Chapter 2. Bacterial Diseases

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Bacteria are very common in the aquatic environment. Most bacterial disease agents are part of the normal flora of the water. They cause disease only when the fish are stressed due to poor environmental conditions, inadequate diet and poor husbandry techniques.

This chapter focuses on the most common bacterial diseases of groupers.

VIBRIOSIS

Vibriosis is also known as *Vibrio* hemorrhagic septicemia and is often associated with another disease, the red boil disease, which is caused by *Streptococcus* sp. The disease has been reported in *Epinephelus malabaricus*, *E. tauvina*, *E. coioides* and *E. bleekeri*. Vibriosis has been recorded in cultured groupers in Brunei Darussalam, Malaysia, Taiwan, Indonesia, Kuwait, Thailand, Singapore and the Philippines.

Causative agents:

The causative agents of vibriosis are *Vibrio parahaemolyticus, V. alginolyticus, V. vulnificus* and *V. carchariae*.

Stages affected:

The bacteria may affect grouper fry, fingerlings, juveniles, adults and broodstocks.

Gross clinical signs:

The first sign of the disease is anorexia or loss of appetite with darkening of the fish coloration. The fish may be lethargic, swimming near the water surface. Affected fish may lose equilibrium and exhibit abnormal swimming behavior. One of the signs of the disease is body ulcer that may be hemorrhagic (Fig. 2-1a). Fin rot, which usually starts with erosion of the tip of the fin and gradually becomes necrotic, may also be observed (Fig. 2-1b). Exophthalmia and corneal opacity are also common signs of the disease. Internally, bloody discharges may be observed in the abdominal cavity due to internal organ hemorrhage (Fig. 2-1c). In the case of *V. carchariae*, gastroenteritis manifested by a swollen intestine containing yellow fluid may be observed.

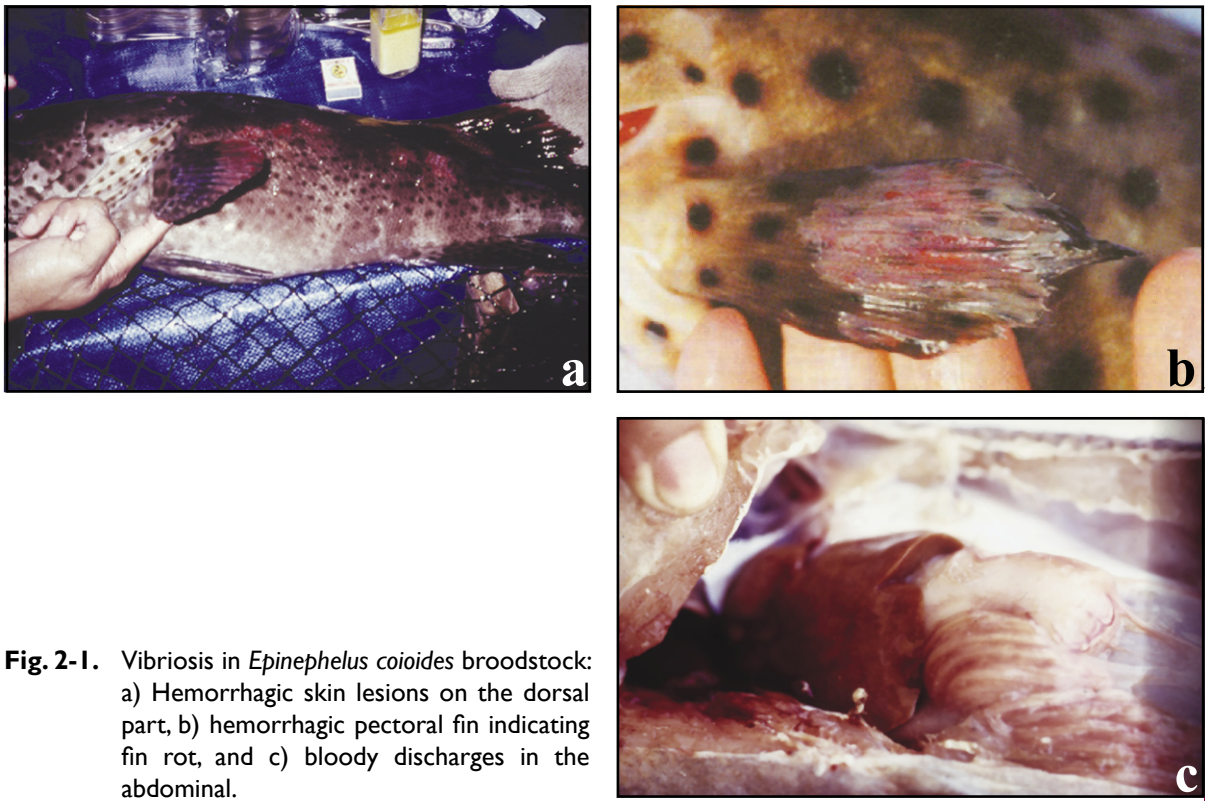

Fig. 2-1. Vibriosis in *Epinephelus coioides* broodstock: a) Hemorrhagic skin lesions on the dorsal part, b) hemorrhagic pectoral fin indicating fin rot, and c) bloody discharges in the abdominal.

Effects on host:

Ten to 50% mortality may be observed in affected populations depending on the farm management. It has also been observed that infected grouper harbor monogeneans and protozoans on the gills and skin. *Vibrio* sp. infection is also associated with red boil disease. In relatively colder countries like Taiwan, outbreaks may occur in summer when ambient temperatures rise.

Transmission:

The spread of the disease has been correlated with high salinity (30-35 ppt). The bacteria enter the fish through damaged areas. The disease may be transmitted through the water and trash fish given to the grouper. Infection is enhanced by parasitic infestation and mechanical injuries during transport, and grading.

Diagnosis:

Squash preparation of affected areas examined under the microscope may reveal the bacteria. Vibrios are Gram-negative straight or curved rods, 0.5-0.8 µm in width and 1.4-2.6 µm in length. The bacteria may be isolated from the infected organ using tryptic soy agar (TSA), nutrient agar (NA), or brain heart infusion agar (BHIA) supplemented with NaCl. Thiosulfate citrate bilesalt sucrose agar (TCBS), a *Vibrio* selective medium, may be used to isolate and primarily identify associated main groups of vibrios. Opening of the abdominal cavity will reveal hemorrhagic internal organs. The kidney may be swollen and filled with yellow fluid.

Preventive methods:

Rough handling of the fish during stocking, sampling, changing of nets, grading and overcrowding should be avoided. Good water quality must be maintained.

Control methods:

The disease may be controlled through freshwater bath for 10-15 minutes. Affected fish may be treated with oxalinic acid mixed with feed at 20 mg/kg of fish. Terramycin added to feed at 7.5 g/kg for 5 days, reduced to 3.75 g/kg for the succeeding 5 days also proved effective. Prefuran bath treatment for 1 hour at 2 ppm may also be implemented (see Appendix 1 for points to consider before using antibiotics and a list of anti-infectives recommended for use in marine food fish together with the withdrawal period).

PSEUDOMONAS INFECTION

The disease is also known as pseudomonad hemorrhagic septicemia. The only reported case of the disease is in cage-cultured *Epinephelus tauvina* in Malaysia.

Causative agent:

The causative agent is *Pseudomonas* sp.

Stages affected:

The bacteria may affect grouper at all stages.

Gross clinical signs:

Infected fish have extensive hemorrhagic erosions of the body. Ulcerations on the skin, fins and tails may also be observed. Other common signs of the disease are exophthalmia and corneal opacity.

Effects on host:

Twenty to 60% mortality may be observed in affected populations. A secondary epibiont fouling may also be observed. Internally, there is renal fragility, and dark red multifocal hepatic discoloration. Histologically, pathological changes consistent with subacute bacterial septicaemia are observed in the internal organs. Diffuse pericarditis and marked multifocal endocardial thrombosis and embolism are observed in the heart (Fig. 2-2a). Thrombosis and embolism are also observed in the hepatic vein (Fig. 2-2b). Diffuse pancreatic acinar cell atrophy and mononuclear cell infiltration are observed in the pancreas (Fig. 2-2c).

Transmission:

Pseudomonas spp. are ubiquitous in the aquatic environment. *Pseudomonas* infects fish when it is subjected to environmental stressors such as extreme water temperature changes, overcrowding, poor water quality and sub-optimal nutrition.

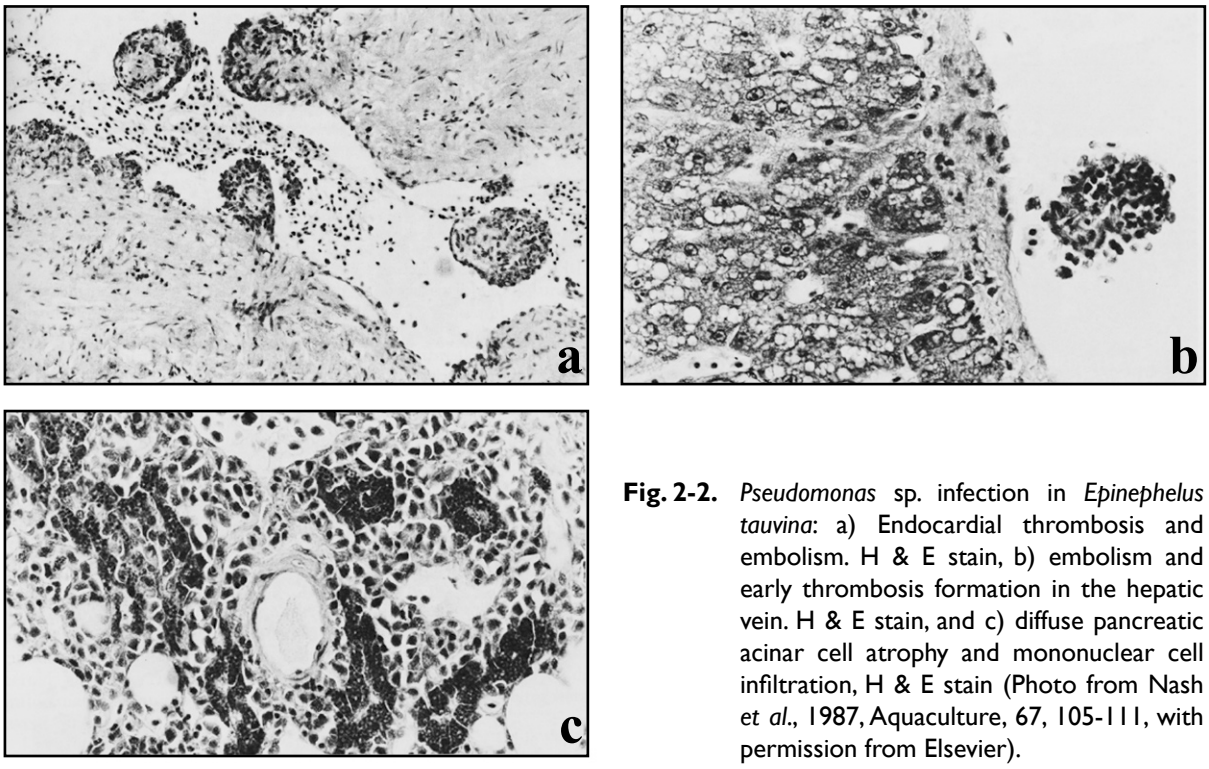

Fig. 2-2. *Pseudomonas* sp. infection in *Epinephelus tauvina*: a) Endocardial thrombosis and embolism. H & E stain, b) embolism and early thrombosis formation in the hepatic vein. H & E stain, and c) diffuse pancreatic acinar cell atrophy and mononuclear cell infiltration, H & E stain (Photo from Nash *et al*., 1987, Aquaculture, 67, 105-111, with permission from Elsevier).

Diagnosis:

Squash preparation of the kidney and other internal organs as well as the affected areas may reveal large colonies of *Pseudomonas* sp. Pseudomonads are Gram-negative, straight or slightly curved rods but not helical in shape, 0.5-1.0 µm in width and 1.5-5.0 µm in length. The bacteria may be isolated from the infected organ and affected areas using glutamate starch phenol red agar (GSP) or *Pseudomonas-Aeromonas* selective agar, a medium that selectively promotes growth of *Aeromonas* and *Pseudomonas* but inhibits growth of other bacteria. Internal examination of affected fish will show renal fragility and dark red multifocal discoloration of the liver.

Preventive methods:

Avoid the predisposing factors such as extreme water temperature changes, overcrowding, poor water quality, and sub-optimal nutrition to prevent pseudomonad infection.

Control methods:

Transferring affected fish into another tank with clean water may control the infection.

STREPTOCOCCAL INFECTION

The disease is also known as red boil disease. The disease is often associated with vibriosis. The disease has been reported in *Epinephelus malabaricus* and *E. bleekeri* in Brunei Darussalam, Malaysia, Singapore and Thailand.

Causative agent:

The causative agent is *Streptococcus* sp.

Stages affected:

Streptococcus sp. could infect grouper at all stages but is common in fry and fingerling stages.

Gross clinical signs:

Affected fish are weak and display disoriented whirling motion. Exophthalmia and hemorrhages on the cornea, operculum, around the mouth and the anus could also be observed. Infected fish have red boils in the skin.

Effects on host:

The red boils on the skin enlarge and eventually burst, exposing the necrotic musculature underneath and form small ulcers, which act as portals of entry for other bacteria. The disease may also cause systemic infections with few external signs. The infection could cause 10% mortality in affected fry but is not fatal to older fish.

Transmission:

The bacterium is ubiquitous in the environment, in the water and in carrier fish. Spread of the disease is associated with the presence of parasites, handling stress and sub-optimal water quality.

Diagnosis:

The bacteria grow well on BHIA, Todd-Hewitt agar, horse agar and TSA supplemented with 0.5% glucose. The colonies on agar plates appear small (0.5-1.0 mm diameter), yellowish, translucent, rounded and slightly raised. *Streptococcus* spp. are Gram-positive bacteria with ovoid or spherical cells, less than 2 µm in diameter, occurring in pairs or chains.

Preventive methods:

Avoid the predisposing factors such as the presence of parasites, handling stress and sub-optimal water quality to prevent disease occurrence.

Control methods:

Affected grouper could be treated with oxolinic acid mixed with feed at 20 mg/kg of fish, and perfuran bath for 1 hour at 2 ppm (see Appendix 1 for points to consider before using antibiotics and a list of anti-infectives recommended for use in marine food fish together with the withdrawal period).

FLEXIBACTER INFECTION

Flexibacter spp. are long rod-shaped, Gram-negative bacteria with parallel sides and rounded ends, typically 0.5 µm wide and 1-3 µm long. The bacteria do not possess flagella and move by gliding, thus are also known as the gliding bacteria. Some *Flexibacter* spp.

are widespread opportunistic bacterial pathogens. Some species of these yellow-pigmented bacteria have been associated with diseased fish, including *Flexibacter columnare* and *F. maritimus*, as well as other unidentified gliding bacteria referred to as *Cytophaga*-like bacteria. *Flexibacter* spp. are reported to cause bacterial gill disease and fin rot in groupers.

1. Bacterial Gill Disease

Bacterial gill disease has been reported in *Epinephelus malabaricus* and *E. bleekeri* in Brunei Darussalam and in *Plectopromus leopardus* in Indonesia. *Cytophaga* sp., *Flexibacter* sp. and *Flavobacterium* sp. cause bacterial gill disease in groupers in Brunei Darussalam. However, no bacteria were isolated from *Plectropomus leopardus* with bacterial gill disease in Indonesia, although histopathological examination showed rod shaped bacteria in the gills, which could possibly belong to the genus *Cytophaga*, *Flexibacter* or *Flavobacterium*.

Causative agents:

The disease is caused by *Cytophaga* sp., *Flexibacter* sp. or *Flavobacterium* sp.

Stages affected:

The bacteria usually attack fingerlings.

Gross clinical signs:

Affected fish become anorexic, lethargic and dark in color. Fish tend to remain near the surface and may be flaring their operculum. The gills produce excessive amounts of mucus and the gill filaments may stick together. The gills of affected fish become yellowish in color indicating gill rot.

Effects on host:

A high mortality rate of >80% may be observed within a week in affected populations. The bacteria attach to the gill surface, grow in spreading patches and eventually cover individual gill filaments that result in cell death. Gill lesion may cause respiratory difficulty and the fish eventually dies. Histologically, fusion of the secondary lamellae, epithelial hyperplasia and presence of rod-shaped bacteria could be observed in the gills.

Transmission:

The disease starts when the water quality deteriorates after a heavy rain. Silt and suspended organic particles from run-offs could irritate the gills and increase susceptibility to the disease. Low dissolved oxygen and high ammonia levels are often observed during disease outbreaks. Stress during grading makes fish susceptible to bacterial infection.

Diagnosis:

The disease is diagnosed by the presence of brown to yellow brown growth of bacteria in the gills (Fig. 2-3). Microscopic examination of wet mounts of the gill will reveal bacteria that are in a slow gliding movement. *Flexibacter* sp. is a thin, long Gram-negative rod with parallel sides and rounded ends, typically 0.5 µm wide and 1-3 µm long, that grows in layers, one on top of the other, giving it the appearance of "columns" or "haystacks" under the microscope.

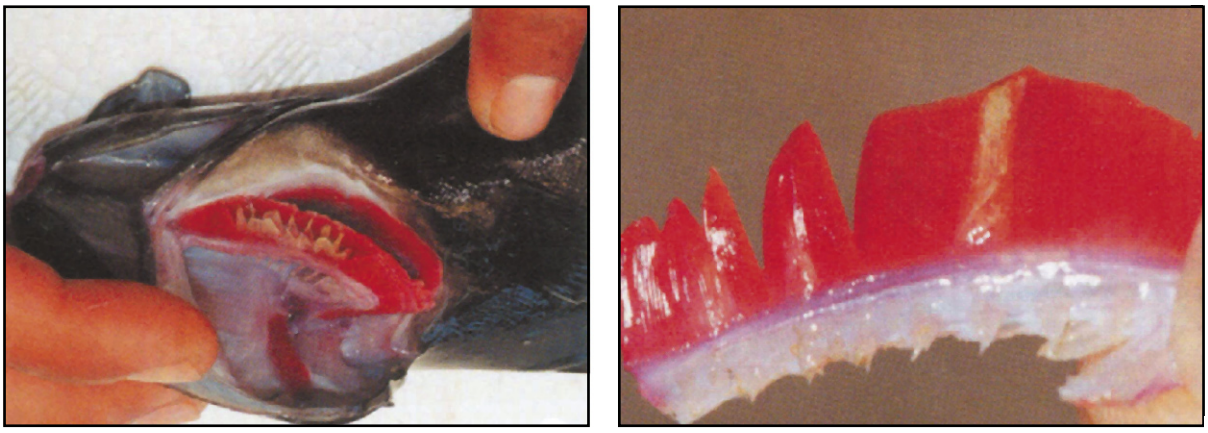

Fig. 2-3. Bacterial gill disease in *Plectropomus leopardus*. Note the presence of brown to yellow brown growth of the bacteria in the gill filaments indicating gill rot (Photos from Koesharyani *et al*., 2001).

The bacteria may be isolated using a selective media such as Selective *Cytophaga* agar and Hsu-Shotts medium. Colonies produced are pigmented yellow with rhizoids or root-like appearance.

Preventive methods:

Maintain good water quality and minimize stress by avoiding overcrowding, low dissolved oxygen and high ammonia levels. The occurrence of the disease could also be prevented by vaccination.

Control methods:

Transferring affected fish into another tank with clean water may control the infection. Affected fish may be treated with oxolinic acid mixed with feed at 20 mg/kg of fish and oxytetracycline at 75 mg/kg of fish/day for 10 days. Acriflavin dip at 100 ppm for 1 minute, and potassium permanganate at 2-4 ppm added to the water and allowed to dissipate over time could also be used to treat diseased fish (see Appendix 1 for points to consider before using antibiotics and a list of anti-infectives recommended for use in marine food fish together with the withdrawal period).

2. Fin Rot

Fin rot with hemorrhages usually affects *Cromileptes altivelis* from the wild in Indonesia. *Flexibacter maritimus* was isolated from the lesions. Secondary *Vibrio* infection may worsen the fish condition if not treated. Fin rot is also observed in fish infected with *Vibrio*.

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 (Figure 2-4a). The lesions progress into fin rot or extensive fin loss (Figure 2-4b). 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 rod-shaped bacteria (0.5 × 2.5 µm) 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 prefuran bath treatment at 1-2 ppm for 24 hours are effective in controlling the disease (see Appendix 1 for points to consider before using antibiotics and a list of anti-infectives recommended for use in marine food fish together with the withdrawal period).

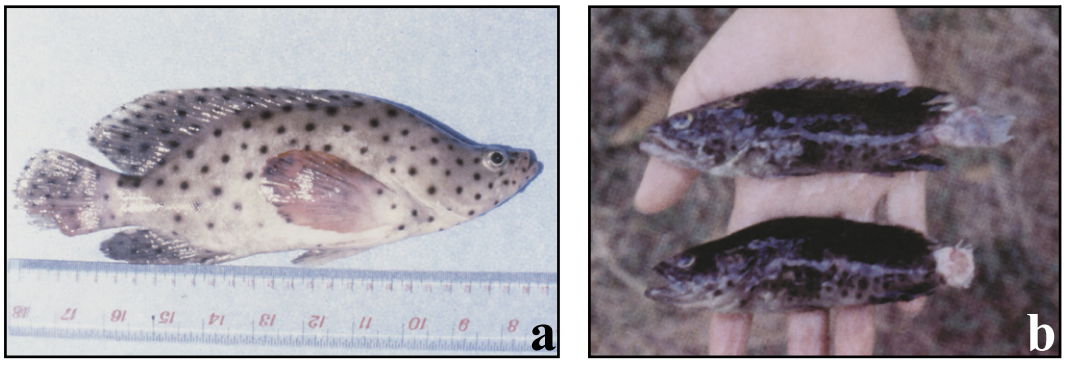

Fig. 2-4. *Cromileptes altivelis* infected with *Flexibacter maritimus*: a) hemorrhagic and eroded caudal and pectoral fins and b) extensive loss of fin (Photos from Koesharyani *et al.*, 2001).

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