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An outbreak of piscine tuberculosis (mycobacteriosis) in an aquarium.

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→ Piscine tuberculosis (mycobacteriosis) occurs in both fresh- and saltwater fish; all species of fish are believed to be susceptible, and the disease is present worldwide.¹ The significance of this disease in natural populations is unknown,² but for the aquarist it is one of the more common chronic bacterial diseases.^{1,2} Clinical signs in infected fish may suggest tuberculosis; however, signs are often nonspecific. This report details an outbreak of piscine tuberculosis in a freshwater aquarium. *Keywords: pH Section, Mycobacteriosis*

Four adult guppies (*Poecilia reticulata* Peters), 2 immature guppies, and 2 dwarf suckermouth catfish (*Otocinclus affinis*) were maintained in a 2 gallon community aquarium with an under-gravel filter. Water (500 ml.) was changed weekly with unaged tapwater. Evaporative loss was replaced as necessary with unaged tapwater. The tank was kept at ambient temperature which generally ranged from 21 to 24 C. The pH of the water ranged from 6.6 to 7.2. The guppies present in the tank were all descended from a single pair of fancy guppies.

We observed the physical condition of an 11-month old female guppy to deteriorate over 72 h. Clinical signs included apparent loss of body weight, dull color, dark eyes, anorexia, scoliosis, and weak, ineffective swimming motions with difficulty in maintaining equilibrium. The fish was euthanized in a moribund condition, and immediately fixed whole in full-strength Zenker's fixative for 1 h, then in 50% Zenker's fixative for 1.5 h. The fish was then washed in running water overnight, post-fixed in 70% ethanol, and processed routinely for histologic examination. Several days later a second adult female guppy (sibling to the first) demonstrated similar clinical signs, was euthanized, and processed for examination in the same manner. Remaining fish in the aquarium appeared normal.

Because of the small size of the fish, necropsies were not performed and gross observations were limited to those clinical observations noted ante mortem.

Histologic lesions present in the 2 guppies were similar and consisted of multifocal granulomas and granulomatous inflammation in the liver, kidney, ovary, skeletal muscle, peritoneum, and spinal cord. Granulomas were typically variably sized and consisted of a nodular accumulation of macrophages having abundant, eosinophilic cytoplasm, with a few lymphocytes located at the periphery. The nodules were surrounded by an indistinct layer of fibrous connective tissue. The centers of granulomas occasionally contained a small amount of cellular debris and/or a small amount of granular, golden-brown pigment interpreted to be hemosiderin (Figure 1). Neither multinucleated giant cells nor mineralization were present within the lesions. Within some areas, particularly in the kidney, there was a more diffuse infiltration of macrophages in the interstitium, with a lesser tendency to form discrete granulomas. Gram-positive, acid-fast bacilli measuring 1-2 by 8-10µm were easily demonstrated in granulomas (Figure 2) as well as in areas of granulomatous inflammation. In addition, intestinal epithelial cells, renal tubular epithelial cells, and epidermal cells occasionally contained intracellular acid-fast bacilli in the absence of any local inflammatory response.

In 1 fish, there was diffuse severe muscle degeneration and necrosis present, characterized by fragmentation and hyalinization of myocytes and loss of cross striations, with infiltration of moderate numbers of lymphocytes and macrophages. No acid-fast organisms were observed associated with the myositis. The other fish had several small foci of mineralization within renal tubules.

After determining the cause of death of the 2 female guppies, we depopulated the tank and the remaining fish were examined histologically. The remaining adult guppies had variably severe lesions similar in appearance and distribution to the first 2 guppies. The testicle of the male guppy was severely affected, with almost complete replacement of parenchymal tissue by granulomas and granulomatous inflammation. The intestinal tract of the remaining adult female guppy was much

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more severely involved, with thickening of the lamina propria by marked numbers of macrophages. Acid-fast organisms were found in the granulomatous lesions of both adult guppies, as well as in the gut lumen and in thrombi adhered to the endocardial surface of the ventricle in the female guppy. No lesions or acid-fast organisms were seen in the 2 dwarf suckermouth catfish or in the 2 immature guppies. Subsequent to the histologic identification of tuberculosis lesions, we cultured water from the aquarium and attempted isolation of acid-fast organisms. An acid-fast bacillus was isolated. Colony growth characteristics and biochemical analysis identified the organism as an "atypical" rapid-grower other than a member of the *Mycobacterium fortuitum* - *M. chelonae* complex.

The causative bacteria of piscine tuberculosis have been identified as *Mycobacterium marinum* (formerly *M. piscium* and *M. platyoeilus*) and the less common *M. fortuitum*.³ The nomenclature is confusing, however, and other organisms (*M. ranae*, *M. anabanti*, and *M. salmoniphilum*) have been incriminated.¹ These organisms are gram-positive, acid-fast, nonmotile, pleomorphic bacilli, 1.5 to 2.0µm long with occasional filaments up to 10µm. They often have a banded or beaded appearance in Ziel-Nielson-stained preparations. The habitat of *M. marinum* is not known, however, carrier fish with subclinical infections are usually responsible for most infections in aquaria.

Clinical signs are variable and generally nonspecific, as was the case in this outbreak. The disease progression may be acute with death prior to the development of lesions, or more commonly, it is chronic and progressive in its course. Affected fish may be cachexic, exhibit a change in color, and show swelling of the abdomen due to ascites. Tropical fish typically lose color with mycobacteriosis while affected salmonids generally appear brighter.² Rapid, shallow breathing, skin ulcerations and hemorrhages, exophthalmos, fin destruction, and skeletal deformities (lordosis and/or scoliosis) are also commonly observed.

At necropsy, miliary, greyish-white tubercles of variable size, which may coalesce to form tumor-like masses, may be found in virtually any organ; the liver, spleen and kidney are especially common sites. While the miliary form is not uncommon, no gross lesions may be present and diagnosis may depend on histologic and/or cytologic examination. Histopathological findings vary, but individual tubercles generally consist of an outer fibrous wall surrounding a central zone of epithelioid macrophages, hemosiderin-containing cells, and tissue debris; many acid-fast bacilli are regularly seen in the cytoplasm of macrophages. Caseation and Langhans giant cells are uncommon but have been reported by some investigators⁴; calcification has not been reported.

The differential diagnosis must include all other granuloma-forming diseases of fish, including infection by various species of flavobacteria (non-acid-fast), systemic fungal infection (identified by PAS-positive mycelia and spore stages), various species of migrating helminths (usually elicit an eosinophilic, granulomatous response), and the microsporidian and myxosporidian protozoa (identified with Giemsa stain). A presumptive diagnosis can be made by demonstrating gram-positive, acid-fast organisms in tissue sections or on impression smears. Definitive diagnosis is made by isolating and identifying the organism grown on bacteriologic media. *M. marinum* is slow growing, difficult to culture, and may require 14 to 21 days for colonies to become grossly visible.⁵ *M. fortuitum* is distinguished by more rapid growth (within 3 days at 30 C), lack of colonial pigment, catalase positivity, and nitrate reduction ability.³

Aquarium fish which exhibit clinical signs of disease should be removed from the community or display tank and isolated in a treatment tank. Once tuberculosis is confirmed, affected fish should be destroyed, as no effective treatment is available. Restocking with new, clean fish should not be accomplished until after thorough disinfection of the aquarium and its contents is completed.

Both *M. fortuitum* and *M. marinum* are zoonotic, and although uncommon, aquaria are a possible source of atypical mycobacterial infection in man. Humans infected with *M. marinum* typically present having experienced a minor abrasion on the hand, obtained when they cleaned an aquarium where many fish had recently died. Infection initially results in a chancriform lesion at the site of inoculation with subsequent nodular abscesses along lymphatics. Regional lymph nodes are usually not enlarged. The condition must be differentiated from several other granulomatous skin diseases, including cutaneous sporotrichosis, pasteurellosis, nocardiosis, and blastomycosis. Treatment is usually difficult, and although single nodules may be surgically excised, chemotherapy is the recommended treatment for patients with multiple lesions.⁶ In contrast, *M. fortuitum* may be associated with progressive lesions in the lung or eye.⁷

The source of the infection in this incident remains undetermined. The organism is usually believed to be transmitted horizontally by ingestion of infective material. Bacterial invasion through damaged skin or gill tissue may occur, and transovarian passage is also possible, at least in viviparous species.³ The aquarium and all of its equipment were new when the aquarium was established, so organisms did not persist from a previous infection. The original parent stock died within 2 weeks of each other approximately 8 months previous to this outbreak. The parent male was examined histologically and a cause of death was not determined; no lesions typical of piscine tuberculosis nor any acid-fast bacilli were seen. The parent female developed cutaneous saprolegniasis and was removed from the community tank and subsequently died. We believed that a sharp drop in the water temperature to 18 C for 24 h was responsible for initiating the fungal infection. Histopathologic examination was not performed. The 2 dwarf suckermouth catfish were added after the death of the parent guppy stock. One possible source, then, is the original female guppy, which was not examined histologically; she may have had a clinically inapparent infection and served as the source of infection for the remaining fish. In addition to the change in

water temperature, mycobacteriosis may have also contributed to her susceptibility to saprologniasis, as infection with this group of fungi generally requires some other stress or injury to become established.

Although no lesions or acid-fast organisms were observed histologically, the 2 dwarf suckermouth catfish may have served as carriers of the infection. It was expected that, of the fish present, and by virtue of their bottom-feeding habits, the catfish would be most likely to be exposed to organisms which were being shed in the excreta of infected fish and to demonstrate typical lesions of tuberculosis. Their lack of infection may be due to a species variation in susceptibility to the causative agent, as is the case with tuberculosis in higher vertebrates. It is possible that they were shedding organisms at the time of introduction to the aquarium, but had not become persistently infected.

Another possible source of the initial infection is through the use of contaminated food as is sometimes the case in commercial fisheries in which infected fish offal may be fed back to the fish. It is unlikely in this instance, however, since a commercial flaked food was used and its processing should have rendered it free of mycobacteria. One additional possible source is the water which was added to the aquarium at regular intervals, and although it was not investigated, this too is unlikely, as the water was from the city water supply.

This case demonstrates the importance of histopathologic examination in the diagnosis of fish diseases. Clinical signs were nonspecific and did not suggest any particular therapeutic approach. Had the diagnosis not been made histologically, the aquarium, in all likelihood, would not have been depopulated and the disease would have been perpetuated in any additions to the aquarium. It is uncertain whether the organism isolated was indeed responsible for the observed lesions as cultures were obtained indirectly by culture of aquarium water rather than directly from the infected fish; the isolated organism may have been an incidental, non-pathogenic saprophyte.

In any event, although the organism isolated was not 1 of the 2 known zoonotic species commonly isolated from cases of piscine tuberculosis, that possibility could not be excluded in this instance and hence further emphasizes the importance of obtaining an histopathologic diagnosis.

1. Dulin MP.: A review of tuberculosis (mycobacteriosis) in fish. Vet Med SAC, May 1979, pp. 731-735.
2. Ribelin WE, Migaki G: *The Pathology of Fishes*. Madison, Wisconsin: University of Wisconsin Press, 1975; 84-87.
3. Roberts RJ: *Fish Pathology*. London: Cassell Ltd., 1978; 201-202.
4. Timur G, Roberts RJ: The experimental pathogenesis of focal tuberculosis in the plaice (*Pleuronectes platessa*). *J Comp Path* 1977; 87:83-87.
5. Leibovitz L: Fish tuberculosis (mycobacteriosis). *J Am Vet Med Assoc* 1980; 178:415.
6. Adams RM, Remington JS, Steinberg J et al.: Tropical fish aquariums. A source of *Mycobacterium marinum* infections resembling sporotrichosis. *J Am Med Assoc* 1970; 211:457-461.
7. Jawetz E., Melnick JL, Adelberg EA: *Review of Medical Microbiology*. California: Lange Medical Publications, 1978; 207.

Figure 1

Multiple granulomas present in the mesentery of a guppy with tuberculosis (mycobacteriosis). H and E stain. 80x original magnification.

Figure 2

Large numbers of acid-fast bacilli within a mesenteric granuloma in a guppy with tuberculosis (mycobacteriosis). Ziel-Nielsen stain. 80x original magnification.