

FUNGAL DISEASES

Fungal agents causes diseases and economic losses occur in different development stages (egg, larvae, fry and adult) in fishs. Fungal disease usually develop with or after other diseases (bacterial, viral, parasitic) and change course of other diseases. Even if there are fungal agents in the water, if the fish healthy, that is, if there is no lesion on the skin, fin and gill, addition to if the mucoid layer of the skin is intact, fungal infections do not develop in fish.

SAPROLEGNIOSIS (WATER MOLD INFECTION / WMI /, WINTER KILL, OOMYCETE INFECTION)

It is the most common external fungal disease in freshwater and salt water fishs and fish eggs. The name of the fungi responsible for the disease are *Saprolegnia parasitica* and *S. invodes*, and the agents settle on the skin, fin, gill, around of mouth, head region and surface of eggs. This disease occurs in fish that live in warm and cold water.

Virtually every freshwater fish is probably susceptible to at least one species, and water molds are classical opportunists that normally feed saprophytically on dead organic matter.

There is increasing evidence that these infections in fish are associated with immunosuppression. Outbreaks often occur after a drop in temperature or when temperatures are near the physiological low end for a particular fish species. Many oomycetes are more active in the cooler months of the year. When fish are exposed to acute confinement stress, their skin can actually slough off and this severe skin loss makes them much more susceptible to water mold infection (or fungal infection).

The excessive use of hormone (corticosteroid and androgen) cause a decrease of mucus cells count in the skin, which in turn reduces the resistance of the skin. In addition, the excessive use of hormone leads to a decrease of Vit. C in fish.

When saprolegniosis affects fish during very cold temperatures it is called winter kill. The disease occurs when pond temperature drop below 15 ° C.

The disease may be caused by immunosuppression because of the rapid temperature drop, possibly in combination with chronically high ammonia levels or exposure to some environmental stress in the prior summer / fall.

Transmission of disease; the water fungi are ubiquitous saprophytes in soil (or sediment and mud) and water. Most transmission is probably by motile zoospores. The zoospores allows dissemination to distant sites. It is important to realize that most fish infections are probably acquired from an animate source (i.e. , water molds sporulating on dead organic matter). There is asexual reproduction. If the infection begins in the skin, micells develop in the epidermis, but , if the zoospores have been taken by oral route, micells firstly develop in the digestive system and then reach the muscle and skin surface.

Pathology and clinical signs :

The first clinical sign is a dermatomycosis. Typical infection present as a relatively superficial, cottony growth on the skin or gills. The first lesions begin on the surface of skin such as focal and small lesions are not realized, and then, the lesions expand and the micellia grow in the skin and gills. If the disease progresses, the lesions grow gradually and begin to spread throughout the body. The lesions progress from epidermis to dermis and from there to the muscle layer. The epidermis layer is destroyed and shed, and then erosion and ulcers develop. The affected fish, the colour of skin are turned dark gray.

As clinical appearance, the lesions consisting of gray-white foci due to fungal micells on the skin and cottony-wool structures extending out of the body are noticeable. Newly formed lesions are white due to presence of the micellia, with them, the lesions often become colored red, brown, or green because of the trapping of sediment, alg, or debris in the micellia mat.

Although the micellia grow rapidly over the skin's surface, the infection rarely penetrates beyond the superficial muscle layers, and the micellia cause necrosis in tissue. If the micellia cover 30-40% of the body, the treatment may not result.

Sometimes, the infection spread from gills to pharynx and esophagus, and then the disease may be observed in liver, spleen kidney, brain, eyes, and swim bladder. Affected fish are exhibited depression, anorexia, weight loss and loss of balance.

The micellia are observed in the abdominal region of fish in the infection originating from the digestive system.

Oomycetes are important pathogen of fish eggs, and infections most often begin in unfertilized or otherwise nonviable eggs. Once establishment they can rapidly spread to healthy eggs, eventually resulting in complete loss of the brood. Affected eggs are opaque. The eggs covered the micellia, in which case there is no turning back.

Diagnosis :

Observation of a cottony, proliferative growth on the skin and gills should alert the clinician to a possible diagnosis of saprolegniosis. Some other pathogens (e.g. *Flavobacterium*, *Epistly*) can cause grossly similar lesions but are easily differentiated microscopically.

Scraping preparations taken from the skin, fins and gills are examined and the definitive diagnosis is made. Avoid skin damage and predisposing stresses. There is usually limited success in treatment. The measures to be taken in hatcheries prevent economic loss in fish farms.

- 1) dead or unfertilized eggs are cleaned
- 2) water temperature must be optimum and stable
- 3) diseased fish should be not kept in the pond
- 4) care and nutrition should be quality
- 5) the skin integrity must be complete

BRANCHIOMYCOSIS (GILL ROT DISEASE)

Brachiomycosis is a fungal disease that has caused acute, often high mortality in freshwater fish. There are two species. The disease is caused by the fungi *Brachiomyces sanguinis* and *B. demigrans*. Both species of fungi are found in fish suffering from an environmental stress, such as low pH (5.8-6.5), low dissolved oxygen, or high alg bloom. The agents grow at temperatures between 14-35 ° C but grow best between 25-32 ° C.

The main sources of infection are the fungal spores carried in the water and detritus on pond bottom. Contamination occurs when the spores in the water enter through the gills. The agents settle and germinate and occur hyphae.

Pathology and clinical signs :

Although the pathology of both species is similar to each other, *B. demigrans* is located in the gills and blood vessels, *B. sanguinis* is located in the filaments of gills and blood vessels. *Brachiomyces demigrans* reproduces outwards by destroying gill tissue, but *B. sanguinis* develops only in blood vessels and no reproduce outwards.

The gills are destroyed, and the difficulty respiration is obvious. The disease increases in the summer in temperate climates, morbidity 100 %, mortality 50 %. The course of the disease is fast in the crowding ponds.

Affected fish are exhibited difficulty of respiration, depression, anorexia, unwillingness movement, stagnation, not taking feed. The desire to take air from the water surface and the rapid movement of the operculum are observed in fish. The affected fish are easily caught by hand.

Brachiomycosis is primarily gill disease, and it causes melting, adhesion and necrosis in the filaments leave their grip and fall. In the later period, the deaths occur due to respiratory failure in affected fish.

In the acute cases, there is a marble appearance due to hemorrhage and infarction formation in the gills (streaked or marble appearance that shows infected and death tissue). Micelles obstruct blood vessels and cause thrombosis. The gills turn gray-white over time.

Diagnosis :

Diagnosis of Brachiomycosis can be made by examining wet mounts or histopathology of lesions. Characteristic hyphae, causing deep brachial infection, are diagnostic.

APHANOMYCOSIS (EPIZOOTIC ULCERATIVE SYNDROME, EUS/ATYPICAL WATER MOLD INFECTION)

EUS occurs in numerous freshwater fish worldwide, and mortality and morbidity can be high, and epidemics can develop rapidly. Interestingly, once an epidemic has occurred in an area, the prevalence and severity of future outbreaks often subside. The disease which is mostly seen in freshwater crayfish, crabs, and lobsters, also creates serious problems in aquaculture.

Aphanomyces invafans (*piscidia = invaderis*) is found in freshwater fish. This agent is localized in freshwater aquaculture and also is seen in river region fishing.

EUS occurs mostly at water temperatures of 18-22 ° C and after period of heavy rainfall. These conditions favor sporulation of *A. invadans* , and temperatures 17-19 ° C have been shown to delay the inflammatory response of fish to oomycete in fish.

The hyphae show thick (> 7 µm) , multinucleated, non-segmented and branching.

Life cycle ; the fungi zoospores can be horizontally transmitted from one fish to another through the water supply. The active spores remain in the water for 5 days, at the end of this period the spores lose their vitality. The zoospores are capable of attaching to the damaged skin of fish and germinating into hyphae. The hyphae invade skin, muscle layers and internal organs. If the spores can not find the susceptible species or encounter unfavorable conditions, they can encyst in the pond environment.

The bacteria (*Aeromonas, Vibrio, Pseudomonas, Micrococcus*), the viral diseases (*Rhabdo, Birna, Reoviral*), some fungal diseases (Saprolegniosis , Achyla) and other factors (low pH degree, rainfall, the water temperature decrease, some toxins, skin destruction, cannibalism) are predisposed to the occurrence of this infection. The encysting property of the *Aphanomyces* pathogen may play an important role in the cycle of outbreaks in endemic areas.

Pathology and clinical signs :

Aphanomyces astaci is found in joints in freshwater crayfish and caused 100 % mortality. Water temperature is an important factor in the course of the disease. If the water temperature is above 10° C acute deaths occur within 3 weeks, but as the water temperature decreases, the development of the agents slows and the deaths occur within 3 months.

The deep ulcerative lesions are observed in chronic form of the disease in fish. These lesions are initially small erythematous foci, and then they progress to muscle layer and are turned into the ulcerative lesions.

The intense granulomatous lesions are occurred due to intensive tissue reactions. The hyphae protruding from the body are typically.

The chronic and heavily stage of the disease, opened ulcerative areas that are large, hemorrhagic and necrotic lesions are occurred, and as the most striking findings, epithelioid granulomatous lesions are observed internal organs.

The disease are known as RED – SPOT DISEASE, MYCOTIC GRANULOMATOSIS /MG/ and ULCERATIVE MYCOSIS /UM/) due to its clinical appearance.

Diagnosis :

A presumptive diagnosis of typical EUS infection is based on the presence of deep ulcers that contain brood (at least 7 µm in diameter) , aseptate hyphae that are usually observed severe and chronic inflammation.

Treatment : there is no known treatment for EUS. The antibiotics and antiseptics have been used, there is no evidence for their efficacy.

ICHTHYOSPORIDIOSIS (SWIMMING DISEASE , ICHTHYOPHONOSIS)

Ichthyosporidiosis is a chronic and granulomatous, and systemic fungal disease that is mostly observed in trout, and also, it is an endo-fungal disease. The agent name is *Ichthyosporidium (Ichthyophonus) hoferi*. The agent is an intercellular fungus, and its shape is oval or spherical forms, and there are many granular nuclei in the cytoplasm. The yellow-brown agents are seen during sporulation or rest period. Endemics have occurred in freshwater and marine fish.

The agent usually develops at 3-20 °C intervals, but the optimal water temperature is 10 °C. The life cycle of this fungus is complicated. Contamination occurs with eating fungal cysts that pass into water with feces, as cannibalism, with parasites containing fungi and consumption of infected foods. The spores germinate to form amoeboblasts in the digestive tract, penetrate the gut mucosa, reach the blood vascular system and spread via the portal system and usually localizes in the liver, spleen, kidney, and particularly in the muscle including the cardiac muscle. Spores are released when hyphae rupture the surface of the gut or penetrate the skin to allow infection to spread via the water.

Ichthyophonus is frequently observed in the resting or spore stage, and this is an oval structure, 10-250 µm in diameter.

Pathology and clinical signs :

Clinical signs are not observed in mild and more recent infections, but the appearance of sandpaper on the skin and tail area due to pathology in the subcutaneous and muscle tissue is noticeable. A large number of nodules develop in the internal organs. The sandpaper effect is due to the loss of epithelium over the proliferating fungal granulomata (ring nodules). These nodules are produced by the severe granulomatous response with a large number of epithelial cells, macrophages and occasional giant cells, along with tips of fungal hyphae or newly developed spores. Gray-white nodules develop in the internal organs, especially liver and kidney.

Affected fish are observed with color darkening, anorexia, exophthalmus, irregular scales, depression, and swimming disorders.

The small nodules that develop in the lateral and ventral region of the diseased fish grow and expand over time. Over time, the skin over these nodules becomes necrotic, turns black in color, falls off, and gray-white ulcerated areas appear in the place.

There are dense fungal cysts within the ulcers, from which the cysts pass into water with discharge.

Neurological signs (swinging disease) are common in freshwater fish because of central nervous system involvement. Fishes may also have spinal curvature and darkening of the skin.

There is no treatment, and treatment is often inadequate; the fish cannot be put on the market due to malodor, nodules, and melting. The sick fish are destroyed.

Diagnosis :

The disease can often be identified from fresh lesion material. Typical, thick-walled spores surrounded by granulomatous inflammation are seen on fresh wet mounts.

CONTROL ---- TREATMENT

- 1) It is impossible to control of fungal disease in natural conditions, and prevention of fungal disease is main goal in aquaculture. Predispose factors should be reduced due to limited success treatment
- 2) sick and dead fishes not be left in ponds. Zoospores pass into water for 24 hours even if the fish die
- 3) there should be no wounds or injuries to the fish body, and ectoparasitic infections should be no in the fishes
- 4) stress factors should be reduced and fish density in the ponds should be balanced
- 5) all materials used must be disinfected
- 6) there should be no feed residue in the pond
- 7) water circulation should be regular and sufficient
- 8) dead and infertilized eggs should be cleaned from pond daily
- 9) the causative agent must be diagnosed correctly and the biology of the agent must be know
- 10) both-sytle medication is commonly used in treatment, and in application, oxygen support must be made to the pond water
- 11) drug applications should be made in the early morning hours when temperature change are not excessive and before feeding
- 12) wounded or injured fish must be seperated before treatment
- 13) general drug dose should be calculated according to “ mg per kg for daily”, and container prepared with medication should be wooden or plastic
- 14) preliminary tolerans and efficacy tests are performed and the main drug application should be done after the short time

DOSE OF DRUGS AND THEIR ROUTE OF ADMINISTRATION

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| H2O2 | 100-500 mg / L for 1 hour, bath 0.71-1.42 ml (35 % H2O2) 15 minutes, for eggs |
| MgCl2 | 25mM MgCl2 , 1 day , bath |
| Malachite green | 0.5 – 5 mg /L for 1 hour , bath 50 -60 mg / L for 10-30 seconds , immersion 10 mg / L for 30-40 minutes for eggs |

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| | 0.5 g / L 1 hour or 0.3 mg / L for eggs, bath |
| NaCl | 20 g / L 1 hour, bath |
| | 1-5 mg / L 1-2 minutes, bath |
| Bronopol | 20 mg / L 1 hour X 14 days, bath |
| Eugenol | 1-10 g / L, bath |
| Proxitane (5 % peracetic acid in H ₂ O ₂) | 100mg / L 5 minutes, bath |
| Azadirachtin+camphor+curcumin | 400-700mg / L for 3 days, bath |
| Formalin | 0.125-0.250 ml / L treat for up to 1 hour, bath |
| | 250 mg/ L treat for up to 1 hour, bath |
| | 1-2 ml / L treat for eggs for up to 1 hour, bath |
| | initially 15 ppm followed by 25 ppm treat for Branchiomyces spp |
| CuSO ₄ | 100 mg / L treat for up to 10-30 minutes, immersion |
| NaOH | 10-25 mg / L treat for up to 30-90 minutes, bath |
| Methylene blue | 2 mg / L treat for up to 1 day for eggs, bath |