

## **Bacterial diseases of fish**

### **6. Fin Rot**

#### **Causative agent:**

The disease is caused by *Flexibacter maritimus* (synonyms: *Cytophaga marina*, *Tenacibaculum maritimum*).

#### **Stages affected:**

The bacteria usually attack fingerlings.

#### **Gross clinical signs:**

Affected fish become anorexic, lethargic and dark in color. Initially, the tip of the fin becomes grayish, and then it becomes eroded and hemorrhagic. The lesions progress into fin rot or extensive fin loss. Eventually, even the muscle fibers will be affected. Effects on host: Mortality rate of 80% may be observed within a few days if the infected fish are not treated. The bacteria could destroy the tail region within 2 days.

#### **Transmission:**

The occurrence of the disease is correlated with water salinity. The disease is observed when the fish are exposed to high salinity of 30-35 ppt. The bacterium infects the fish through damaged area on the fin region.

#### **Diagnosis:**

Squash preparation of affected areas examined under the microscope will reveal long rodshaped bacteria ( $0.5 \times 2.5 \mu\text{m}$ ) gliding slowly without flagella. The bacteria may be isolated from the infected tissue using *Cytophaga* agar prepared with seawater forming yellowish colonies.

#### **Preventive measures:**

Avoid rough handling of fish to minimize lesions, which could be portals of entry for the bacteria, due to mechanical damage.

#### **Control methods:**

Treatments should be implemented before secondary *Vibrio* infection sets in. Freshwater bath for 10-15 minutes and pefuran bath treatment at 1-2 ppm for 24 hours are effective in controlling the disease.

### **7. Motile Aeromonas Septicemia**

Motile *Aeromonas* septicemia (MAS) is caused by any of three species of the genus *Aeromonas*: *A. hydrophila*, *A. caviae* and *A. veronii* biovar *sobria*. These species are commonly referred to as motile aeromonads. One strain of *Aeromonas hydrophila* causes particularly devastating MAS in channel catfish and channel x blue hybrid catfish. This strain has distinct characteristics and will be referred to as "virulent *Aeromonas hydrophila*" (VAh).

#### **Geographical Range:**

Motile *Aeromonas* septicemia occurs worldwide, especially in freshwater and brackish water systems. The devastating MAS caused by *VAh* has a more limited distribution. It was first reported in Alabama in 2009 (Pridgeon and Klesius, 2011) and has since been found in Mississippi and Arkansas.

#### **Host Species:**

Most fish and many aquatic invertebrates are susceptible to disease caused by motile aeromonads. These bacteria also cause disease in amphibians, reptiles, birds and mammals, including humans.

#### **Epizootiology:**

The epizootiology of MAS is complex, only partially understood and likely varies substantially based on the host and water system. The environmental distribution of motile aeromonads has been well studied, with a focus on public health due to concerns of human infections acquired from drinking water, contaminated food and physical contact. These bacterial species are widely distributed in virtually any relevant freshwater or marine environment, including chlorinated tap water (due the ability to form protective biofilms). The density of potentially pathogenic motile aeromonads in natural waters is generally correlated with temperature and organic load. These species can associate with algae, biofilms, and zooplankton and are natural components of the gut flora of many vertebrates and invertebrates. These organisms have the potential to harbor and spread motile aeromonads.

The motile aeromonads appear to be transmitted primarily by horizontal means. Uptake may be by oral or dermal routes with the later occurring after disruption of mucosal defenses. Asymptomatic carriers likely shed the bacterium in feces. Diseased individuals may shed the bacterium from open lesions and are often preyed upon by other fish. Furthermore, motile aeromonads can be saprophytic and likely persist and multiply in decomposing fish.

Most cases of MAS can be correlated to a predisposing factor such as injury, infection with another agent, or an immunosuppressive event such as environmental stress. Motile *Aeromonas* septicemia is often a complicating factor in columnaris disease, saprolegniosis, and several diseases of viral etiology. Furthermore, MAS is sometimes associated with nutritional stress, low dissolved oxygen and handling stress. Typical MAS outbreaks are most common in spring and fall when fluctuating temperatures compromise the immune system.

Many severe outbreaks of MAS caused by *VAh* have been documented in cultured channel catfish and channel x blue hybrid catfish since 2009, without any identified predisposing primary pathogen or obvious condition that would cause immunosuppression. Severe MAS outbreaks due to *VAh* in the catfish industry occur primarily in the warm summer months. Losses can progress rapidly, with a large percentage (~20-30%) of the catfish in the pond succumbing to disease within a week from when losses are first observed. These outbreaks frequently occur in larger fish that are at or near market size. In addition, other fish species may succumb to MAS if present in a pond with *VAh*-associated MAS in the resident catfish population.

#### **Disease Signs:**

Gross pathology of MAS can range from few external or internal signs in peracute cases, to hemorrhagic septicemia in acute cases, to abscesses and large ulcers in chronic cases. Severe MAS outbreaks often display a range of lesions indicating variation in the progression of disease in individuals. External signs seen in fish with typical septicemia include reddened fins, inflammation of

the anus, diffuse hemorrhages on the skin, exophthalmia and abdominal swelling. Scaled fish may display protruding scales (lepidorthosis) due to edema of the scale pockets, also fin erosion, focal scale loss and ulcers may be seen. One sign that is commonly seen in catfish with MAS caused by VAh is iridial hemorrhages in the eye. Internal signs include bloody ascites, diffuse hemorrhages in the intestines, connective tissue, visceral fat and musculature, swollen friable kidney and spleen.

#### **Disease Diagnostic Procedures:**

**Presumptive diagnosis:** The presumptive diagnosis of MAS is usually based on clinical signs and the isolation and identification of *A. hydrophila*, *A. veronii biovar sobria*, or *A. caviae* using traditional biochemical tests or with the aid of commercial biochemical assays such as API 20E, API 20NE (BioMérieux, Durham, NC), Sensititre (TREK Diagnostic Systems, Inc., Cleveland, OH) or BBL Crystal Enteric/Nonfermenter (E/NF; BD Diagnostics, Sparks, MD). However, biochemical characteristics are variable and often result in the misidentification at the species level (Solar et al. 2003, Lamy et al. 2010). These tests may also misidentify at the genus level, with *Vibrio* being the most common misidentification. It is suggested that resistance to the vibriostatic agent O/129 be used as a supplemental test when using these system.

**DNA extraction and controls:** DNA is extracted from affected fish tissues (lesion, posterior kidney) or bacterial cells using a commercially available kit such as DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. The DNA can be quantified and evaluate for purity spectrophotometrically. Alternatively, a suspension of approximately 2 µl of an individual bacterial colony can be suspended in 100 µl of sterile PBS heated to 95°C for 5 min and used as PCR templates. All PCR assays should use a representative negative control (a non-*Aeromonas* or non-VAh bacterial isolate, on non-infected tissue) prepared using the same reagents. Likewise a positive control should be included in each analysis to rule out assay failure.

#### **8. Bacterial kidney disease (BKD)**

##### **Causative agent:**

The bacterium causing BKD is a small Gram-positive diplobacillus named *Renibacterium salmoninarum*.

##### **Signs of infection:**

##### **External signs:**

1. Infected fish may appear normal. In rainbow and brown trout, there may be a "buckshot" appearance due to the presence of numerous small, open ulcers in the skin that expose the underlying musculature.
2. In brook trout and coho salmon fingerlings and yearlings, large "boils" filled with a pinkish, creamy fluid that contains massive numbers of *Renibacterium salmoninarum* bacteria can be found on the sides of the fish.
3. Exophthalmia (popeye), due to osmo-regulatory disruption, is a common sign of BKD. However, this sign is not diagnostic since it may also be due to other causes, such as gas supersaturation (gas bubble disease), enteric redmouth (ERM) infections, certain viral diseases.

##### **Internal signs:**

1. As indicated by its name, BKD severely affects the kidneys, and, to a lesser extent, the spleen and liver. The kidneys are usually swollen, convex, and have a corrugated or lumpy surface in sharp contrast to the smooth, concave surface of healthy kidneys. Creamy, soft, off-white cysts represent massive colonies of the causative organism. Such cysts are common in the posterior kidney and may vary in size and number. They should not be confused with normal stannous bodies located in the mid-kidney or with nephrocalcinosis (kidney stones) that may fill excretory tubules of the kidney.
2. A bloody, turbid, or yellow-brown fluid often accumulates in the abdominal cavity and around the heart.
3. Other internal organs and visceral fat may appear normal or appear unusually white. The intestinal tract may contain a white or yellow viscous fluid.

#### **Diagnosis:**

The diagnosis of BKD in salmonids can be confirmed by the detection and identification of *Renibacterium salmoninarum* organisms in the tissues. Fluorescent antibody techniques applied to fresh, frozen, or formalin-fixed infected posterior kidney tissue will usually reveal the presence of large numbers of small "bean-like" organisms (Mitchum and Sherman 1981; Paterson et al. 1979; Bullock and Stuckey 1975). Gram stains of similar material will reveal many tiny, Gram-positive diplobacilli which often occur in pairs. Fish that are apparently healthy can carry small numbers of the causative organism that are difficult to detect in Gram-stained smears (Sanders *et al.* 1978). Smears prepared from scrapings of the posterior intestinal tract of asymptomatic carriers may contain more organisms than smears of kidney tissue taken from the same fish (Mitchum and Sherman 1981). Diagnosis of BKD cannot be based, with any certainty, on the observation of a few isolated BKD-like organisms seen in a few microscope fields (Fryer and Sanders 1981). If this situation is encountered, more detailed sampling of the population in question should be initiated with particular attention to yearling trout or the oldest salmon fingerlings available at the site (Paterson et al. 1979; Snieszko *et al.* 1955).

#### **9. Furunculosis:**

##### **Causative agent:**

The disease is caused by gram-negative, short bacilli called *Aeromonas salmonicida*, which is classified into two strains.

1. Typical *A. salmonicida*, isolated from salmonids only.
2. Atypical *A. salmonicida*, isolated from salmonids & non salmonids species

It is aerobic but is capable growth as facultative anaerobe and not spore-forming. Non-motile *A. salmonicida* produce brown-pigment on culture media as trypticase soy agar, furunculosis agar and brain heart infusion agar. The microorganism was isolated from skin lesions, liver, Kidney and blood of infected fish. Colonies develop within 48 hours at 22-25 °C as small circular, raised and translucent. Colonies will not grow at 37 °C.

##### **Susceptible species:**

All species of the family salmonidae are considered to be susceptible to furunculosis. *A. salmonicida* has been isolated from fishes other than the salmonids such as carp, catfish, and other fish species. Young fish are more susceptible to the disease than old fish

**Predisposing factors:****These include:**

1. Physical damage of the skin or gills.
2. Poor water quality.
3. Presence of ectoparasites and other diseases.
4. Both smolting and high temperature.
5. High stock density.
6. Rough handling

**Course of the disease:**

1. Per-acute form: This form is restricted in young fish and leads to rapid death among fish.
2. Acute form: This form is characterized by sudden increase in mortality with few or no symptoms within 2-3 days.
3. Subacute form: This form characterised by gradual decrease in mortality. With furuncles formation and hemorrhages at the base of fins.
4. Chronic form: In this form mortality rate is low with intestinal inflammation and variable lesions.
5. Latent form: No mortality. But the pathogen present systemically.

**Mode of transmission:**

1. The infection was transmitted by ingestion of contaminated food.
2. Water was found to be vehicle for spreading the infection.
3. A carrier is important sources for the infection.
4. The infection occurs through eggs.
5. The infection may occur through injuries of the skin.

**Clinical signs:**

These are different forms showing on diseased fishes:

**1. Per-acute form:**

This form is characterized by rapid death of fish especially young fish, whose defenses against a severe bacterial septicemia will be poor Darkening of skin, rapid breathing and slight exophthalmos may be observed on infected fish. Cardiac damage is a possible cause of death.

**2. Acute form:**

Fish with an acute infection show signs of hemorrhagic septicemia, including body and vents. Skin lesions may be haemorrhagic patches along the side or on the dorsal body surface, hemorrhages at base of fins. Darkening of skin and sluggishness in movement. Hemorrhages scattered over abdominal walls, viscera and heart. Soft and friable or liquefied kidney is observed. Enlarged spleen with round edges. Pale liver with subcapsular haemorrhages. Stomach & intestine may contain bloody mucous. Swim bladder is hyperaemic. Raised furuncles, which usually develop in the dermis due to localization of bacteria rather than the hypodermis. Fish may die within 2-3 days.

### 3. Subacute and chronic form:

In this form mortality rate is low & more common in older fish. The clinical includes. Slight darkening of skin, inappetance, and lethargy and congested blood vessels at base of fins. Slight exophthalmos & fish may have pale or congested gills. The furuncle may be small and compact or large & soft. They contain dark red pus with numerous bacteria. The mature furuncle bursts leaving deep ulcer or healed furuncle may leave scar tissue. The furuncle may be found in liver, kidney and spleen.

### 4. Intestinal form:

This form is associated with low mortality. Prolapse of anus and intestinal inflammation may occur.

#### Diagnosis:

1. Case history.
2. Clinical signs and postmortem findings.
3. Isolation and identification of the causative agent.
4. Serological identification of the etiology by:
  - a. Agglutination test.
  - b. Precipitation test.
  - c. Fluorescent antibody technique.
  - d. ELISA.

#### Treatment:

1. Sulfamerazine: 150-220 mg/kg fish weight/day for 10-14 days.
2. Oxytetracycline: 50-75 mg/kg fish weight/day for 10 days.
3. Furazolidone: 25-100 mg/kg fish weight/day for 10 days.
4. Oxolinic acid: 10-mg/kg fish weight/day for 10 days.

## 10. Columnaris disease

#### Caused by:

*Flavobacterium columnare*

#### Signalment:

A huge range of fish hosts can be infected including the trout and salmon family, eels, goldfish, Siamese fighting fish, catfish and bass species. Cultured fish, free ranging fish and aquarium fish can all be affected although cultured fish reared in ponds or raceways are the primary concern. It is not recognised as a problem in wild fish populations.

Columnaris disease can occur in cool and warm water fish, but is most prevalent in air temperatures above 12-14°C.

Skin abrasions, husbandry stressors such as overcrowding, poor nutrition, underlying diseases and warm water all increase the risk and prevalence of infection.

**Clinical signs:**

The skin and fins of infected fish may darken or lighten in colour and circular yellow-grey opalescent necrotic patches or erosions are visible on the skin and/or gills. The gill filaments may have whitish spots on their tips. Mucus often also accumulates on the gills, head and dorsal regions. In advanced disease, erythematous spots are widespread. Inappetance and lethargy are common, as are mortalities. Gill lesions cause respiratory distress and its associated clinical signs – gulping air, sinking, erratic behaviour etc. Morbidity can approach 100% and mortalities are highest in younger fish.

In catfish specifically, severe tissue necrosis develops into “saddleback” lesions on the dorsum.

**Diagnosis:**

Bacteria can be isolated from gills, skin and sometimes internal organs, particularly the kidneys.

The pathogen can then be cultured on reduced nutrient agar, such as cytophaga agar. Culture is often more successful by inhibiting contaminant growth on the agar by adding neomycin or polymyxin B. The bacterium will also grow at 37°C which will inhibit most other pathogens of interest so can be used as an inhibitory factor. Colonies are small, 3-4mm in diameter and grow within 24h. They are characteristically and uniquely rhizoid in structure and pale yellow in colour, and adhere to the agar surface. They stain pink when exposed to potassium hydroxide. This pathognomic culture finding can be used as a definitive diagnosis for columnaris disease, especially where clinical signs are present.

Phenotypic tests can then be carried out on colonies if in any doubt.

Microscopy of wet tissue mounts from gill or skin scrapings often reveals haystacks or columns of bacteria. Cells can also form chains, giving the impression of a longer, single prokaryotic cell.

**Treatment:**

- Potassium permanganate, copper sulphate and hydrogen peroxide can be applied externally to adult fish and fry but can be toxic at high concentrations.
- Terramycin can also be added to feed for adults, fry or broodstock, but resistance is emerging.
- Vaccines can also be given in the face of an outbreak.