Expanding the dermis, elevating the ulcerated scaled epidermis and infiltrating underling skeletal muscle, there is a mesenchymal neoplasm arranged in long streams and bundles. (HE, 5X)

SOX-10 may be applied for NST diagnosis. However, very few antibody clones have already been tested in fish, and among them, only the S-100 clone was available in our lab. The specific immuno-labeling obtained for S-100 pointed out the nervous origin of the neoplastic cells, while the possible melanocytic origin was ruled out by the use of Fontana-Masson staining, which proved to be negative. This, together with the Alcian-blue positivity of the loosely arranged matrix (a feature which is not reported for melanocytic neoplasms), reinforced the diagnosis of a myxoid NST.

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JPC Diagnosis:
Skin and dermis: Pigmented malignant neoplasm.

JPC Comment:
Nerve sheath tumors (and text suggestive thereof) are rarely described in ancient historical writings, likely attributable to their relatively low frequency of occurrence as well as an overall lack of anatomic understanding by ancient physicians. Hippocratic writings from 460-375 BC highlight this lack of understanding as

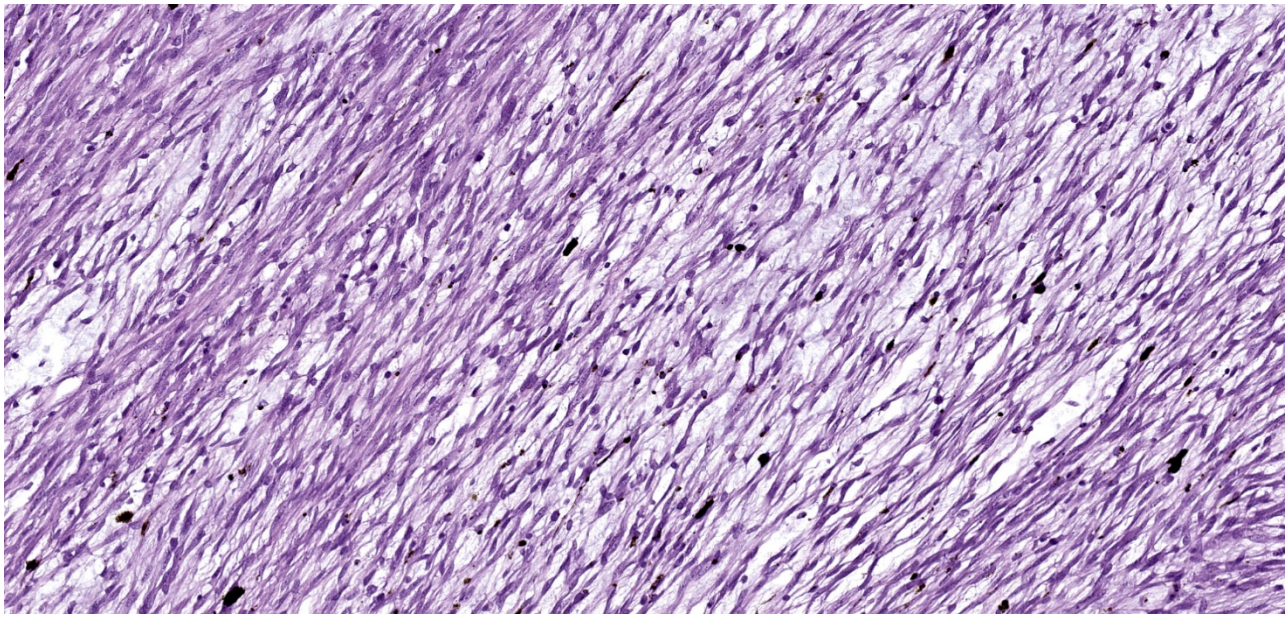

Figure 4-4. Scaled skin, trout. Neoplastic cells are spindled, with elliptical to spindled nuclei. The neoplastic cells are separated by abundant myxomatous matrix. Small numbers of neoplastic cells contain variable amounts of cytoplasmic melanin. (HE, 189X)

ambiguous words are used to describe both nerves and tendons. Galen of Pergamon (129-200 AD) was one of the first to provide detailed descriptions of peripheral nerves extending from the spinal cord to the digits, largely based on dissections of pigs and apes. Interestingly, Galen did not believe peripheral nerves were capable of repair following transection and a wounded nerve connected to the brain would result in convulsions. Galen recommended a salve composed of ground earthworms to treat nerve injury rather than surgical incision.[9]

The notion peripheral nerves are incapable of regeneration remained unchallenged until Cruickshank's experiment investigating canine survival following vagal nerve transection in 1776. Cruickshank found dogs survived following unilateral transection vagal nerve but died when the contralateral nerve was incised in two weeks later. While examining the site of the first transected nerve, Cruickshank noted the presence of a white substance inconsistent with scar tissue and postulated it was regenerating nerve tissue. In subsequent experiments, he found dogs survived when the time interval between transections was increased. Despite this convincing evidence of nerve regeneration, the Royal Society rejected his thesis for publication based on over 1700 years of belief to the contrary. Haighton took the experiment one-step further 20 years later, transecting the initially transected vagal nerve shortly after the contralateral nerve, resulting in the death of the animal. Cruickshank and Haighton's manuscripts were subsequently published in 1795.[9]

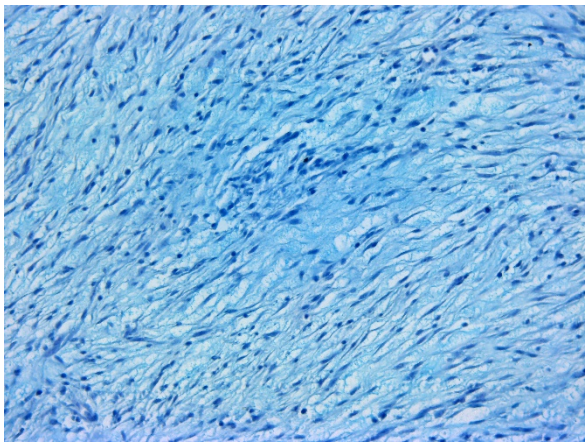

Figure 4-5. Scaled skin, trout. Neoplastic cells are surrounded by abundant myxomatous matrix which stains positively with Alcian Blue 2.0. (Photo courtesy of Dept. Comparative Biomedicine and Food Science – University of Padova, AGRIPOLIS – Viale dell'Università 16, 35020 Legnaro (PD) - Italy). (Alcian blue 2.0, 400X)

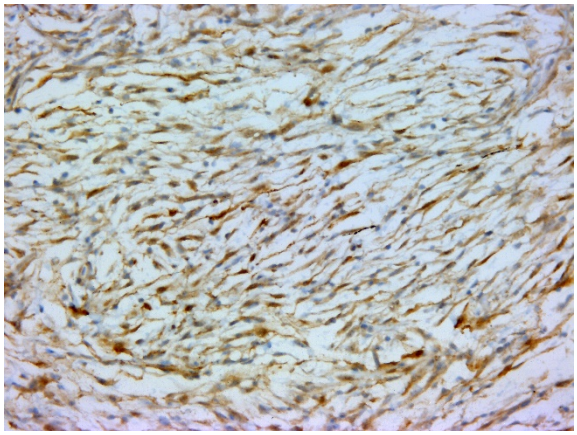

Figure 4-6. Scaled skin, trout. Neoplastic cells demonstrate moderate cytoplasmic immunoreactivity for S-100. (anti-S100, 400X)

Odier of Geneva first used the term "neurom" to describe tumors formed by diseased enlarged nerves in 1811. Over the ensuing decades, authors applied this term to describe both traumatic neuromas (as likely observed by Cruickshank) as well as both primary and metastatic tumors involving nerves. In 1864, Virchow separated peripheral nerve tumors of into "true" and "false" neuromas, with the former containing both nerve fibers and nerve sheath elements while the latter only contained elements of the nerve sheath (now known as nerve sheath tumors).[9]

Significant debate ensued over the next century about the cell of origin with Genersich first proposing "false" neuromas (i.e. nerve sheath tumors) arose from Schwann cells while others argued they were composed of endoneurium. Harkin and Reed

expanded the classification of peripheral nerve sheath tumors in 1969 by identifying schwannomas and neurofibromas as separate entities. Their use of electron microscopy confirmed of Schwann cell involvement in schwannomas but they were unable to determine the neurofibroma cell of orgin.[9]

Subsequent advances in the fields of molecular and cellular biology have generated substantial evidence that both schwannomas and neurofibromas originate from Schwann cells lacking expression of tumor suppressor gene known as *NF1*. This gene is responsible for the production of NF1 protein, which inactivates Ras by promoting the autohydrolysis of GTP to GDP. Notably, only Schwann cells in schwannomas are deficient in *NF1* gene expression whereas all the supporting cells in neurofibromas are *NF1* deficient with Ras hyperactivity, with an increased rate of proliferation in response to growth factors released from tumorigenic Schwann cells.[9] As noted by the contributor, additional nerve sheath tumors including perineurinoma, and malignant nerve sheath tumor have also been described.[8]

Neoplasia may present in fish in a similar manner as in mammals, with reports of metastatic disease. Farmed species are not as commonly affected as others, largely as the result of inherently prohibitive rapid production cycles that reduce the incidence neoplasia development in those populations as a whole. However, older fish (as in this case) are also encountered in hatcheries, such as when utilized as broodstock and are more likely to present with neoplasia. Compared to farmed species, the rate of detection is much higher in ornamental and wild species with longer lifespans. Neoplasia is most commonly associated with highly proliferative tissues such as the skin, gills, liver, gastrointestinal tract, in addition to numerous reports of nerve tumors as previously noted by the contributor.[1]

In this particular case, additional sections submitted to the WSC underwent a battery of additional stains including vimentin, Sox10, OLIG2, muscle specific actin, and Fontana-Masson; however, these stains and their associated procedures have not been verified in fish. The neoplastic cells demonstrate diffuse, moderately strong nuclear and cytoplasmic immunoreactivity for S100, indicating the cells are of neural crest origin. Neoplastic cells are immunonegative for muscle specific actin. Vimentin, Sox10, OLIG2, and Fontana-Masson are non-contributory. In addition, participants noted approximately 10% of the cells within the neoplasm have cytoplasmic melanin, which generated additional discussion in regard to a differential diagnosis of a melanocytic neoplasm, which would also be expected to be immunoreactive for S100. Unfortunately, the additional stains required to make this differentiation were non-contributory in this case. Given an inability to definitively identify this neoplasm via histomorphologic characteristics and available histochemical and immunohistochemical stains, we prefer a more general diagnosis of pigmented malignant neoplasm.

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