Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2024-2025

Conference #20 12 February 2025

CASE I:

Signalment:
Approximately 5-month-old, female, cherry shrimp (*Neocaridina davidi)*

History:
This shrimp was one of 10 ornamental dwarf shrimps housed in a 28-gallon freshwater aquarium and was found dead with no noted premonitory signs. The death of this shrimp occurred one month after a complete water change from tap water to distilled and re-mineralized water although standard water acclimation procedures were followed.

Gross Pathology:
External examination revealed numerous fine, light green to yellow, elongate to club-shaped, up to 1 mm long structures projecting outward along the interpleopodal spaces.

Figure 1-1. Presentation, shrimp. Numerous fine, light green to yellow, elongate to club-shaped, up to 1 mm long structures projecting outward along the interpleopodal spaces (*Photo courtesy of:* Department of Pathobiological Sciences and Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA. https://www.lsu.edu/vetmed/laddl/; https://www.lsu.edu/vetmed/pbs/index.php)

Microscopic Description:
Sagittal sections of the entire shrimp were available for histologic review. Many thalli were protruding externally within the pleopodal regions and consisted of cuticular perforating multinucleated basal cells that measured 20 µm in diameter and externally branching erect filaments that ranged from 8 to 15 µm in diameter with numerous terminal zoosporangia of varying maturational stages. Fully sporulated zoosporangia measured up to 800 µm in length and 100 µm in diameter and consisted of numerous peripherally located zoospores. The perforating basal cells were associated with a branching network of hyphal-like rhizoids that infiltrated the epidermis and subcutis, ranged from 4 to 8 µm in diameter, were uninucleated and partitioned, and occasionally contained granular material highlighted by a Grocott methenamine silver stain (GMS). The subcutaneous tissue was markedly expanded by hemolymph and heavily infiltrated by predominately granulated hemocytes. Rhizoids and associated inflammatory infiltrates extended to and surrounded the ventral nerve cord and multifocally (but minimally) invaded the ad

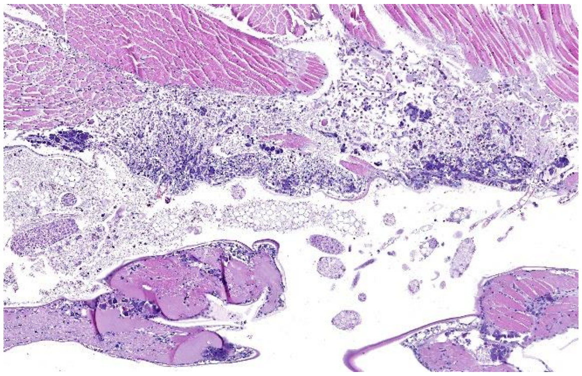

Figure 1-2. Pleopodal region. Many thalli were protruding externally within the pleopodal regions and consisted of cuticular perforating multinucleated basal cells that measured 20 µm in diameter and externally branching erect filaments that ranged from 8 to 15 µm in diameter with numerous terminal zoosporangia of varying maturational stages. (HE, 200X) (*Photo courtesy of:* Department of Pathobiological Sciences and Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA. https://www.lsu.edu/vetmed/laddl/)

jacent abdominal flexor musculature. All observed algae forms were strongly highlighted by a GMS stain. Similar uninucleated and partitioned, short rhizoids were also circulating within the hemolymph of the ventral cephalothorax, hepatopancreas, and around the midgut. Within these areas, pooled hemolymph contained numerous circulating hemocytes.

Contributor's Morphologic Diagnosis:
Interpleopodal subcuticular invasive and circulating rhizoids, numerous, with external zoosporangia and infiltrating granulated hemocytes

Contributor's Comment:
The external thallus portion consisting of cuticular perforating basal cells, branching erect filaments, and terminal zoospores as well as subcutaneous rhizoids within the pleopod area are morphologically consistent with *Cladogonium* sp. *Cladogonium* sp. is a parasitic epibiotic green algae of East Asian freshwater shrimp. It was first described in *Paratya improvisa* in 1950[1], then described in *Macrobranchium longipes* in 1971[2], and since then has been well characterized in *Neocaridina davidi*.[4,5] *Cladogonium* sp. is an important cause of epibiont-related morbidity and mortality in aquacultured and captive *Neocaridina davidi*. Classification of *C. ogishimae* and *C. kumaki* sp. nov. has primarily been based on morphology with limited molecular characterization grouping *Cladogonium* sp. within the order Trentepohliales although still widely accepted as belonging to the order Cladophorales and family Pithophoraceae.[1,5]

Neocaridina davidi are freshwater dwarf shrimp that are native to Taiwan and are heavily bred for the aquatic pet trade industry due to their popular small size, intense coloration, and large diversity. The lack of readily available veterinary care and scientific literature on this agent has resulted in widespread misinformation amongst shrimp hobbyists.

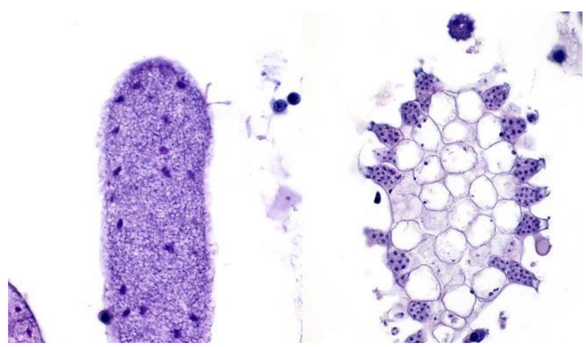

Figure 1-3. Pleopodal region. Fully sporulated zoosporangia measure up to 800 µm in length and 100 µm in diameter and consisted of numerous peripherally located zoospores. (HE, 1000X) (*Photo courtesy of:* Department of Pathobiological Sciences and Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA. https://www.lsu.edu/vetmed/laddl/)

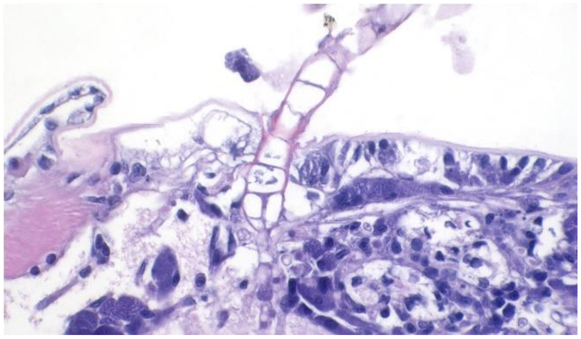

Figure 1-4. Pleopodal region. Algal basal cells perforated the cuticle, which was thin. Many granulated hemocytes infiltrated the epidermis and subcutis. (HE, 400X) (Photo courtesy of: Department of Pathobiological Sciences and Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA. https://www.lsu.edu/vetmed/laddl/)

The common misnomer for *Cladogonium* sp. is "green shrimp fungus" and has widely been confused with Ellobiopsidae. Ellobiopsidae is a small diverse group of protists that are closely related to dinoflagellates and are primarily ectoparasites of marine pelagic crustaceans (krill) and freshwater copepods and cladocerans (water fleas).[3] Other well recognized epibionts of *Neocaridina davidi* and their preferred anatomical location include 1) Rotifera found along the rostrum and antennas; 2) *Stentor* sp. along the rostrum and antennas; 3) *Scutariella japonica* along the rostrum, pleopods, and pereiopods; 4) *Monodiscus kumaki* sp. nov. along the rostrum, pleopods, and pereiopods; 5) *Saprolegnia* sp. along the pleopods and uropods; 6) *Holtodrilus truncates* along the rostrum, branchial chambers, pleopods, and pereiopods; and 7) *Vorticella* sp. along the chelipeds, pereiopods, pleopods, and uropods. [4,5] Of these, *Cladogonium ogishimae*, *Saprolegnia* sp., and *Scutariella japonica* are known to exhibit parasitic lifestyles. [4]

Importation of *Neocaridina davidi* and their epibionts is considered the main route of spread.[4,5] It is suspected that following rhizoid colonization of the cuticular epidermis and subcutis along the interpleopodal region, basal cells perforate the cuticle and branch externally as erect filaments with terminal zoosporangia.[1] Ciliated zoospores are then released into the environment by the mature zoosporangia. These contain chloroplasts, which impart the grossly observed green coloration and is evident in the advanced stage of infection. [6] All algal forms stain strongly with Grocott methenamine silver stain. Death is thought to be due to debilitation, potentially related to involvement of the ventral nerve cord, molting complications, and/or secondary infections.[1] Systemic infections, as was seen in this case, have not been previously described. The possibility of a systemic coinfection with *Saprolegnia parasitica* was considered; however, the circulating short rhizoid segments are morphologically identical to those within the pleopod area with branching, partitioning, and uninucleation and are morphologically distinct from *Saprolegnia* sp. filaments.

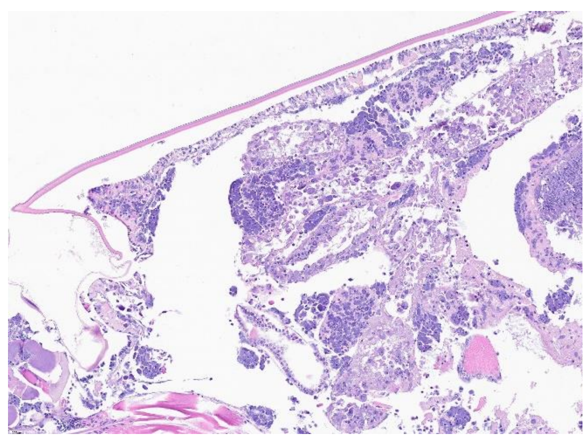

Figure 1-5. Dorsal cephalothorax, shrimp. The coelomic cavity contains numerous hemocytes, which are markedly distended by large, granular, cytoplasmic, bacterial inclusions. Similar bacterial inclusions are in the epidermis and hepatopancreatic interstitium. (HE, 100X)

Diagnosis of *Cladogonium* sp. infections is largely based on observation of the characteristic light green elongate club-shaped structures projecting outward in the pleopodal area, which are, again, evident in advanced infections as the erect filaments and immature zoosporangia largely lack chloroplasts. The prognosis is poor for heavily parasitized shrimp, which constitute the source of infection for other shrimp in the environment. While algaecides are effective at eliminating this agent by substituting copper for magnesium within chlorophyll, shrimp are exquisitely sensitive to copper toxicity and would be unlikely to survive treatment doses required to kill algae.[1] Water quality management, quarantining, and reduction of stressors such as temperature fluctuations are all important in preventing and managing outbreaks of this disease.[1] In our case, this disease manifested approximately one month after a 100% water change from tap water to distilled and re-mineralized water. During the water acclimation period, there was an ammonia spike in the temporary holding tank, which may have predisposed this shrimp to succumb to a preexisting parasitic infection. Considering that there were no additions or other changes to the tank since acquisition of the shrimp, an asymptomatic carrier state was suspected although could not be proved. No additional deaths were observed following prompt removal of the dead shrimp, and the remaining shrimps have prolifically bred under stable water and temperature parameters.

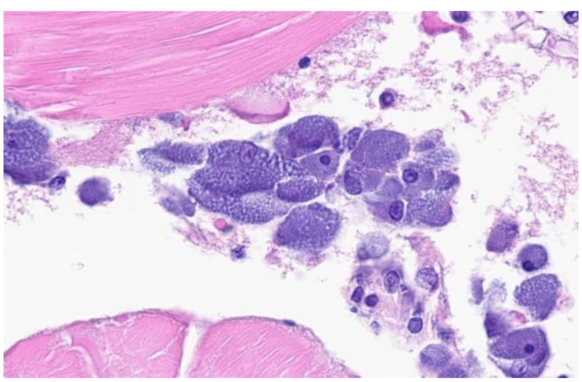

Figure 1-6. Coelom, shrimp: Hemocytes are distended up to 27 µm by granular, basophilic, cytoplasmic, bacterial inclusions, consistent with rickettsia-like organisms. (HE, 1000X)

Contributing Institution:

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https://www.lsu.edu/vetmed/laddl/
https://www.lsu.edu/vetmed/pbs/index.php

JPC Diagnosis:

1. Body as a whole: Hemocytosis, chronic, diffuse, severe with hemocytic and multisystemic cytoplasmic bacterial inclusions (rickettsia-like organisms)
2. Ventral carapace: dermatitis, necrotizing, acute, regionally extensive, mild with invasive algae

JPC Comment:

This week's moderator was Prof. Karen Terio from the University of Illinois who selected four exotics/wildlife cases to test conference participants on classic entities as well as several new ones. This first case features an ouotstanding, meticulously prepared sagittal section of a cherry shrimp. Preservation of major anatomic features[7] such as the eye, anterior ganglion, antenna, gills, tail fin, and pleopods facilitates slide orientation and interpretation of lesions.

We enjoyed discussing the pathogenesis of this case. The most substantial finding for mortality was likely the rickettsia-like organism (RLO) infection, which resulted in numerous bacterial inclusions within the cytoplasm of hemocytes throughout the hemolymph. Other cells (particularly the epidermis) also had inclusions, though less frequently. Rickettsia-like organisms are primary pathogens of invertebrates and given the severity of inflammation, RLOs were likely the main cause of the decline seen in this case. Rickettsia are obligate intracellular bacteria. Infections with RLOs are widely reported in crustaceans. Regardless of the crustacean host, disease caused by RLOs has typical histologic findings, namely bacterial inclusions in hemocytes throughout the body and less commonly in other tissues, as seen in this case.

Given the limited distribution of the algal infection, participants suspected that the algal lesion was likely secondary to generalized debilitation from RLO infection, though two separate primary pathogenic processes are possible. We did not speculate on the exact algae involved (lacking ancillary confirmation) as microhabitat and epibiont-host relationships are not entirely exact,[5] though the shape and distribution is appropriate for *Cladogonium*.

Finally, participants also discussed the nature of epibiosis. Epibiotic organisms live on the surface of another organism and this relationship can be harmless (neutral), commensalistic (both benefit), or parasitic (one benefits at the others expense). Such relationships have the potential to directly impact organismal coloration, health, and reproduction which all have significant economic impacts.[5] In the present case, this relationship bests fits with a parasitic one. It is possible that entry of thalli (direct penetration) and previous areas of carapace damage both facilitated *Cladosporium* development. That the nine other conspecifics of this shrimp were not affected points to this host-epibiont relationship being fairly muted (or at least tolerable), though *Cladogonium* should still be viewed as a pathogen nonetheless.

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CASE II:

Signalment:
Adult female betta fish (*Betta splendens*)

History:
This animal was wild-caught in Thailand and singly housed at the Wake Forest University Reynolda Campus for use in an experimental study evaluating differences in personality, behavior, and locomotion between wild and domestic betta fish. About 5 weeks after arrival from Thailand it appeared thin, but was observed consuming normal amounts of food. The animal hid in the tank and was found dead approximately 8 hours later. Three other female wild-caught betta fish in this cohort were also found dead. All domestically purchased male and female betta fish and male wild-caught betta fish in this cohort had no clinical signs.

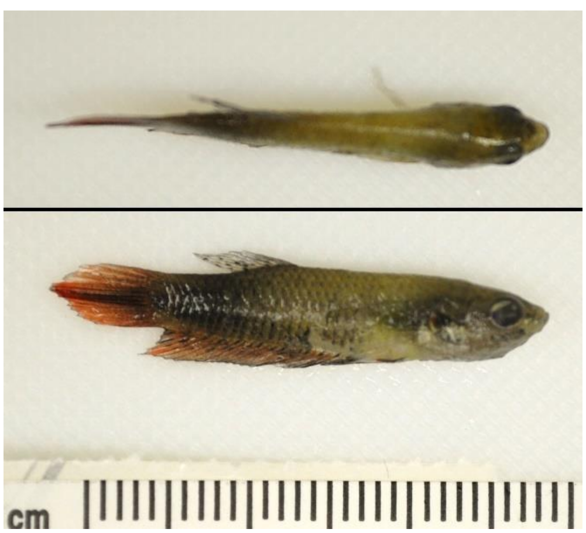

Figure 2-1. Whole body, betta. The betta was emaciated, evidenced by concavity of the middle abdomen. (*Photo courtesy of* Wake Forest Baptist Medical Center, Winston-Salem, NC 27157)

Figure 2-2. Whole body, betta. A sagittal section of the whole fish minus tail is submitted. At subgross magnification, granulomas in the liver, kidney and gills, as well as numerous cysts within skeletal muscle are evident. (HE, 9X)

Gross Pathology:
Examined was a 0.25g female betta fish (*Betta splendens*) in emaciated body condition, evidenced by concavity of the middle abdomen.

Microscopic Description:
Whole body longitudinal section: Numerous round to oval, 40-400um diameter granulomas were present in the kidneys, liver, intestine, ovary and in the gill chamber. The granulomas were composed of central cores of brightly eosinophilic material admixed with karyorrhectic debris (necrosis) surrounded by concentric layers of epithelioid macrophages. Abundant acid-fast bacterial rods were present within the cytoplasm of the macrophages and in the necrotic cores of the granulomas. The intestinal mucosa contained variably-sized, up to 200um diameter, round microsporidian xenomas**.** The xenomas had thin eosinophilic walls, contained numerous eosinophilic, 2-4 um, acid-fast oval spores, and some had central hyperplastic host nuclei. Many 0.4-0.6mm diameter round cysts with up to 3um thick eosinophilic hyaline walls were present in the skeletal musculature and body cavity that contained cross sections of trematode larvae. The larvae had 5um thick

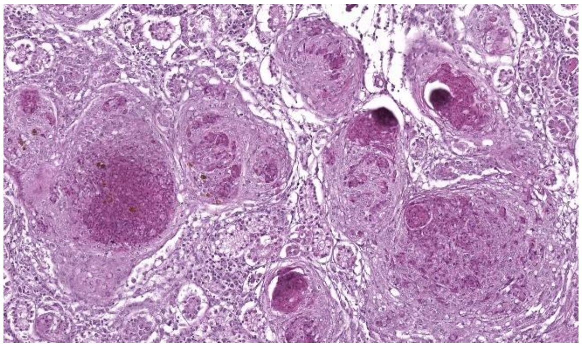

Figure 2-3. Kidney, betta. Numerous well-formed granulomas are scattered throughout the renal parenchyma (HE, 301X).

teguments, lacked body cavities, had abundant parenchymatous matrix, paired ceca, and prominent suckers. Some of the cysts contained only clear space or degenerate cells. Few aggregates of macrophages with intracytoplasmic brown granular pigment (melanomacrophages) sat adjacent to some cysts and within some granulomas.

Contributor's Morphologic Diagnosis:

1. Nephritis, hepatitis, enteritis, branchitis, and oophoritis, multifocal to coalescing, chronic, severe, granulomatous with intralesional acid-fast bacteria
2. Trematodiasis, multifocal, skeletal muscle and body cavity
3. Microsporidial xenomas, multifocal, intestinal mucosa

Contributor's Comment:

Mycobacteriosis and nocardiosis are well-recognized diseases of wild and aquacultured fishes in which actinomycetes cause severe chronic granulomatous systemic disease.[4] Mycobacteriosis is much more common in fish than nocardiosis, but both produce analogous gross and histologic lesions.[10] Bacterial morphology can help to differentiate these, as *Mycobacteria* spp. are non-filamentous, non-branching while *Nocardia* spp. are branching filamentous organisms that may resemble Chinese letters.[11] Further, the Ziehl-Neelsen acid-fast stain is useful for discrimination as *Nocardia* spp. stain poorly or not at all with this stain, and are best visualized with modified acid-fast stains, such as Fite's modified acid-fast stain[10]. Therefore, the bacteria in this case were consistent with *Mycobacteria* sp. and bacterial culture or PCR could provide species-level characterization.

Many non-tuberculous *Mycobacterium* species have been reported to cause disease in fish, although *M. marinum*, *M. chelonea* and *M. fortuitum* are the most common isolates.[6] Transmission occurs principally through ingestion of either contaminated feed, organic debris, or infected carcasses.[14] Prompt removal of dead or moribund fish within a tank is, therefore, of the utmost importance in preventing transmission. The clinical manifestations of mycobacteriosis in zebrafish are often non-specific and may include emaciation, lethargy, abdominomegaly, and changes in swimming behavior.[14] In many cases fish die without exhibiting clinical signs.[14] Chronic stress in zebrafish exacerbates the susceptibility to mycobacteriosis and increases the severity of the disease.[12] This likely played a role in this case as the fish was transported from a natural environment in Thailand to an experimental setting about 5 weeks prior to death. Notably, aquatic mycobacteria such as

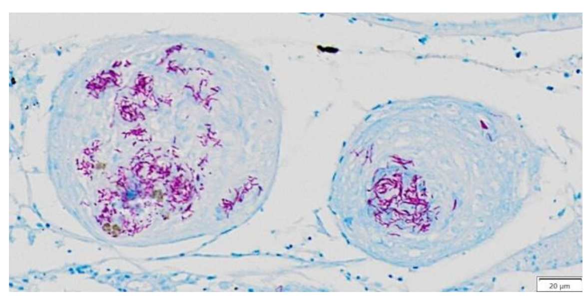

Figure 2-4. Kidney, betta. Numerous acid-fast bacterial rods are present within the granulomas (Ziehl-Nielsen, 301X). (*Photo courtesy of* Wake Forest Baptist Medical Center, Winston-Salem, NC 27157)

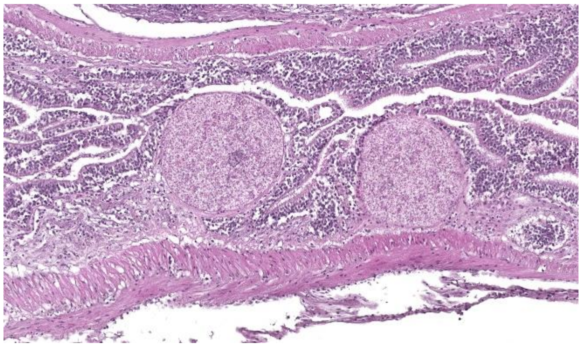

Figure 2-5. Intestine, betta. Two large microsporidial xenomas are present in the intestinal mucosa. A hypertrophied host nucleus is present within the xenoma on the left. (HE, 361X).

M. marinum pose significant zoonotic concerns including cutaneous granulomas and protracted illness, especially in immunocompromised individuals.[6]

Microsporidia are obligate intracellular eukaryotic parasites that can infect a wide range of hosts.[4] Taxonomic classification of microsporidia has shifted over time, and this group is currently considered to be most closely related to fungi based on molecular studies[4]. Fish are hosts to over 150 species of microsporidia in 14 different genera[4]. The genera can be divided according to their ability to form xenomas (*Glugea*, *Tetramicra*, *Loma*, *Ichthyosporidium*, *Jirovecia*, *Microfilum*, *Microgemma* and *Nosemoides* spp.) or not (*Nucleospora*, *Pleistophora*, *Heterosporis* and *Thelohania* spp.). Xenomas are cyst-like structures composed of hypertrophied host cells within which reside microsporidians at various life stages. The infected host cell increases in size due to the proliferating parasites and hypertrophy of the infected cell, including the host cell nucleus as seen in this case.

Coinfection of microsporidia with mycobacteria in fish is not uncommon as microsporidosis is more prevalent in immunosuppressed individuals[12] and mycobacteriosis suppresses immunity.[2] Microsporidial infestation of the gastrointestinal tract when confined to the intestinal mucosa is relatively inconsequential, as was likely in this case, as the surrounding inflammation was minimal.[3] Two microsporidians that cause significant disease in laboratory-reared zebrafish are *Pseudoloma neurophilia* and *Pleistophora hyphessobryconis.*[1] Infection with these can cause subclinical disease or overt illness and may impact experimental outcomes[1]. Extensive surveillance methods and strict biosecurity measures have been outlined to control or prevent microsporidial infection in zebrafish research facilities.[8,13] Further, zebrafish lines that are specific-pathogen free for *Pseudoloma neurophilia* have been developed.[7]

The encysted parasites were consistent with metacercariae, the larval form of digenetic trematodes.[1] The life cycle of most trematodes involves an aquatic snail intermediate host, in which asexual reproduction occurs leading to the release of cercariae into the water.[5] These penetrate the skin or gills of the fish intermediate hosts and often migrate to skeletal muscle for development into encysted metacercariae.[5] These are infective to definite hosts after consumption of the fish.[5] Natural infection is not associated with clinical disease in zebrafish and the inflammatory response is usually minimal due to the parasitic capsule, but the presence should be noted as infection may impact experimental outcomes.[1] Several species of digenean trematodes have metacercarial forms, and common species in freshwater fish in Thailand include *Haplorchis pumilo* and *Centrocestus formosanus.*[9]

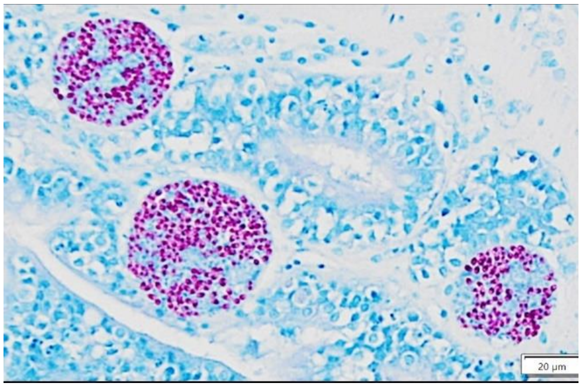

Figure 2-6. Intestine, betta. A Ziehl-Neelsen stain highlights the spores within the xenomas in the intestinal mucosa. (Ziehl-Nielsen, 400X). (*Photo courtesy of* Wake Forest Baptist Medical Center, Winston-Salem, NC 27157)

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JPC Diagnosis:

1. Liver, heart, anterior kidney, posterior kidney, coelom, ovary: Granulomatous hepatitis, myocarditis, coelomitis, oophoritis, and nephritis, chronic, multifocal, severe
2. Skeletal muscle and coelom: Granulomas, multiple with encysted metazoans
3. Intestines: Microsporidial xenomas, multiple

JPC Comment:
This second case showcases several classic fish entities while offering some additional points for consideration. We agreed with the contributor that the multifocal granulomatous inflammation best represented mycobacteria, though we did note small numbers of filamentous, slender bacteria on Fite-Faraco that could reflect *Nocardia* spp. that could be differentiated via PCR or culture. Within affected tissues, the presence of pigmented macrophages (melanomacrophages) likely reflects sequestration of iron which is a host defense mechanism against mycobacteria. Although termed "melanomacrophages" in many texts, recent studies have demonstrated that these macrophages contain multiple pigments, to include iron and melanin, and may be more appropriately termed "pigmented macrophages."

The microsporidial xenomas within the intestine were characteristic and easy to identify in this case. Although H&E was sufficient to make the diagnosis, acid-fast stains also sharply outlined spores within affected enterocytes. With the Fite-Faraco stain, morphology was helpful to not confuse a xenoma

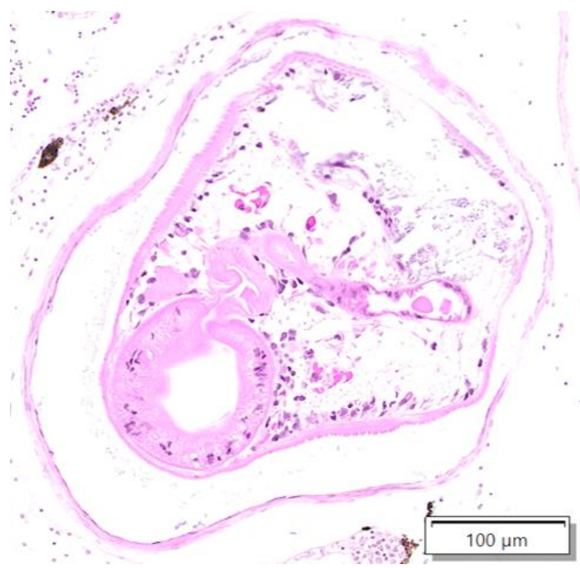

Figure 2-7. Skeletal muscle, betta. Numerous metacercariae are present within the skeletal muscle. (HE, 400X). (*Photo courtesy of* Wake Forest Baptist Medical Center, Winston-Salem, NC 27157)

with adjacent mycobacterial foci given the overlapping distribution of these lesions and staining characteristics. Conference participants also reviewed the anticipated features of non-xenoma forming microsporidial infections which characteristically have wider distribution of spores and associated inflammatory cells instead.

The metazoan parasites of this case gave conference participants pause. The encysted metazoans within skeletal muscle resemble metacercaria, though the metazoans within the coelom appear morphologically distinct and contain 10um round amphophilic to basophilic bodies (possible calcareous corpuscles), consistent with a larval cestode (i.e. a plerocercoid). Tapeworms are a common finding in both free ranging and cultured fish,[15] opening up the possibility that this case is in fact a 'four-fer'.

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<u>CASE III</u>:

Signalment:
11-year-old, female cockatiel (Nimphicus hollandicus).

History:
This cockatiel was first obtained by the

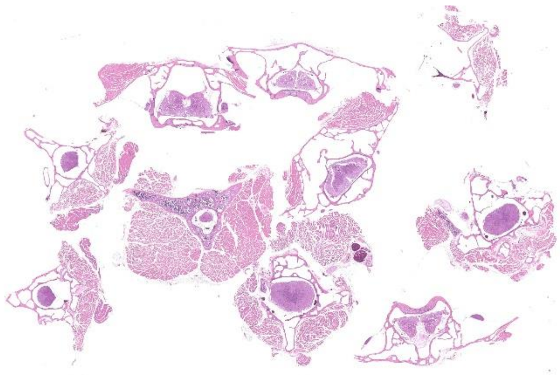

Figure 3-1. Spinal column, cockatiel. Numerous sections from all levels of the spinal column, including spinal cord and surrounding vertebral bodies consisting of pneumatic or hematopoietic bone are submitted for examination. (HE, 8X)

owner in September 2019, approximately 5 months prior to euthanasia. Initial veterinary consultation was sought with the complaint of frequent trembling and shaking. Diagnostic evaluation was initially declined, except for *Chlamydophila* testing pursued at a different clinic (unspecified), which was reported as negative. At subsequent presentation to the submitting veterinarian, the bird was thin (66 g) could barely perch, and seemed painful, with wings drooped forward. On manipulation, the wings were stiff and could not be returned to normal anatomical resting position. Euthanasia was elected.

Gross Pathology:

Examined is the body of a female, 11-month-old, 60 g, Cockatiel with a body condition score of 2 out of 5 (North Carolina Zoo Keel Score) and moderate autolysis. No significant lesions were noted.

Microscopic Description:

Examined are ten cross-sections of spinal column. Throughout all sections of the spinal cord, including cervical, thoracic, lumbar, and sacral regions, virtually all neurons within the gray matter and in the dorsal root ganglia contain large, intracytoplasmic, lamellar to globular, 5-35 µm in diameter, basophilic to eosinophilic structures. These intracytoplasmic structures often have a central, finely granular, pale center (Lafora bodies). Consistently surrounding the structures is a 2-5 µm wide band of finely granular, amphophilic cytoplasm (peripheral lysed axoplasm). Affecting approximately 20% of the axial muscles, muscle fibers are shrunken in multifocal random areas. Affected fibers have hypereosinophilic sarcoplasm with poorly defined cross striations and enlarged nuclei with open chromatin.

Period acid-Schiff staining with and without diastase applied to formalin-fixed, decalcified (formalin/formic acid solution), paraffin-embedded spinal column tissue highlights intensely PAS-positive, 5-35 µm intraneuronal cytoplasmic inclusions which are resistant to diastase digestion.

Staining of the same tissue using Luxol fast blue with cresyl violet and Acian blue (pH2.5) reveals bright violet and deep blue staining of the described inclusions, respectively.

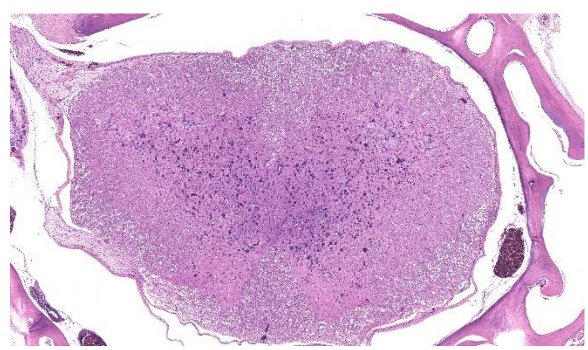

Figure 3-2. Spinal cord, cockatiel. There are numerous 10-30um deeply basophilic intracytoplasmic inclusions within neurons (which obviously results in the grey matter being disproportionately affected) (HE, 8X)

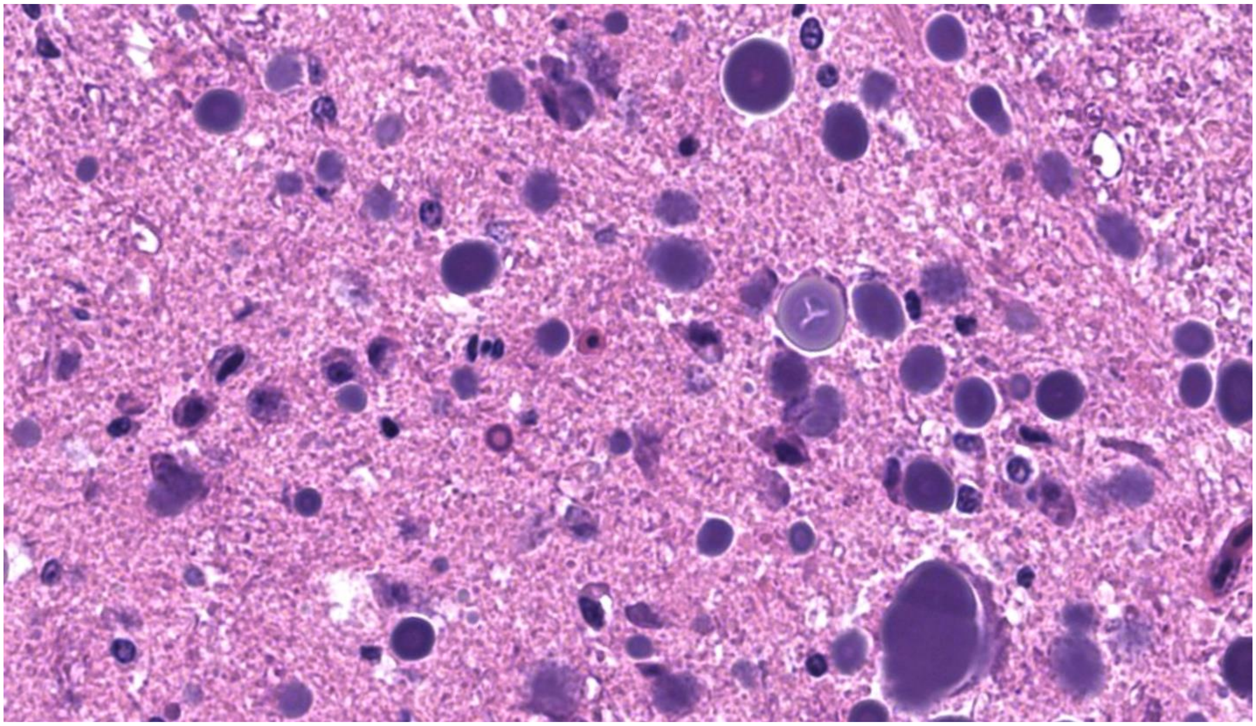

Figure 3-3. Spinal cord, cockatiel. Grey matter neurons contain numerous amphophilic to basophilic lamellated intracytoplasmic polyglucosan (Lafora) bodies. (HE, 381X)

Contributor's Morphologic Diagnosis:
Spinal cord: Numerous polyglucosan bodies consistent with Lafora disease

Contributor's Comment:
Lafora disease (neuronal glycoproteinosis) is a rare condition variably characterized by myoclonus, ataxia, weakness, behavioral abnormalities, and seizures in affected animals.[1,3-6,9,10] These clinical signs result from abnormal carbohydrate metabolism and accumulation within the perikaryon, dendrites, and axons of neurons as well as freely in the neuroparenchyma of both central and peripheral nervous tissue.[2] Inclusion bodies can also be found in other organ systems including skeletal and cardiac muscle, skin, and liver. In this case, inclusions were present in the cerebrum, spinal cord, dorsal root ganglia, perirenal ganglia, and myenteric ganglia.

These inclusion bodies, also called Lafora bodies, are spherical, basophilic, 5-35 µm, PAS-positive and diastase-resistant, and also display staining with Cresyl violet, Alcian blue pH 2.5, Bests carmine, methenamine silver, and Weils (6). Lafora bodies are composed of polyglucosan, an abnormal form of glycogen with low numbers of branches and very long glucose chains.[8] On electron microscopy, Lafora bodies are composed of radiating, branched filaments surrounding a dense central core.[9]

Disease associated with Lafora body accumulation has been described in humans, dogs, cockatiels, cattle, a fox, a cat, and a flying fox,[1,3-6,8,9] with associated genetic mutations identified in humans, miniature wirehaired dachshunds, a basset hound, and beagles.[4,7] In humans, Lafora disease is a fatal autosomal-recessive condition resulting from loss of function sequence variations in the genes *EPM2A* (encodeing laforin) or *NHLRC1* (also *EMP2B*, encodes malin),[4] encoding glycogen synthesis regulators laforin and malin, and

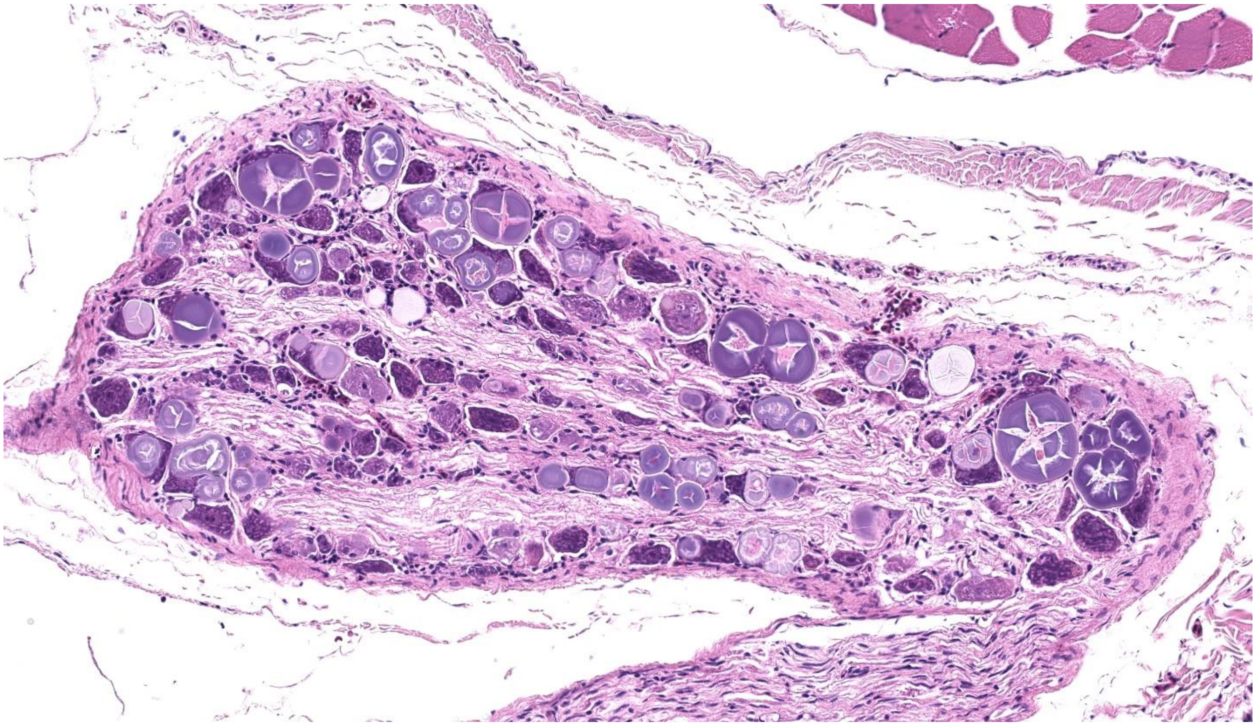

Figure 3-4. Dorsal root ganglion, cockatiel. Neurons in the dorsal root ganglion also contain prominent intracytoplasmic polyglucosan bodies.

the latter of which has also been implicated in dogs with a massive expansion of a 12 bp repeat in the gene.[4,7]

Contributing Institution:

Cornell University College of Veterinary Medicine, Department of Biomedical Sciences, Section of Anatomic Pathology

https://www.vet.cornell.edu/departments/biomedical-sciences/section-anatomic-pathology

JPC Diagnosis:

1. Spinal cord, spinal nerves, dorsal root ganglion: Intracytoplasmic polyglucosan (Lafora) bodies, numerous, with mild spongiosis
2. Spinal muscles: Atrophy, multifocal, mild

JPC Comment:

We have seen Lafora disease several times in conference, to include in an ox (WSC 2017-2018, Conference 17, Case 4) and in multiple dogs (WSC 2021-2022, Conference 7, Case 3; WSC 2012-2013, Conference 22, Case 4). The present case in a bird is interesting, though as the contributor notes this condition has previously been described in a cockatiel.[1] There is also a recent case report in a toucan from Brazil with features that mirror this case.[11]

Inclusion bodies were numerous across the provided sections of spinal cord and spinal nerves and stained readily with PAS, Alcian blue, and Luxol fast blue. We considered whether these inclusions might also be similarly present within skeletal muscle given that select myocytes also contain amphophilic globoid material. However, these myocyte inclusions did not reliably stain with PAS or Alcian blue (or at all with LFB), so we were unable to confirm this impression. As these stains are non-specific, it is possible that inclusions in myocytes may represent an entirely different material.

We added a separate morphologic diagnosis of muscular (neurogenic) atrophy that corresponds to the decreased body condition and inability of this animal to perch. This is best observed from subgross as shrunken, condensed myofibers. Additionally, there is also medullary bone (i.e., endostosis) present which is a normal/expected finding for a mature female bird. This amphophilic to basophilic bone is deposited on the outer aspects of the medullary trabeculae and represents a dynamic calcium depot for the egg-laying cycle. Medullary bone has also been described as a premonitory sign of testicular neoplasms in male birds.[12]

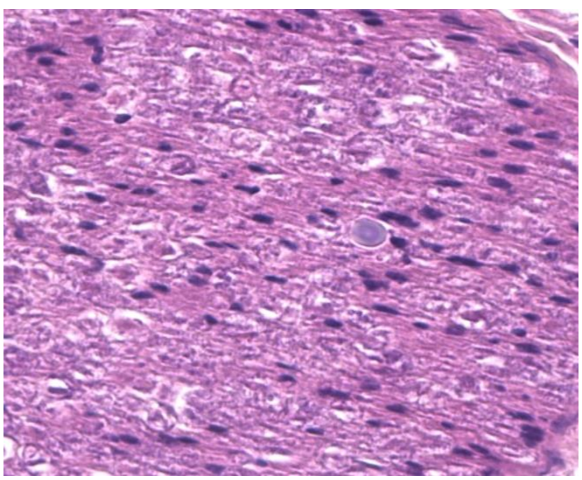

Figure 3-5. Spinal nerve, cockatiel. Rare polyglucosan bodies are present in spinal nerves. (HE, 1446X)

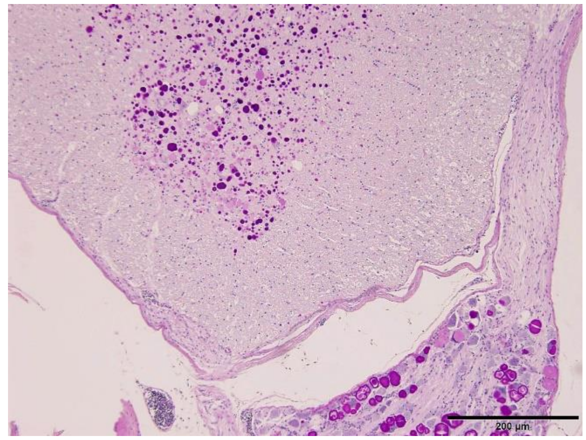

Figure 3-6. Spinal cord and dorsal root ganglia. Polyglucosan bodies stain strongly positive on periodic acid-Schiff preparations. (PAS, 100X)

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CASE IV:

Signalment:
Juvenile, male, *Megaptera novaeangliae,* humpback whale.

History:
A juvenile male humpback whale, measuring 11 meters in length, was found stranded alive in the coast of southern Brazil. Due to its deteriorated health and unsuccessful attempts to return the animal to the ocean over two days, it was euthanized.

Gross Pathology:
At necropsy, the humpback whale was in poor body condition. Multiple marine crustaceans (*Thoracica* sp.) were adhered to the skin in the submandibular and ventral neck region. Upon internal examination, significant changes were noted in the liver. The hepatic capsula appeared opaque and whitish. The right hepatic lobe was moderately reduced in size and with multifocal and elevated areas, which upon sectioning corresponded to biliary ducts with thickened walls and dilated lumen. The lumen was filled with bile and with numerous trematodes measuring 1.5-4 cm in length, 0.3-0.4 cm in width, with flattened and leaf-shaped morphology, containing oral and ventral suckers. Morphological and molecular characterization classified these parasites as *Brachycladium goliath*. Inside the renal veins, a high number of nematodes of up to 30 cm in length, morphologically compatible with *Crassicauda* sp., were observed. Additional macroscopical findings included the multiple accessory spleens measuring from 1.5 to 5 cm in diameter. Furthermore, the small intestine showed multifocal to coalescent areas of hemorrhages (petechias and ecchymosis) and the mucosa was markedly red with cestodes measuring approximately 80 cm in length in the intestinal lumen. The gross examination of remaining organs was unremarkable.

Figure 4-1. Cerebrum, humpback whale. One section of telencephalon is submitted for examination. (HE, 6X)

Laboratory Results:
RT-Nested-PCR: positive for cetacean morbillivirus (CeMV).

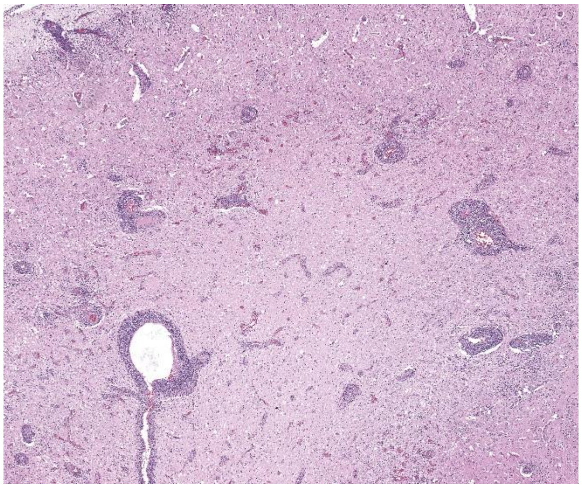

Figure 4-2. Cerebrum, humpback whale. Multifocally, there are prominent cuffs of inflammatory cells within Virchow-Robins spaces as well as gliosis and rarefaction of the adjacent neuropil. (HE, 50X)

Immunohistochemistry (IHC): IHC anti-*Morbillivirus* (anti-canine distemper virus, monoclonal) revealed moderate cytoplasmic immunolabelling in astrocytes, neuronal cell bodies, and axons).

Microscopic Description:
Brain: in the telencephalic cortex, mainly in the grey matter, there is marked and multifocal inflammatory infiltration of lymphocytes and plasma cells surrounding blood vessels (perivascular cuffings), frequently extending into the adjacent neuropil where moderate vacuolization is also frequent. Additionally, there are multifocal areas of moderate gliosis. Mild to moderate neuropil rarefaction (malacia) is evident in some areas, where there is neuronal necrosis, characterized by neurons with hypereosinophilic and retracted cytoplasm and absent or pyknotic nucleus. Moderate infiltration of Gitter cells and occasional axonal spheroids is also present. Occasional intracytoplasmic and intranuclear eosinophilic inclusion bodies are seen in neurons within affected areas. Similar lesions are also present in the brainstem and white matter of the cerebellum (slides not submitted).

Contributor's Morphologic Diagnosis:
Brain: encephalitis, lymphoplasmocitic, multifocal, marked, subacute, with mild and multifocal malacia, Gitter cell inflammatory infiltration, neuronal necrosis and occasional intracytoplasmic and intranuclear eosinophilic inclusion bodies.

Contributor's Comment:
Viruses from the genus *Morbillivirus* belong to the family Paramyxoviridae and order Mononegavirales. These are pleomorphic but frequently spherical, approximately 150 nm in diameter, RNA viruses with a nonsegmented and single-stranded linear genome.[11] Morbilliviruses can affect different species of mammals, including humans. The measles virus (MeV) is a human pathogenic *morbillivirus* that causes measles, a disease with high rates of morbidity and significant childhood mortality. Rinderpest, also known as "cattle plague", the peste des petits ruminants, and canine distemper are other notable diseases caused by morbilliviruses in mammals.[16]

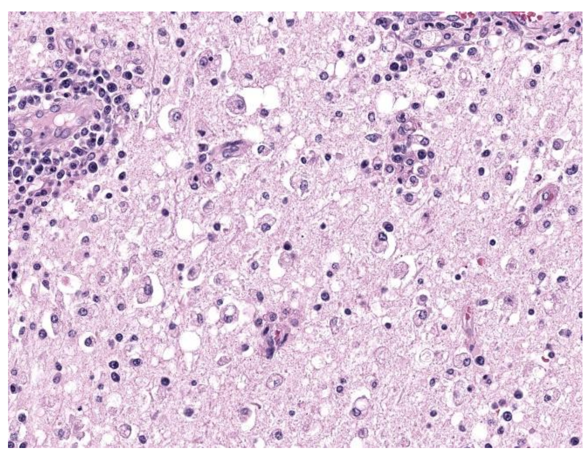

Figure 4-3. Cerebrum, humpback whale. Higher magnification of the inflammatory changes in the cerebral cortex (HE, 450X)

Morbillivirus infections resulting in massive strandings have been reported in aquatic mammals in recent decades.[3] The first description occurred in pinnipeds during 1988 and 1989 in northwest Europe, where nearly 18,000 harbor seals (*Phoca vitulina*) and hundreds of grey seals (*Halichoerus grypus*) died due to the infection by a morbillivirus identified as *Phocine Distemper Virus* (PDV).[7,9] Meanwhile, in cetaceans, the first report of a *morbillivirus* infection was described in six harbor porpoises (*Phocoena phocoena*) in Ireland in late 1988. This infection was related to the *Porpoise Morbillivirus* (PMV), a strain from the *Cetacean Morbillivirus* (CeMV). A couple of years later, infections by this virus were reported in harbor porpoises from various countries across Europe.[9,17] Since then, many strains of CeMV have been recorded causing stranding and deaths in odontocetes. However, reports in mysticetes are rare and include few cases in fin whales (*Balaenoptera physalus*)[8] and more recently, in two humpback whales in Brazil.[1]

Morbilliviruses are known to be lymphotropic and epitheliotropic, and studies have indicated that the pathogenesis associated to the CeMV infection resembles what is commonly seen in other morbillivirus infections in animals and humans.[10] When infected by CeMV, cetaceans can develop a marked interstitial pneumonia and/or non-suppurative encephalitis. Animals that survive this acute stage may acquire opportunistic infections due to the immunosuppression caused by the virus. Some animals may still have lesions of meningoencephalitis associated with demyelination. However, animals that clear out the systemic infection may develop a neurological disease with lesions restricted to the brain. In these cases, the virus is detected only in the CNS, and cytoplasmic or nuclear eosinophilic inclusion bodies are occasionally found in neurons, and astrocytes.[3] In a similar way, in humans with measles, the MeV infection can lead to encephalitis with inclusion bodies, particularly in immunosuppressed individuals, or to panencephalitis in persistently infected children.[5] Neurological involvement is common in carnivore morbilliviruses, as in the canine distemper, which can affect not only domestic dogs, but a broad range of domestic and wild carnivores.[4] In dogs, a strong immune response typically clears the canine distemper virus (CDV) from the tissues, resulting in fully recovery. On the other hand, with a weak immune response, the virus can penetrate epithelial tissues and the CNS, leading to clinical signs that typically appear around 20 days post-infection.[17] Neurologic signs can also develop later, due to a demyelination induced by the CDV.[14]

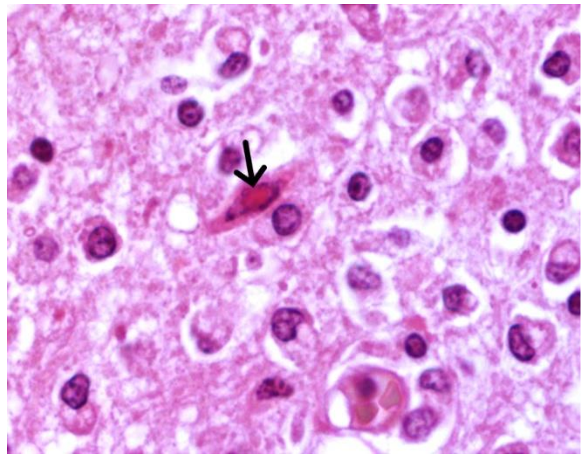

Figure 4-4. Cerebrum, humpback whale. An intranuclear eosinophilic inclusion body (arrow) is seen in a neuron. (HE, 400X) (*Photo courtesy of*: Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, http://www.ufrgs.br/patologia)

In this humpback whale, no macroscopic changes were seen during the evaluation of the encephalon, corroborating to previous studies on morbillivirus infections affecting the CNS of other species, such as dogs, in which neurological involvement is frequent.[15] On histological examination, CeMV infection in cetaceans resembles the lesions described in humans and animals infected by morbilliviruses, primarily characterized by multiple mononuclear perivascular cuffings, microgliosis, neuronophagia and demyelination in the cerebral cortex. The white matter may also show areas of malacia. Intracytoplasmic and/or intranuclear eosinophilic inclusions bodies are frequently seen.[6] Furthermore, when IHC anti-*Morbillivirus* is applied, a marked immunolabelling in neurons, axons, and dendrites, as well as in astrocytes and microglial cells is described in cetaceans.[6] This is similar to what has been reported in dogs infected by CDV.[15] In the humpback whale from the present case, immunolabeling was evident in astrocytes and neurons from the telencephalon, cerebellum and brainstem, corroborating the aforementioned studies.

Differential diagnoses for mononuclear meningoencephalitis in cetaceans are limited, but they include infections caused by herpesvirus[13] and flaviviruses such as the West Nile Virus.[12] However, the Cetacean Morbillivirus remains as the most common cause of viral meningoencephalitis in cetaceans.

Contributing Institution:

Faculdade de Veterinária

Universidade Federal do Rio Grande do Sul

Setor de Patologia Veterinária

http://www.ufrgs.br/patologia

JPC Diagnosis:

Cerebrum: Meningoencephalitis, lymphoplasmacytic and necrotizing, chronic, diffuse, moderate, with neuronal necrosis, Gitter cells, and rare intracytoplasmic and intranuclear viral inclusions.

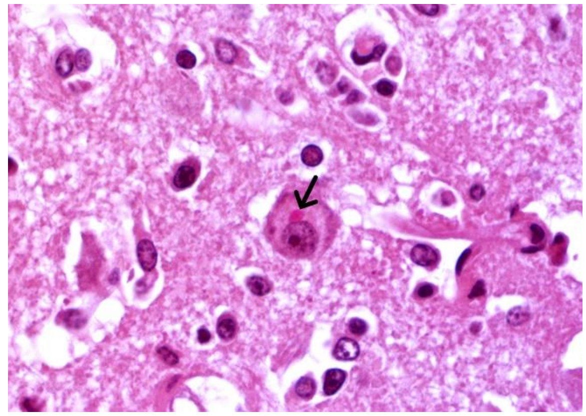

Figure 4-5. Cerebrum, humpback whale. An eosinophilic inclusion body (arrow) is evident in the cytoplasm of a neuron (HE, 400X) (*Photo courtesy of*: Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, http://www.ufrgs.br/patologia)

JPC Comment:

The final case of this conference is a (rarely seen) well-preserved whale brain. Dr. Terio noted that the brain condition in this case is very good – that this animal was euthanized and immediately necropsied is helpful for appreciating the microscopic features that the contributor nicely captures. Difficulty in accessing the brain (especially in a stranding event) combined with carcass deterioration typically confound examination in wild whales.

Microscopic features of this case mirrored morbillivirus infection in other species. Perivascular cuffing, gliosis (glial nodules), and neuronal necrosis were readily apparent, though viral inclusions within the cytoplasm and nucleus were rare which Dr. Terio confirmed is usually the case in the brain. Inclusions may be more prominent within bronchiolar epithelium in animals with respiratory changes. Viral syncytial cells were also infrequent in our section, though they too are typically more prominent in other tissues. This can be a helpful ancillary finding in cases with marked lymphoid depletion and autolysis as syncytial cells may still be discernable.

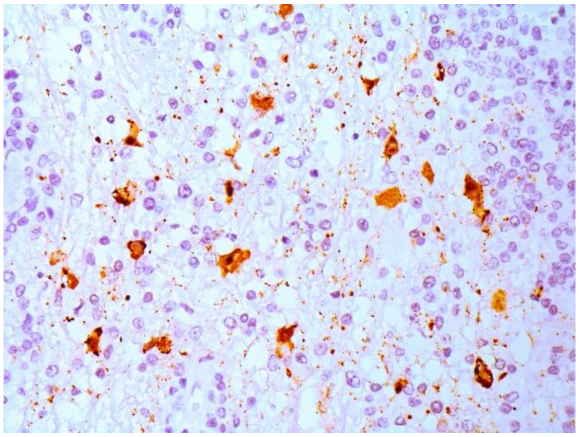

Figure 4-6. Cerebrum, humpback whale. Marked immunolabelling is seen in the cytoplasm of neurons and astrocytes in the IHC (anti-*Morbillivirus* , 400X) (HE, 400X) (*Photo courtesy of*: Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, http://www.ufrgs.br/patologia)

Conference participants discussed potential comorbidities in cetacean morbillivirus cases. Coinfection with *Brucella* (e.g. *B. ceti*) also causes neuropathology[18] and may be aided by concurrent lymphoid depletion arising from CeMV infection. Joint infection is another common sequalae of *Brucella*. Microscopically, there is marked infiltration of the meninges by lymphocytes; in acute cases, bacteria and neutrophils may be appreciated in Dr. Terio's experience. Fungal infections are also abetted by lymphoid depletion, though there were no characteristic features in this case and we did not pursue any special stains.

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