Pathology in practice. Severe, chronic, focal, granulomatous, nodular and ulcerative stomatitis with myxosporidia (Myxobolus sp.).

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Pathology in Practice

Figure 1—Photograph of a 7-month-old Oranda goldfish that was evaluated because of rapidly developing left-sided facial deformity and exophthalmia associated with a mass that affected the oral cavity and left operculum.

History

A 7-month-old 25-g (0.055-lb) male calico Oranda goldfish (*Carassius auratus*) was evaluated because of a rapidly enlarging mass that affected the oral cavity and left operculum. Associated clinical signs included left opercular flaring, progressive gill pallor, left-sided exophthalmia, and suppressed appetite. The fish had been acquired from a retail vendor 1 month earlier; it appeared thin but otherwise outwardly healthy prior to development of these signs a week before the evaluation. The goldfish had been housed in a tank with a sexually mature female fancy goldfish; the fish had spawned twice in the month preceding onset of the male's clinical signs. The female fish remained healthy.

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Clinical and Gross Findings

Physical examination revealed gross facial disfigurement (**Figure 1**). Upon exploration of the oral cavity, a soft, nodular, pink mass was detected in close association with the ventral aspect of the left gill arch; the mass partially occluded the mouth and caused deviation of the oropharynx to the right. Moderate gill pallor was observed bilaterally. The goldfish was anesthetized with buffered tricaine methanesulfonate[a] (75 mg/L), and a sample of the mass was retrieved via incisional biopsy. Unstained impression smears of the excised tissue, smears stained with a modified acid-fast stain[b] or a Romanowsky-type stain,[c] and formalin-fixed tissue samples were submitted to the pathology service at the Virginia-Maryland Regional College of Veterinary Medicine for evaluation.

Formulate differential diagnoses from the history, clinical findings, and Figure 1—then turn the page →

Cytologic and Histopathologic Findings

The impression smears prepared from the oropharyngeal biopsy specimen were examined microscopically. Examination of the smears that were stained with a Romanowsky-type stain revealed moderate cellularity with a high degree of cellular disruption (evident as poor demarcation between nuclear and cytoplasmic staining, free nuclei, and nuclear streaming). Epithelial cells of uniform size and shape predominated; these cells were most often in small clusters. A moderate number of mature heterophils were present. There was a large number of myxosporean spores. These spores were 8 to 10 µm in length and 5 µm in width and had a 1-µm-thick basophilic teardrop-shaped capsule. Each spore had 2 intensely basophilic, pyriform, apical polar capsules with occasionally extruded polar filaments and 1 basally located sporoplasm. Examination of the acid-fast stained smears revealed similar cellular composition (**Figure 2**). In those preparations, the myxosporean spores were clearly visible against the background of epithelial and inflammatory cells because of their intense red (acid-fast positive) staining but internal features of the spores were difficult to discern.

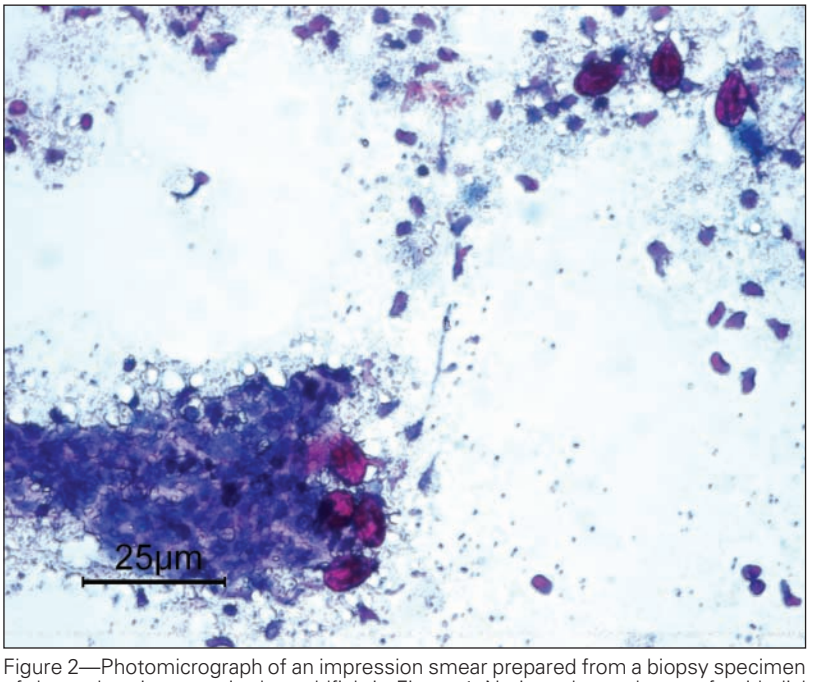

Figure 2—Photomicrograph of an impression smear prepared from a biopsy specimen of the oral cavity mass in the goldfish in Figure 1. Notice a large cluster of epithelial cells (blue) and numerous mature *Myxobolus* sp spores (red). Modified acid-fast stain; bar = 25 µm.

Histologic examination of sections of the oropharyngeal biopsy specimen revealed multifocal erosion and ulceration of the oral mucosa. The remaining epithelium was hyperplastic. Moderate and multifocal infiltration of the mucosa by mature heterophils, often in association with mats of basophilic bacilli, was evident. The submucosa was markedly expanded by large numbers of macrophages and well-vascularized immature connective tissue, moderate numbers of eosinophils, and fewer lymphocytes. Within the submucosa, multiple large (1- to 3-mm-diameter) polysporic plasmodia were visible surrounded by a thin rim of macrophages. The plasmodia each had a thick eosinophilic capsule that surrounded multiple sporogonic stages contained within the endoplasm; smaller, round, multicellular and basophilic developmental stages of these histozoic plasmodia were present and had elicited minimal host reaction (**Figure 3**). Large numbers of mature spores were scattered randomly throughout the submucosa. The spores were 8 to 10 µm in length with a thick, refractile, teardrop-shaped capsule; each contained 2 spiral, eosinophilic polar filaments that were contained within equally sized polar capsules and occasionally had a spiral appearance. The polar capsule-to-spore length ratio was approximately 1:3. Small lakes of fibrin, which contained varying amounts of cell debris, parasitic cysts, and RBCs and that were surrounded by macrophages and heterophils, were also identified in the submucosa. Mild and multifocal submucosal hemorrhage was evident.

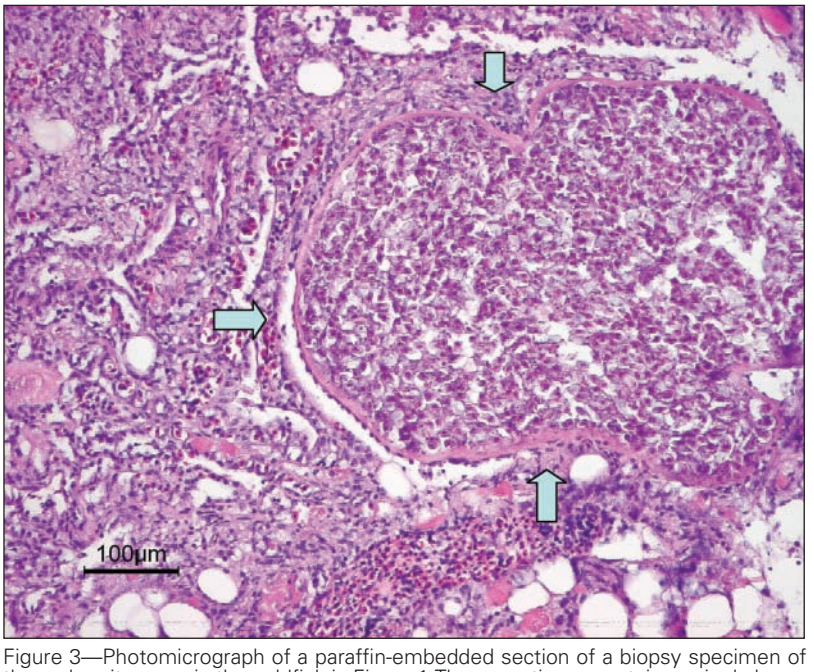

Figure 3—Photomicrograph of a paraffin-embedded section of a biopsy specimen of the oral cavity mass in the goldfish in Figure 1. The mass tissue contains a single large, late-stage, polysporous, histozoic plasmodial pseudocyst (borders of this structure are demarcated by arrows) and evidence of inflammation. H&E stain; bar = 100 µm.

Morphologic Diagnosis

Severe, chronic, focal, granulomatous, nodular, and ulcerative stomatitis (oropharyngeal region) with myxosporidia (*Myxobolus* sp).

Comments

Approximately 2,200 species of myxosporean parasites have been described, and although myxosporean infections in amphibians, reptiles, invertebrates, and birds have been reported,[1,2] most of these parasites affect fish. Myxosporean parasites cause disease in a variety of fish, including freshwater, brackish-water, saltwater, warmwater, coldwater, bony, and cartilaginous fish; despite this broad host range, host specificity varies widely among the individual species of myxosporidia.

One of the most important myxosporean parasites is *Myxobolus cerebralis*, the causative agent of whirling disease. This is a devastating disease of juvenile cultured and wild salmonids that has a complicated indirect life cycle involving an oligochaete worm, which acts as an intermediate host. The worms release infective actinosporean spores into the water column, where they remain viable for as long as 2 weeks. The spores penetrate the skin of fish, migrate through nervous tissue as trophozoites, and eventually reach their target tissue, cartilage. In cartilage, these trophozoites develop into plasmodia; when the affected fish dies, mature myxosporean spores are released into the water. The life cycle is completed when these spores are ingested by the intermediate host. Axial skeletal deformities, behavioral changes, and hyperpigmentation of the caudal peduncle and tail (so-called blacktail) are the clinical hallmarks of whirling disease.[3]

Various species of the genus *Myxobolus* infect goldfish. However, we are not aware of any reports of infection with *M cerebralis* in goldfish. Nevertheless, the natural history of this parasite illustrates the complex nature of myxosporidean infections, which always involves an invertebrate intermediate host that releases actinosporean spores, which in turn develop into myxosporean spores in the definitive vertebrate host. To date, myxozoans are known to infect the finrays, liver, and biliary epithelium of goldfish[4–8]; to our knowledge, this is the first report of *Myxobolus* sp infection in the oral mucosa of a goldfish.

In evaluation of this case, important differential diagnoses for the goldfish's nodular oral mass included granulomatous disease resulting from infection with organisms such as *Mycobacterium* spp (common bacterial pathogens that cause internal and external granulomatous disease in fish) and neoplasia. Detection of myxosporidean spores during cytologic examination of biopsy specimen impression smears facilitated the etiologic diagnosis. Histologically, vegetative stages (early and late polysporous histozoic plasmodia) and mature spores were identified in the oral submucosa. These findings enabled localization of the infection (primary stomatitis) and provided evidence of asynchronous sporogenesis.

In most ornamental fish, the source of infective actinosporean spores is unknown. Invertebrate intermediate hosts are unlikely to be cohabitating with fish in home aquaria. As such, it seems likely that aquaculture facilities serve as the source of infection in many instances. In both the United States and abroad (other important producers include China and Japan), culture of cyprinid species is often pond based; these ponds have muddy bottoms, which provide ideal substrate for the growth of worms, snails, and other small invertebrates. In this setting, fish are infected during growth periods and maintain subclinical infection until the stress of transport (to either a retailer or home aquarium) blunts their immune system response and allows development of clinical disease.[9] Poor feeding practices may also result in infection; tubifex worms, which are readily available to aquarists in live, frozen, and freeze-dried forms, may be a source of infection. Continued use of a feedstuff that contains the infective organisms would perpetuate the problem.

Treatment of myxosporidiosis is difficult; furazolidine, proguanil, and fumagillin have been used with limited success.[3,10] Efficacy of such treatments is thought to be limited by the 2 resistant spore stages that occur during the parasite's life cycle. For the goldfish of this report, management of the disease involved frequent water changes; the intent was to disrupt the parasitic life cycle by limiting reexposure of the intermediate host to myxosporean spores shed by the fish and by limiting reexposure of the fish to infective actinosporean spores shed by the intermediate host. Enrofloxacin (5 mg/kg [2.3 mg/lb], IM, q 72 h) was administered to treat secondary bacterial stomatitis. Despite this treatment, the goldfish died 9 days after the initial evaluation. Necropsy was not performed; however, it was suspected that the myxosporean infection also affected underlying cartilage, which contributed to the facial deformity.

In ornamental fish, myxosporidiosis should be considered as a differential diagnosis for ulcerative and nodular oral masses. A diagnosis can be achieved via microscopic evaluation of wet-mount or stained cytologic preparations of scrapings or impression smears of the affected tissues or via evaluation of stained sections of paraffin-embedded biopsy specimens. Attempts should be made to identify the source of infection to minimize the risk of reinfection. Treatment options are limited and include disinfection, quarantine, and supportive care.

a. Finquel, Argent Laboratories, Redmond, Wash.
b. Fites acid-fast method, Polyscientific Research & Development Corp, Bayshore, NY.
c. Diff-Quik, Andwin Scientific, Addison, Ill.

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