Pathology in Practice

In collaboration with the American College of Veterinary Pathologists

Figure 1—Gross image of the frontal (A) right lateral (B) views of a 2-year-old telescope butterfly goldfish (*Carassius auratus*) evaluated because of buphthalmia of the right eye [oculus dextrus (OD)] and decreased vision of 2 weeks' duration. A—There is marked buphthalmos OD compared with the left eye [oculus sinister (OS)]. B—The OD has buphthalmos, perilimbal vascularization, and cloudy media in anterior chamber. Note the artifactual white speckled corneal opacities observed in this image are due to water spotting on tank walls.

History

A 2-year-old telescope butterfly goldfish (*Carassius auratus*) was presented to the Colorado State University Veterinary Teaching Hospital (CSU VTH) Avian, Exotic, and Zoological Medicine and Ophthalmology services because of buphthalmia of the right eye [oculus dextrus (OD)] and decreased vision of 2 weeks' duration. Vision deficits were noted based on the fish bumping into plants within its tank 2 weeks prior to presentation.

Allison M. Cleymaet, DVM, PhD¹; Michala de Linde Henriksen, DVM, PhD^{1*}; Samantha L. Pederson, DVM, MS¹; Miranda J. Sada, DVM²; Matthew Johnston, VMD²; Gretel Tovar-Lopez, DVM²; Joshua Daniels, DVM, PhD³; Leslie Sharkey, DVM, PhD⁴; Leandro B. C. Teixeira, DVM, MS⁵

¹Comparative Ophthalmology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO

²Avian, Exotic, and Zoological Medicine, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO

³Veterinary Diagnostic Laboratory, Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO

⁴Department of Clinical Sciences, College of Veterinary Medicine, Tufts University, North Grafton, MA

⁵Comparative Ocular Pathology Laboratory of Wisconsin, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI

*Corresponding author: Dr. de Linde Henriksen (Michala.Henriksen@colostate.edu).

<https://doi.org/10.2460/javma.19.10.0547>

Clinical and Clinicopathologic Findings

The fish was anesthetized with 70 ppm tricaine methanesulfonate (MS-222) and 140 ppm sodium bicarbonate for evaluation. A physical examination revealed mild elevation in opercular rate and listing to the right, which were suspected to be due to the small size of the transport bag and excessive waste that was present within it. Buphthalmos was also noted, prompting consultation with Ophthalmology. No other abnormalities were noted on the general physical examination. No parasites or abnormalities were noted upon microscopic evaluation of skin scrape or gill clip.

Ophthalmic examination performed by a board-certified veterinary ophthalmologist (MdLH) confirmed OD buphthalmos (**Figure 1**) and also noted severe periocular swelling and chemosis, conjunctival hyperemia, episcleral injection, marked irregular diffuse corneal edema, and mild circumferential perilimbal vascularization. Anterior uveitis with 3+ flare (according to the standardization of uveitis nomenclature flare scale from 0 to 4+),¹ fibrin, hyphema, and hypopyon were present in the anterior chamber. The remainder of OD could not be visualized due to anterior segment pathology. The left eye [oculus sinister (OS)] had a deep anterior chamber with 2+ flare, fibrin, and minimal hypopyon surrounding clotted hyphema, multiple posterior uveal cysts, and a dorsally subluxated lens with hypermature cataract.

Rebound tonometry (TonoVet, icare) revealed intraocular pressures (IOPs) of 12 mm Hg OD and 14 mm Hg OS (reference ranges for zebrafish,² 10.5 to 21.7 mm Hg). Standard transcorneal ocular ultrasonography was performed on both eyes [oculus uterque (OU)] using a 12-m Hz ocular ultrasound probe (UltraViewXL Rev 2.16, Capistrano Labs Inc), which confirmed buphthalmia OD and revealed a hyper-echoic and enlarged posterior segment with cone-like deformation of the posterior globe. The lens was not visualized OD, and a dorsally subluxated lens was noted OS.

Formulate differential diagnoses, then continue reading.

Additional Clinical Findings

Differential diagnoses for the source of ocular inflammation included ocular manifestation of systemic disease, lens-induced uveitis secondary to cataract, or intraocular neoplasia.³ Consequently, treatment was initiated with ceftiofur crystalline free acid (Excede, Zoetis Inc; 200 mg/mL; 60 mg/kg, IM, once) and meloxicam (1.5 mg/mL; 0.1 mg/kg, IM, q 24 to 48 h for 5 days). One week later, examination revealed improved anterior uveitis OS (persistent 2+ flare with resolution of fibrin, hypopyon, and hyphema) but static intraocular findings OD. Coelomic ultrasonography performed by CSU VTH's Radiology service revealed a normal coelomic cavity. Therapeutic and diagnostic enucleation OD was recommended, and systemic meloxicam was continued at the same dose and frequency as on initial presentation.

Routine subconjunctival enucleation⁴ OD was performed 7 days after initial presentation under general anesthesia (MS-222, 50 to 100 mg/L, immersion) and, immediately after globe removal, with a 0.5% ropivacaine (Naropin, Fresenius Kabi LLC; 0.5 mg) topical splash block. Enrofloxacin 2.27% (0.175 mg/kg, IM) was administered, and meloxicam (0.25 mg/kg, PO, q 24 h for 5 days) was continued as an oral formulation via gel-feeding cubes.

Cytologic, Histopathologic, and Microbiological Findings

Vitreous cytology was obtained via vitreous paracentesis immediately after enucleation and revealed high cellularity fluid with a mix of granulocytes and vacuolated macrophages in a coarsely granular proteinaceous background with no organisms appreciated (**Figure 2**). The enucleated globe was submitted to the Comparative Ocular Pathology Laboratory of Wisconsin. Grossly, the globe was buphthalmic, the lens was posteriorly luxated, and the vitreous was liquefied (**Figure 3**). Histologically, the superficial corneal stroma was nearly completely vascularized. Large numbers of lymphocytes and plasma cells infiltrated the iris and ciliary body stroma.

Figure 2—Photomicrographs from cytologic examination of an OD vitreous paracentesis sample of taken immediately after OD enucleation, showing 4 vacuolated macrophages (black arrows) and 2 granulocytes in a coarsely granular proteinaceous background. No organisms were appreciated. Wright-Giemsa stain; bar = 20 μ m.

There was a thick epiretinal fibrovascular membrane admixed with abundant histiocytes, lymphocytes, and plasma cells covering most of the retina. Abundant histiocytes and fewer heterophils infiltrated the choroid, and there were multifocal granulomas in the choroid composed of cores of melano-macrophages surrounded by eosinophilic lamellar material. Melano-macrophages were also scattered around the granulomas throughout the choroid. The optic nerve head was cupped and infiltrated by heterophils, lymphocytes, and plasma cells. The use of Ziehl-Neelsen acid-fast stain, Fite acid-fast stain, and Gram stain revealed abundant acid-fast and gram-positive bacilli in the cytoplasm of macrophages in the choroid and epiretinal membrane. The bacilli also were faintly positive under Gömöri methenamine silver stain.

A culture swab specimen from the vitreous sample was submitted for microbiology at CSU's Diagnostic Laboratory. The organism was cultivated on Columbia Blood Agar supplemented with 5% sheep's blood, incubated in 10% CO₂ at 35 °C and confirmed to be *Mycobacterium chelonae*. The isolate was identified using partial sequence of *HSP65* as having a 99.72% sequence identity to *Mycobacterium chelonae*.

Diagnosis and Case Summary

Diagnosis of mycobacterial endophthalmitis and secondary glaucoma in a telescope butterfly goldfish (*Carassius auratus*) via vitreous paracentesis and culture and globe histopathology.

Comments

Three species of mycobacteria are predominantly recognized as pathogenic in fish: *Mycobacterium fortuitum*, *M marinum*, and *M chelonae*. These 3 mycobacteria species are aerobic, acid-fast bacilli and gram positive.⁵ *Mycobacterium chelonae* may be isolated

Figure 3—Photomicrographic images of the enucleated OD from the fish described in **Figure 1**. A—There is retinal detachment (arrows) and the cupping of the optic nerve (ON). H&E stain; bar = 0.5 cm. B—Higher magnification of the area delineated by the box in panel A. The inner retina (R) is markedly infiltrated by lymphocytes, plasma cells, and histiocytes. To the right of the image, an epiretinal membrane (arrows) is admixed with large numbers of macrophages (asterisk). H&E stain; bar = 100 µm. C—Higher magnification of the macrophages in the epiretinal membrane in panel B. H&E stain; bar = 20 µm. D—Large numbers of gram-positive bacilli are in the cytoplasm of macrophages and free floating in the background (arrow). Gram stain; bar = 20 µm. E—Large numbers of acid-fast bacilli are the cytoplasm of macrophages and free floating in the background (arrow). Ziehl-Neelsen stain; bar = 20 µm. AC = Anterior chamber. C = Cornea. R = Retina. V = Vitreal space.

from terrestrial and aquatic environmental samples^{6,7} and is an infrequent cause of opportunistic infections in both animals and human beings.^{5,7-11} Piscine mycobacteriosis can be acquired from the environment secondary to shed by other infected fish^{5-7,12} or via vertical transmission.^{13,14}

Uni- or bilateral exophthalmos and keratitis have been previously reported in mycobacteriosis of fish, with low vision to blindness secondary to corneal opacity.^{5,8,15,16} It is also interesting to note a report of necrotizing scleritis with abscess formation secondary to disseminated *M. chelonae* in an immunocompromised man.¹⁷ However, to the authors' knowledge, this is the first report of mycobacterial endophthalmitis and secondary glaucoma, presumed to be an ocular manifestation of systemic sepsis, in a fish. Secondary glaucoma is a type of glaucoma that occurs as a result of (secondary to) an acquired condition including inflammation (as in this case), infection, trauma, drug reaction, or neoplasia. Although elevated IOP is typically associated with glaucoma, in this case the IOP within reference limits was in fact inappropriately elevated given the degree of intraocular inflammation, which would otherwise be

expected to decrease IOP below or near the lower reference limit.

Systemic clinical signs of piscine mycobacteriosis include lethargy, inappetence and emaciation, abdominal swelling, scale loss, skeletal deformation, pigmentation changes, reproductive problems, and visceral (largely renal, splenic, and hepatic) granulomatous lesions.^{5,8,15,16,18} Morbidity in aquaria with persistent infection of the habitat may approach 100%.¹⁹ Death is a consequence of direct mycobacterium action, starvation, or malnutrition.⁵ Chronic infection with *M. chelonae* may lack clinical manifestation, as has been demonstrated in Atlantic Salmon experimentally inoculated with the pathogen,¹² rendering most ornamental fish potential carriers of the disease and underscoring the need for reliable antemortem screening in the epizootiology of this disease.⁵

Previously, only lethal methods have been described for detection of piscine mycobacteriosis. This entails examining viscera obtained postmortem for granulomatous lesions and subsequently treating the tissue with special stains (Ziehl-Neelsen) for acid-fast bacilli and culturing harvested tissue.⁸ This report suggests an alternative, minimally invasive,

antemortem method of diagnosis (ie, vitreal cytology and culture).

Culture is more sensitive for identification of mycobacterium in aquarium fish than direct microscopy, with a high prevalence of infection noted independent of macroscopic lesions and microscopy identifying organisms in as few as 37.1% of culture-positive cases.^{9,10} *Mycobacterium chelonae* is considered to be a rapid grower (growth observed < 7 days) in comparison to other mycobacteria pathogenic to fish, such as *M. marinum*, though like all mycobacteria it is fastidious and readily overtaken if contaminants are present.⁸ The use of PCR assay in combination with restriction fragment length polymorphism analysis for the identification of pathogenic mycobacterium including *M. chelonae* in the fish has been described as a rapid diagnostic modality with greater sensitivity and specificity than cytologic or histopathologic organism identification,^{5,9,12,20} but was not pursued in this case study in light of the positive culture result.

Commercially or in the laboratory, quarantine and diagnostic screening are recommended to prevent introduction of *Mycobacterium* spp.-infected fish into established healthy colonies.²⁰ Effective treatment of piscine mycobacteriosis involves typically culling of infected fish and disinfection of exposed environmental surfaces, as mycobacterium can persist as microcolonies in environmental biofilms.^{20,21} Antimicrobial treatment of the housing water (kanamycin sulfate, 50 ppm, q 48 h for 4 doses) has been shown to be clinically effective in a report using guppies; however, culture-negative status was not established in that case.¹⁴ In humans, resolution of persistent infection requires surgical debridement and lengthy treatment with appropriate antimicrobials.^{17,22,23} Given that the fish in this report was a pet fish, a similar course of treatment (enucleation, prolonged antimicrobial regimen) resulted in clinical resolution of disease, and the fish was doing well 68 days postoperatively.

References

1. Chang JH, McCluskey PJ, Wakefield D. Acute anterior uveitis and HLA-B27:2 what's new? In: Kriegelstein GK, Weinreb RN, Pleyer U, Mondino B, eds. *Uveitis and Immunological Disorders*. Berlin, Germany, Springer-Verlag; 2009:9–18.
2. Link BA, Gray MP, Smith RS, John SWM. Intraocular pressure in zebrafish: comparison of inbred strains and identification

- of a reduced melanin mutant with raised IOP. *Invest Ophthalmol Vis Sci*. 2004;45:4415–4422.
3. Bartlett SL, Peters RM, Lombardino IM, Bowser PR. Bilateral intraocular malignant neuroectodermal tumors in a telescope goldfish (*Carassius auratus*). *Vet Ophthalmol*. 2010;13:3–8.
4. Diehl KA, McKinnon J-A. Eye removal surgeries in exotic pets. *Vet Clin North Am Exot Anim Pract*. 2016;19:245–267.
5. Belas R, Faloon P, Hannaford A. Potential applications of molecular biology to the study of fish mycobacteriosis. *Annu Rev Fish Dis*. 1995;5:133–173.
6. Clark HF, Shepard CC. Effect of environmental temperatures on infection with mycobacterium marinum (balnei) of mice and a number of poikilothermic species. *J Bacteriol*. 1963;86:1057–1069.
7. Beran V, Matlova L, Dvorska L, Svastova P, Pavlik I. Distribution of mycobacteria in clinically healthy ornamental fish and their aquarium environment. *J Fish Dis*. 2006;29:383–393.
8. Francis-Floyd R. Mycobacterial-infections-of-fish. *S Reg Aquac Cent*. 2011;4706:1–12.
9. Pate M, Jenčič V, Žolnir-Dovč M, Očepek M. Detection of mycobacteria in aquarium fish in Slovenia by culture and molecular methods. *Dis Aquat Organ*. 2005;64:29–35.
10. Zanon RG, Florio D, Fioravanti ML, Rossi M, Prearo M. Occurrence of *Mycobacterium* spp. in ornamental fish in Italy. *J Fish Dis*. 2008;31:433–441.
11. Kušar D, Zajc U, Jenčič V, et al. Mycobacteria in aquarium fish: results of a 3-year survey indicate caution required in handling pet-shop fish. *J Fish Dis*. 2017;40:773–784.
12. Bruno DW, Griffiths J, Mitchell CG, et al. Pathology attributed to *Mycobacterium chelonae* infection among farmed and laboratory-infected Atlantic salmon *Salmo salar*. *Dis Aquat Organ*. 1998;33:101–109.
13. Conroy DA. Observaciones sobre casos espontaneos de tuberculosis ictica. *Microbiol Esp*. 1966;19:93–113.
14. Conroy G, Conroy DA. Acid-fast bacterial infection and its control in guppies (*Lebistes reticulatus*) reared on an ornamental fish farm in Venezuela. *Vet Rec*. 1999;144:177–178.
15. Dulin MP. A review of tuberculosis (mycobacteriosis) in fish. *Vet Med Small Anim Clin*. 1979;74:731–735.
16. van Duin C. Tuberculosis in fish. *J Small Anim Pract*. 1981;22:391–411.
17. Metta H, Corti M, Brunzini R. Disseminated infection due to *Mycobacterium chelonae* with scleritis, spondylodiscitis and spinal epidural abscess. *Braz J Infect Dis*. 2008;12:260–262.
18. Lansdell W, Dixon B, Smith N, Benjamin L. Communications: Isolation of several mycobacterium species from fish. *J Aquat Anim Health*. 1993;5:73–76.
19. Post G. *Textbook of Fish Health*. Neptune City Inc; 1987.
20. Astrofsky KM, Schrenzel MD, Bullis RA, Smolowitz RM, Fox JG. Diagnosis and management of atypical *Mycobacterium* spp. infections in established laboratory zebrafish (*Brachydanio rerio*) facilities. *Comp Med*. 2000;50:666–672.
21. Schulze-Röbbecke R, Janning B, Fischeder R. Occurrence of mycobacteria in biofilm samples. *Tuber Lung Dis*. 1992;73:141–144.
22. Levendoglu-Tugal O, Munoz J, Brudnicki A, et al. Infections due to nontuberculous mycobacteria in children with leukemia. *Clin Infect Dis*. 1998;27:1227–1230.
23. Hoyen HA, Lacey S, Graham T. Atypical hand infections. *Hand Clin*. 1998;14:613–634.