Fish Diseases

(Source: J. Matthews from Zebrafish Book 5th Edition)

Several of the most common diseases that affect zebrafish are discussed briefly below. Additional information of the diseases that affect laboratory zebrafish can be found in the on-line manual, "Diseases of Zebrafish in Research Facilities," available at:


Diagnostic services are provided by the Zebrafish International Resource Center:

http://zebrafish.org/zirc/health/index.php

Mycobacteriosis

Mycobacteriosis, often incorrectly called fish tuberculosis or fish TB, is a common disease of laboratory zebrafish as well as wild and captive fishes worldwide. Mycobacteria are nonmotile, weakly staining Gram-positive, pleomorphic rods that are acid-fast. Many species of atypical (non-tuberculosis) mycobacteria are found ubiquitously in water and biofilms. Multiple species of Mycobacterium have been identified as infectious to zebrafish including M. marinum, M. fortuitum, M. chelonae, M. abscessus, M. haemophilum, and M. peregrinum/septicum.

Clinically, mycobacteriosis can manifest in a wide variety of signs. These include lethargy, anorexia, skin inflammation and ulceration, fin loss, edema/dropsy, peritonitis, and granulomatous nodules in internal organs and muscle. Deformities may occur with muscle and skeletal involvement.

Diagnosis is based on clinical signs, characteristic granulomatous inflammation and the presence of acid-fast bacteria in tissue sections or smears. Culture of the microorganism is considered definitive but can be difficult due to slow growth and special media requirements. PCR tests for the identification of mycobacteria infecting fish have been described.

As with most bacterial pathogens of fish, mycobacteria infections in zebrafish are most often opportunistic in nature. Poor water quality, high stress or other type of husbandry failure will commonly precede outbreaks. The virulence of a particular species or strain of mycobacterium may also affect the severity of the disease. Mycobacteria infecting fish typically respond poorly to antimicrobial treatments. Control should be focused on the removal of infected fish, optimizing water quality and husbandry practices and the use of strict sanitation and quarantine procedures. In severe outbreaks with highly virulent strains of mycobacteria, control may require the eradication of infected stocks and subsequent disinfection of the system.

Mycobacterium Zoonotic Considerations

Fish-pathogenic mycobacteria can infect humans. The disease is commonly referred to as fish tank granuloma or swimming pool granuloma. Humans are typically infected by contamination of lacerated or abraded skin with aquarium water or fish contact. A localized granulomatous nodule may form at the site of infection, most commonly on hands or fingers. The granulomas usually appear approximately 6-8 weeks after exposure to the organism. They initially appear as reddish bumps (papules) that slowly enlarge into purplish nodules and nonhealing ulcers. The disease can be difficult to treat due to drug resistance. The infection can spread to nearby lymph nodes. A physician should be consulted if lesions are noted. Individuals who have an immune-compromised medical condition or are taking medications that impair immune function (steroids, immunosuppressive drugs, or chemotherapy) are at a greater risk for disseminated forms of the disease and should consult their physician. It is also possible for these species of mycobacteria to cause some degree of positive reaction to the tuberculin skin test. If you have any cuts or abrasions on your hands or arms, you should wear sturdy, impervious gloves and always wash hands and arms after handling fish and aquarium water.

Additional reading


Microsporidiosis

Microsporidia are well-recognized pathogens of fish. A microsporidian infecting the central nervous system of zebrafish was first reported in 1980 by a group in France. The parasite has been more recently described as Pseudoloma neurophilai, and is found commonly in zebrafish from both laboratory facilities and commercial suppliers.

Microsporidia are obligate intracellular parasites of eukaryotes with a complicated life cycle. The life cycle concludes with the production of an infectious and resistant spore. The formation of giant host cells filled with spores (a xenoma) is common for microsporida species infecting fish. Spores have a characteristic posterior vacuole and polar tube apparatus, which function to transmit the spore contents and genetic material into the host cell. With most microsporidia, direct horizontal transmission occurs via the ingestion of the infective spore; however, vertical transmission has also been demonstrated in some species of microsporidia.

The primary site of infection with P. neurophilai zebrafish is the central nervous system (spinal cord, ventral nerve roots and hindbrain) where xenomas are commonly found. Free spores or small xenomas can also be found occasionally in the ovary, skeletal muscle and viscera. Mild to severe, chronic myelitis (inflammation of the spinal cord) and myositis (inflammation of muscle) can be associated with infections. Inflammatory changes are most common when free spores are present. Currently, only the spore can be readily identified in zebrafish, and very little is known about the complete life cycle and mode(s) of infection of the parasite. The only confirmed host for P. neurophilai is the zebrafish, however, we have detected a morphologically identical microsporidian in a neon tetra (Paracheilodon innesi).
Clinical signs of microsporidiosis in zebrafish can include chronic wasting or emaciation, lethargy, spinal deformities and dorsal darkening of the skin. Zebrafish can also be infected with *P. neurophila* and show no abnormal clinical signs. It is often an incidental finding on routine histological exam. Severe infections are commonly associated stressful husbandry conditions and immunosuppression.

Diagnosis can be made by finding characteristic spores upon examination of dissected spinal cord tissue in wet mount preparation. Spores are approximately 3 µm x 5 µm, oval to pyriform in shape and have a large posterior vacuole. Xenomas (up to 200 µm), spores and associated inflammation can also be readily identified in histological sections.

Currently there is no treatment for microsporidiosis in zebrafish. Control should focus on optimizing husbandry conditions and removal of all emaciated and moribund fish to prevent cannibalism. Several drug treatments (e.g. flubendazole, albendazole, furamidin) have been tried in other species of fish with some success, but at this time they would be considered experimental in zebrafish.

**Additional reading**


**Velvet Disease**

Zebrafish are highly susceptible to velvet disease. The causative agent of velvet disease in zebrafish is *Piscinoodinium sp.*, a dinoflagellate that contains chlorophyll, which imparts a yellow-gold color to the parasite. This oval- to round-shaped parasite attaches to the skin and gills of the fish. The parasite life cycle is direct and may be completed in 10 to 14 days under optimal conditions (23-26°C). The parasitic stage, the trophont, feeds on the host's epithelium. After feeding for several days, the trophont detaches from the host and becomes a tomentum. The tomentum undergoes a series of divisions to produce the motile and infective dinospores. The dinospores attach to a fish host, differentiate into a trophont, and continue the cycle.

Infected fish show general signs of discomfort, flashing or rubbing behavior, clamped fins (fins held close to the body), increased respiration, lethargy and decreased feeding. The parasite can be identified microscopically on wet mount examination of skin scrapings or fin and gill biopsies. Trophonts are almost round when mature, non-motile and can vary in size from 10-50 µm. In histological sections, trophonts appear as oval to round organisms on the gill and skin surface, with numerous cytoplasmic (often refractile) granules and a large nucleus. On heavily infected fish, the skin may have a dusty appearance (velvet) when illuminated directly (i.e. with a flashlight).

Successful treatment has been previously described using Atabrine (Quinacrine hydrochloride), however, this drug is no longer available in the United States or Canada. Chloroquine diprophosphate (prolonged immersion at 10 mg/L) has been used successfully to treat the marine counterpart, *Amyloodinium* (Noga, 1996). The drug is reported to be relatively nontoxic to fish; however, it is toxic to algae and invertebrates. Strict quarantine practices are an important preventive measure. Infections can be easily avoided if only surface sanitized (bleached) embryos are introduced into a system. There is a greater chance of encountering this parasite in zebrafish acquired from commercial sources such as pet suppliers.

**Additional reading**


**Intestinal Nematodes**

Intestinal nematodes are frequently found in freshwater tropical fish. An intestinal nematode found infecting zebrafish has been identified as *Pseudocapillar ius tomentosa*, which is a common nematode of cyprinid and other fishes. Infections in zebrafish have been associated with chronic wasting disease, decreased reproductive potential and growth rate and intestinal neoplasms.

Capillarids are thin, transparent worms that are typically found within the lumen of the intestine and are locally tissue invasive. The life cycle of intestinal nematodes can either be direct or involve intermediate or paratenic (transport) hosts. Precise identification of Capillarid nematodes to the species level requires careful examination of sexual organs of the male worm and is typically not necessary in a clinical setting.

Clinically, infected zebrafish may be darker in color, emaciated and lethargic. Infected fish can also appear normal or show only subtle abnormalities such as decreased fecundity. Diagnosis is made by finding adult worms in the intestine of fish on necropsy or histological exam. Worms can be identified grossly in infected fish by dissecting fresh intestine and examining in a squash or wet mount preparation. The adult worms are motile, thin (~50 µm) and 4-12 mm in length. The eggs of parasitic nematodes have a distinctive oval shape with a cap or plug-like structure at either end. Characteristic ova (~30 x 60 µm) can be seen within gravid female worms or found free within the intestinal contents or feces. Histological sections reveal the worms within the lumen or wall of the intestine and can be associated with significant tissue reaction.

Nematode infections in zebrafish can be difficult to eliminate. Because direct transmission can occur between fish, the infection can spread within a population if not controlled. Infections can be prevented with the use of strict quarantine procedures that allow only the introduction of surface sanitized (bleached) embryos. Oligochaete worms (e.g. *Tubifex*) can also carry the parasite and thus should be avoided as a food source. If fish are not highly valuable, the most effective treatment may be to cull the infected population and disinfect the system.

There are reports of anthelmintic drugs used to treat intestinal nematodes in fish but these have not been extensively tested in zebrafish. Pack et al. (1995) reported that a mixture of trichlorfon and mebendazole in the form of Fluke-Tabs (Aquarium Products, Glen Burnie, MD) added to water (at the manufacturer's recommended dose, one tablet/38 L) eliminated the infection in zebrafish. Treated fish gained weight and regained fertility, and when examined later the infection was not observed. No adverse effects of the treatment were reported, however, caution should be used with this treatment in zebrafish brood stock because mebendazole is reported to be embryotoxic and teratogenic and trichlorfon is a neurotoxic organophosphate. Levamisole has been reported to be ineffective against intestinal nematode infections (Pack et al., 1995) and to cause sterility in zebrafish brood stock.
Oral fenbendazole (Panacur) has been used to treat intestinal nematodes in fish (Noga, 1996). Fenbendazole is added to food at a concentration of 0.25% and fed at a rate of 1% of body weight/day for three days. Treatment should be repeated in 14-21 days. The drug can be mixed with commercial food enhanced with cod liver oil and bound with gelatin. Fenbendazole can also be dosed as a water-borne formulation. Fenbendazole in a prolonged immersion is added to aquarium water at 2 mg/L and repeated once per week for three weeks (Noga, 1996). The efficacy of fenbendazole against intestinal nematodes in zebrafish has not been documented.

Additional reading

