3

CHAPTER THREE Bacterial diseases

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Diseases caused by bacteria may cause heavy mortality in both wild and cultured fish and crustaceans. Bacteria are found everywhere in the aquatic environment. Most bacterial disease agents are part of the normal microflora of the marine environment and are generally considered as secondary or opportunistic pathogens. Almost all fish bacterial pathogens are capable of independent existence outside the fish. There are only a few obligatory pathogens. Even these, however, are capable of living for a long time in the tissues of their host without causing injury. Clinical infections and disease usually occur only after the onset of some major changes in the physiology or body of the host. Thus, to understand bacterial diseases of fish, one must understand the relationship of bacteria with their host and with their environment.

As in all animal production systems, bacterial disease is one of the major problems facing production, development and expansion of the aquaculture industry. The control of disease is particularly difficult because fish are often farmed in systems where production is dependent on natural environmental conditions. Changes or deterioration in the aquatic environment cause most of the bacterial diseases encountered, and environmental effects give rise to many other adverse conditions. A second major constraint on disease control is the relatively limited number of therapeutic agents available for the control of bacterial disease agents. Even recommended therapies and preventive measures pose limitations. Their application to aquatic animals is often difficult in actual practice, and sometimes impossible to implement.

Outbreaks of major bacterial diseases in aquaculture can be significantly reduced if proper attention is paid to good husbandry practices and the maintenance of optimum environmental conditions, especially water quality. Another important consideration involves identifying the predisposing factors that may lead to a disease state. Once predisposing factors are identified, appropriate corrective measures should be initiated in the culture system.

Most bacterial disease show similar signs, especially in fishes. Bacterial infection may appear on the skin or fins of fish, exoskeleton or appendages of crustaceans, in the muscles and in the internal organs. In nearly all cases, red spots, brown or black spots, or necrotic tissues can be observed. Inflammation may also occur. Proper identification of the causative agent is important to ensure successful treatment.

WHAT ARE BACTERIA?

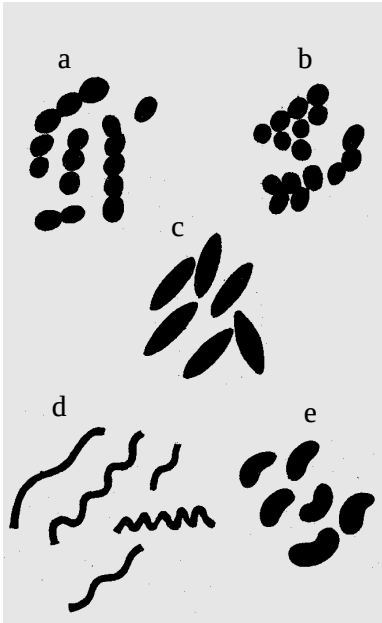

Figure 3-1. The different shapes and arrangements of bacteria: (a) cocci in streptococcal arrangement, (b) cocci in staphylococcal arrangement, (c) bacilli, (d) several kinds of spirilla, and (e) comma-shaped vibrios

Bacteria are not visible to the naked eye. These microorganisms are of very small dimensions, usually between 0.5 and 10 microns (µm). But, when bacteria multiply in great numbers on a solid medium, they form visible colonies representing millions or billions of individual cells. The cells can be seen only under a microscope from a smear stained with a dye on a microscope slide.

Bacteria differ from other cells in that they are prokaryotic (lacking a nuclear membrane). The nucleus occupies the center of the cell. All its genetic material is linked in a single chromosome. The cytoplasm is densely packed with RNA and is finely granular because of the presence of ribosome. The nucleus/ cytoplasm complex is packaged in a complex envelope or integument. Its innermost layer is the thin cytoplasmic membrane (plasmalemma). Outside the membrane is a rigid cell wall. Some bacterial pathogens develop a capsule outside of the cell wall, which is usually associated with the virulence or infective ability of the organism. Many of the pathogenic bacteria are flagellated and a few have no flagella for locomotion. Some move by body flexing or gliding.

Some bacteria produce enzymes called extracellular products or ECP, which are associated with the microorganism's virulence. Extracellular products are highly toxic to fish and crustaceans and they contain proteases, hemolysins, exohemagglutinins and cytotoxins.

Bacteria reproduce asexually by binary fission. That is, they multiply by an elongation of the cell followed by a division.

The most common method used to detect the presence of bacteria is by gram stain. The gram stain classifies bacteria into two groups: the gram positive and the gram negative. Gram-positive bacteria are those that possess a thick peptidoglycan cell wall which will retain the initial crystal violet stain during washing with 95% alcohol. Gram-negative bacteria are those that possess a unimolecular peptidoglycan cell wall bounded on one side by the cytoplasmic membrane and on the other side by the outer membrane; such cells are decolorized by 95% alcohol and take up the secondary stain. To identify bacterium, a pure culture should be obtained, containing a single species and not a mixture of different kinds of bacteria.

In classifying bacteria, one needs to pay attention to the cell shape. There are three distinct cell forms: cocci, baccili and spiral (Fig.3-1). Cocci are spherical cells and exist in several patterns or groupings which are specific to the genus. Paired cocci are called diplococcus, while those in long chains are called streptococcus. Irregularly grouped cocci are called staphylococcus. Longer and cylindrical bacteria are known as bacilli or rods. Cells that are between the coccus and the bacillus in shape are called coccobacilli. The short, curved rods are the vibrios. When more than one curvature is observed, it is called spirilla. Most bacteria that cause disease in fish and crustaceans are rod-shaped. Figure 3-1 shows the different shapes and arrangements of bacteria.

The shape, size and color of a given colony are also important in identification. The bacterial colony surface texture, whether rough, smooth or mucoid, should

also be observed. The same species can form rough or smooth colonies, depending on environmental conditions and the virulence or infective ability of the strain. Smoother colonies are often more virulent or harmful. Considering the large number of bacteria known to exist, gram stain reaction and morphological form are not enough to identify bacterial species. To identify a bacterium, the different physiological or bodily and biochemical properties it possesses must first be known through a series of tests.

IDENTIFYING THE REAL CAUSE OF A DISEASE; KOCH'S POSTULATES

Not all bacteria present in the body of a fish or crustacean are pathogenic or may cause disease. Some bacteria may be harmless or even beneficial. By carefully noting the observations suggested here, one might be able to tell if the isolated bacterium suspected of causing the disease is the causative organism.

Koch, a German physician and bacteriologist, enunciated the following criteria in 1891 to establish unequivocally a causal relationship between an organism and a specific disease.

- The organism should be found in all cases of the disease in question, and its distribution in the body should be in accordance with the lesions observed.
- The organism should be cultivated outside the body of the host, in pure culture, for several generations.
- The organism so isolated should reproduce the disease when introduced into other susceptible animals.
- The organism must be reisolated from the experimentally-infected animal.

Virulence Determinants

To produce disease, microorganisms must be able to:

- Enter the host;
- Multiply under the physical and chemical conditions of the host tissues;
- Interfere with the action of humoral (in body fluids) and cellular defense mechanisms of the host; and
- Damage tissues thereby producing the unpleasant and possibly lethal effects.

Bacteria are ubiquitous. This means that they can be found or are present almost everywhere in the aquatic environment. The actual role of these microorganisms may vary from being beneficial to that of being a secondary opportunistic invader, attacking only when the host is weakened or injured, or a primary pathogen that may cause the death of the species.

In this chapter, the important bacterial diseases of fish and crustaceans are discussed as well as the different diagnostic methods, gross signs and preventive measures and treatments of these diseases.

IMPORTANT BACTERIAL DISEASES OF FISH

Columnaris Disease

CAUSATIVE AGENT:

Flavobacterium columnare (previously named as *Flexibacter columnaris*)

SPECIES AFFECTED:

Ayu *(Plecoglossus altivelis)*, tilapia *(Oreochromis niloticus)*, carp *(Cyprinus* sp.*)*, channel catfish *(Ictalurus punctatus)*, goldfish *(Carassius auratus)*, rohu *(Labeo rohita)*

GROSS SIGNS:

The first indication of infection is generally the appearance of a white spot on some part of the head, gills, fin or body. A zone with a distinct reddish tinge usually surrounds this. The lesions are circular as if spreading from a single focus towards all directions at the same rate. On the gills, the lesions are more necrotic. On the skin, they develop into hemorrhagic ulcers, with an overlying seroma of bacterial cell and necrotic tissue. Histologically, there is epidermal spongiosis, necrosis, and ulcerations.

EFFECTS ON HOST:

F. columnare is an opportunistic pathogen widely distributed in the water. The disease does not usually occur as spontaneous infection but results from injuries to the fish, or physical and nutritional deficiencies. Outbreaks are affected by factors such as temperature and stress. Dissolved cations such as sodium, potassium, calcium and magnesium enhance their infectivity. *F. columnare* attacks fish primarily through the gills or abraded epidermal areas. Once the pathogen is established, proteolytic enzymes break down the skin and muscle to open necrotic lesions. The bacterium appear systematically after extensive tissue necrosis. Gill lesions may cause respiratory difficulty and the fish eventually dies. Body lesions are subject to secondary infection by other microorganisms. Fish that survive the infection may become carriers of the disease.

DIAGNOSIS:

Columnaris disease can be presumptively diagnosed from disease signs on the skin and gills of the host and from squash preparations made from scrapings of the affected areas. In wet mount preparations of infected tissues, the bacteria show a slow gliding movement, gathering into characteristic column-like masses that give the disease its name. Microscopic examination of lesions shows the presence of long, slender, possibly filamentous, rod-shaped, gram-negative bacteria. The growth of *F. columnare* on solid media is usually characterized by yellow-green, flat and rough spreading colonies that adhere to the media. *F. columnare* can also be detected from fish and water using indirect fluorescent antibody technique (IFAT).

PREVENTION AND CONTROL:

- vaccination
- environmental manipulation like lowering water temperature
- addition of competitive bacteria like *Citrobacter fecundii* and *Aeromonas hydrophila*
- copper sulfate dip at 40 mg/L for 20 min or 500 mg/L for 1 min
- oxolinic acid dip at 1 mg/L for 24 h
- sulphamerazine and oxytetracycline at 220 mg/kg/day for 10 days followed by 50 to 75 mg/kg/day for 10 days.

Edwardsiella Septicaemia or Edwardsiellosis

CAUSATIVE AGENT:

Edwardsiella tarda

SPECIES AFFECTED:

Tilapia *(Oreochromis niloticus)*, channel catfish *(Ictalurus punctatus)*, mullet *(Mugil cephalus)*, carp *(Cyprinus carpio)*

GROSS SIGNS:

Edwardsiella tarda infection manifests itself by the presence of small, 3-5 mm cutaneous or skin lesions located dorso-laterally (from along the back to the side) on the body. These lesions progress into abscesses and develop obvious convex swollen areas. The skin loses pigmentation. A foul smelling gas is emitted when the skin is incised. The lesion contains large amounts of necrotized or dead tissue. Internally, there is generalized hyperemia (Fig. 3-2) and enlargement of the liver and kidney. Histologically, the lesion is characterized by focal necrosis, often extending from muscle, haemopoietic tissue and liver parenchyma (Fig. 3-3) to perforate the abdominal wall.

Figure 3-2. *Oreochromis niloticus* with *Edwardsiella tarda* infection showing white nodules on the liver

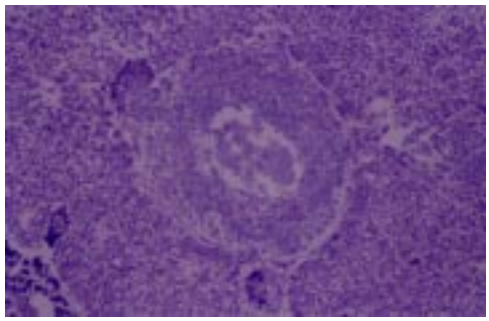

Figure 3-3. Focal necrosis in the liver of *O. niloticus* infected with *Edwardsiella tarda*. (Hematoxylin and Eosin stain, 25X)

EFFECTS ON HOST:

E. tarda infection usually occurs during the warm, summer months. The source of *E. tarda* is presumably the intestinal contents of carrier animals such as snakes, fish (eel and catfish), and some amphibians and reptiles. High temperature, poor water quality and crowding may contribute to the onset and severity of the disease. Affected fish lose mobility of the caudal or tail portion of the body. *E. tarda* infection may cause lesions in the dermis, musculature and visceral organs of the host. Skin lesions when incised emit a foul smelling gas. The lesion contains large amount of necrotized or dead tissue.

DIAGNOSIS:

The bacterium is easily isolated from muscle and internal organs of clinically diseased fish on most general-purpose media such as brain heart infusion agar (BHIA) and tryptic soy agar (TSA). Small punctate colonies develop in 24-48 h on inoculated media.

PREVENTION AND CONTROL:

- Improve water quality.
- Reduce stocking density.
- Apply oxytetracycline at 55 mg/kg fish for 10 days.

Vibriosis

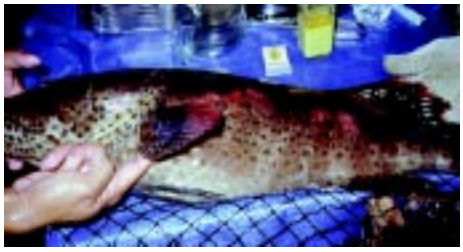

Figure 3-4. *Vibrio* infected grouper with hemorrhagic and necrotic fins. Hemorrhagic lesions on the dorsal body part can also be observed

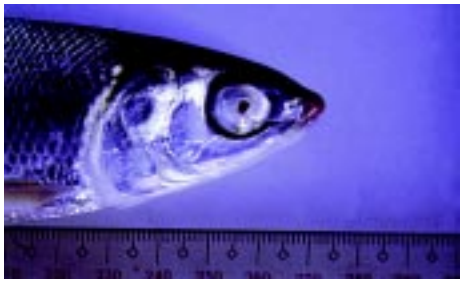

Figure 3-5. Eye opacity in milkfish fingerlings infected with *Vibrio parahaemolyticus*

Figure 3-6 Bilateral exophthalmia in fish with bacterial infection

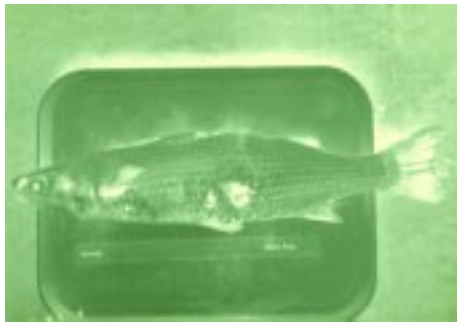

Figure 3-7a. Dermo-muscular lesion in mullet with vibrio infection

CAUSATIVE AGENT:

Vibrio alginolyticus, V. anguillarum and *V. vulnificus*

SPECIES AFFECTED:

Grouper *(Epinephelus* sp.*)*, rabbitfish *(Siganus* sp.*)*, milkfish *(Chanos chanos)*, seabass *(Lates calcarifer)*, sea bream *(Sparus aurata)*

GROSS SIGNS:

The first signs of the disease are usually anorexia or loss of appetite, with darkening either of the whole fish or of particular areas of the dorsum or back. Other common signs of vibriosis are hemorrhagic spot on different parts of the body including necrotic fins (Fig. 3-4), eye opacity (Fig. 3-5) and exophthalmia (Fig. 3-6). The perachute condition results in death without any other clinical signs except occasional periorbital or abdominal oedema. Chronically infected fish generally exhibit very pale gills and large granulating lesions deep in the muscle (Fig. 3-7a; 3-7b). In hatcheries, the presence of red spots in tanks is a sign of *Vibrio* infection.

EFFECTS ON HOSTS:

Vibriosis usually occurs in the warm summer months, especially when the stocking densities are high, and the salinities and organic loads are also high. Stressed fish are more prone to *Vibrio* infection. When an outbreak occurs, mortalities of 50% or higher can be observed in young fish. In older fish, losses may be lower, but infected fish do not feed or grow. When harvested, fish may have large necrotic lesions in the middle of the muscle mass.

DIAGNOSIS:

Squash preparations of kidney, liver, spleen, necrotic muscle tissue and other organs reveal the bacterium. The pathogen can usually be isolated from infected organs in pure culture using standard bacteriological media, such as BHIA, nutrient agar (NA) and TSA, provided they contain 1-2% sodium chloride. Thiosulphate citrate bile salt agar (TCBS) is a medium that selectively promotes growth of pathogenic vibrios while inhibiting other bacteria.

PREVENTION AND CONTROL:

- Maintain good water quality, good husbandry procedures and lower stocking densities.
- Apply oxytetracycline at 77 mg/kg of fish or nitrofurazone at 56 mg/kg of fish for 10 days.
- Vaccinate.

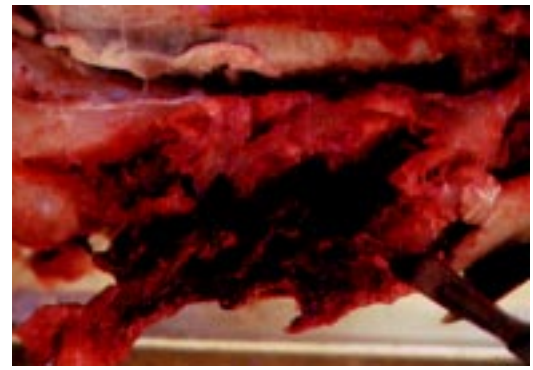

Figure 3-7b. Hyperemia and blood clot in the abdominal cavity of mullet with vibrio infection

Motile Aeromonad Septicemia

CAUSATIVE AGENT:

Aeromonas hydrophila, A. caviae, and *A. sobria.*

Species affected:

Tilapia *(Oreochromis niloticus)*, milkfish *(Chanos chanos)*, goldfish *(Carassius auratus)*, catfish *(Clarias batrachus)*, snakehead *(Ophicephalus striatus)*, goby *(Glossogobius guirus)*, climbing perch *(Anabas testudineus)*, gourami *(Trichogaster* sp.*)*, mullet *(Mugil cephalus)*

GROSS SIGNS:

External signs of motile aeromonad disease vary from darkening in color, enlargement of the abdominal area (Fig. 3-8a) to an extensive superficial reddening of a large area of the body (Fig. 3-9), often with necrosis of fins or tail and extensive ulceration over a considerable portion of the flanks or dorsum. The ulcers are usually shallow and the surface may go brown as it necrotizes or decays (Fig. 3-10). Other disease signs are scale loss, mouth sores,

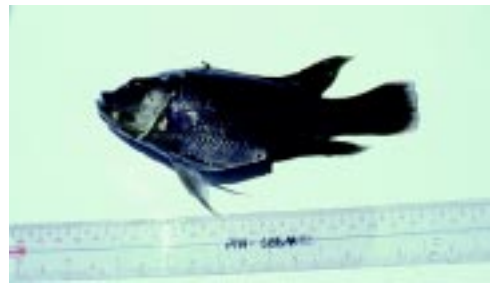

Figure 3-8a. *Oreochromis niloticus* with *Aeromonas hydrophila* infection. Note the enlarged abdominal cavity

Figure 3-8b (far right). The same *O. niloticus* with hyperemia and enlarged abdominal cavity due to *A. hydrophila*

Figure 3-9. Hemorrhagic lesion in milkfish infected with *Aeromonas hydrophila*

Figure 3-10 (far right). Dermo-muscular lesion in catfish experimentally infected with *A. hydrophila* obtained from EUS fish

exophthalmia, and eye opacity. Internally, there may be dropsy (Fig. 3-8b), hyperemia, and the congestion of the internal organs.

EFFECTS ON HOST:

The organisms are usually transmitted through the mouth but may also enter through the skin or gill abrasions. The organisms multiply in the intestine or at the site of invasion and are spread throughout the body by the bloodstream. Internally, there may be ascitic fluid, anemia and damaged internal organs which may lead to mortalities. Mortality as high as 80% may occur among physically stressed, nutritionally deficient, anoxious or injured young fish. Older fishes are less susceptible to motile aeromonads, although 20 to 35% mortalities are not common.

DETECTION AND CULTIVATION:

Squash preparation of the kidney is useful when searching for the etiological agent of the disease. The organisms appear as rod-shaped bacteria, a few are coccoids or short rods in form, usually in single or pairs but rarely in short chains or filaments. They grow well on most common laboratory media such as BHIA, TSA and NA.

PREVENTION AND CONTROL:

- Avoid overcrowding of fish in holding facilities.

Pseudomonad Septicemia or Red Spot Disease

CAUSATIVE AGENT:

Pseudomonas fluorescens, P. anguilliseptica, and *P. chlororaphis.*

SPECIES AFFECTED:

Milkfish *(Chanos chanos)*, goldfish *(Carassius auratus)*, tilapia *(Oreochromis niloticus)*

GROSS SIGNS:

The external disease signs of pseudomonad septicaemia are similar to those caused by other gram-negative bacterial pathogen of fish. The disease causes small hemorrhages in the skin around the mouth and opercula and along the ventral or abdominal surfaces. The body surface may ooze blood and slime in severe cases but there is no reddening of the fins and anus.

EFFECTS ON HOST:

Pseudomonas spp. enters the host either through the oral route or through broken or abraded skin and damaged gills. The organism is carried throughout the fish body by the blood stream. The bacteria and their toxin destroy body tissues, organs and functions. Dysfunctions of the different body organs may lead to mortality of up to 70%.

DIAGNOSIS:

The organisms can usually be isolated from the kidney and other internal organs of affected fish, as well as from the lesion. They grow well on most common laboratory media such as BHIA, TSA and NA. GSP agar or *Pseudomonas-*

Aeromonas Selective Agar is a medium that selectively promotes growth of *Aeromonas* and *Pseudomonas*, and inhibits growth of other bacteria.

PREVENTION AND CONTROL:

- Maintain proper stock management procedures, ensure water quality and reduce stocking density.
- Transfer in a tank and raise temperature to 26-27°C and maintain for about 2 weeks.

Streptococcal Infection

CAUSATIVE AGENT:

Streptococcus sp.

Species affected:

Seabass *(Lates calcarifer)*, tilapia *(Oreochromis niloticus)*, rabbitfish *(Siganus guttatus)*, ayu *(Plecoglossus altivelis)*

GROSS SIGNS:

Clinical signs vary among species of affected fish. However, erratic swimming, darkening of body color, unilateral or bilateral exopthalmia, corneal opacity, hemorrhages on the opercula and the bases of the fins and ulceration of body surface are the most common clinical signs. The hemorrhagic lesions, which gradually extend and ulcerate to release decayed material, are generally raised and have a darkened zone around them. The lesions are more superficial than in furunculosis or vibriosis.

EFFECTS ON HOST:

Infected fish have difficulty ventilating, and lose the ability to orient themselves in the water. The eye becomes opaque and necrotic, conditions that can result to blindness. Fish swim in a spiralling motion. Fish are able to respond to stimuli, but have little control over movements. The spleen and kidney become enlarged. Dysfunction of the damaged internal organs may lead to mortalities.

DIAGNOSIS:

The pathogen grows easily on tryptic soy agar supplemented with 0.5% glucose, brain heart infusion agar, Todd-Hewitt broth agar and horse agar. Colonies develop after 24-48 h of incubation at 20-30°C. The colonies on agar plates appear small (0.5-1mm diameter), yellowish, translucent, rounded and slightly raised.

PREVENTION AND CONTROL:

- Avoid overcrowding, overfeeding and unnecessary handling or transport.
- Remove and slaughter promptly all moribund fish in ponds or net cages at early stage of infection to prevent outbreak or reduce severity of disease.
- Apply erythromycin at 25-50mg/kg body weight of fish for 4-7 days.

Mycobacteriosis or Piscine Tuberculosis

CAUSATIVE AGENT:

Mycobacterium marinum, M. fortuitum and *M. chelonae*

SPECIES AFFECTED:

Siamese fighting fish *(Betta splendens)*

GROSS SIGNS:

Piscine mycobacteriosis is a systemic, chronic, progressive disease presenting various clinical features depending upon species and ecological conditions. Listlessness, anorexia, emaciation, exopthalmia, skin discoloration and external lesions ranging from scale loss to nodules, ulcers and fin necrosis are signs of advancing infection. Gross internal pathology of mycobacteriosis show gray-white lesions of various sizes in most organs and tissues, but the kidney and liver are most often involved.

EFFECTS ON HOST:

Mycobacteriosis is a chronic progressive disease. The disease may take several years to progress from the asymptomatic state to clinical illness. Initially the pigment will fade, and the fish appear sluggish with loss of appetite. Skin ulcers will develop. Fin and tail rot and loss of scale may also be seen. Nodules may form in the muscle and internal organs, which may lead to emaciation or edema or peritonitis. Infection may spread to the skeleton, in which deformities become apparent. Mortalities will then be observed.

DIAGNOSIS:

Primary isolation of fish mycobacteria is best achieved using Ogawa and Lowenstein-Jensen media. Subcultures develop at 28°C within 3-5 days on these media. On Ogawa medium, the cultures appear creamy in the dark but brilliant yellow color when exposed to light. Cultures may not always be obtained even from fish showing unequivocal evidence of infection. Mycobacteria may also be isolated occasionally on general-purpose bacteriological media such as tryptic soy agar, or brain heart infusion agar, provided that a large inoculum is used. All fish mycobacterium have been cultured at 20-30°C for 2 to 30 days. The isolates are strongly acid-fast, rod-shaped, weakly gram-positive, cord forming, non-motile and non-spore forming. Optimum temperature for bacterial growth is between 15°C to 37°C, but the isolates grow best at 28°C.

PREVENTION AND CONTROL:

- Sanitation, disinfection, and destruction of carrier fishes are the primary methods of controlling mycobacteriosis.
- Avoid feeding fish with contaminated fish products. Pasteurize food before use.
- Apply chloramine B or T at 10 mg/l for 24 h.

BACTERIAL DISEASES OF CRUSTACEANS

Bacterial infections of cultured crustaceans occur as: bacterial fouling of surfaces, cuticular or subcuticular localized infections, and internal or systemic infections.

Bacterial Fouling of Surfaces

Filamentous Bacterial Disease

CAUSATIVE AGENT:

Leucothrix sp., *Thiothrix* sp., *Flexibacter* sp., *Cytophaga* sp., *Flavobacterium* sp.

SPECIES AFFECTED:

Penaeus monodon, P. merguiensis, P. indicus

GROSS SIGNS:

Presence of fine, colorless, thread-like growth on the body surface (Fig. 3-11) and gills as seen under a microscope.

EFFECTS ON HOST:

Infected eggs show a thick mat of filaments on the surface, which may interfere with respiration or hatching. In larvae and postlarvae, normal respiration, feeding, locomotion, and molting may be seriously impaired, resulting in slower growth rates, retarded development and eventually death. However, larval shrimps are less prone to infestations due to rapid succession of molts throughout the different larval stages. Frequent molting does not allow adequate time for the bacteria to accumulate on the exoskeleton. In larger shrimps, it may result in respiratory distress. Mortality is due to hypoxia. Disease onset is associated with high organic loads in culture water, low dissolved oxygen levels and added stress from molting. If left untreated in intensive culture systems, accumulative mortality may reach 80% or more within a few days to a few weeks of onset of disease signs.

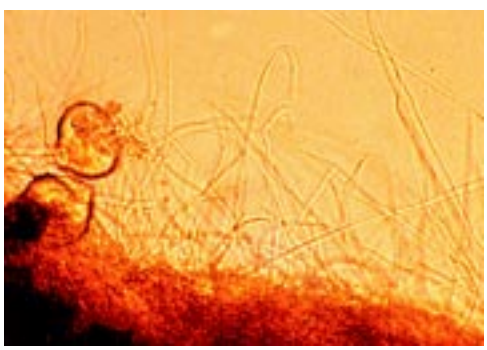

Figure 3-11. Strands of filamentous bacterium *Leucothrix* sp. on heavily infested gills of juvenile *Penaeus monodon*. At left is the protozoan *Zoothamnium* (fresh mount, 200x)

DIAGNOSIS:

Direct microscopic examination of wet mounts of larvae or postlarvae, appendages and gill filaments excised from juvenile or adult shrimp, and of filamentous organisms attached to external surfaces of the cuticle.

PREVENTION AND CONTROL:

- Maintain good water quality with optimum dissolved oxygen and low organic matter levels.
- Apply Cutrine Plus at 0.15 ppm copper in 24 h flowthrough treatments
- Apply 0.5 ppm copper in 4 to 6-h static treatments for PL 2 and older.

Cuticular or Subcuticular Localized Infections

Shell Disease, Brown/Black Spot, Black Rot/Erosion, Blisters, Necrosis of Appendages

CAUSATIVE AGENT:

Shell-degrading bacteria belonging to *Vibrio, Aeromonas, and Pseudomonas* groups

Species affected:

Penaeus monodon, P. merguiensis, P. indicus

GROSS SIGNS:

Figure 3-12a. Shell disease on the carapace of *Penaeus monodon*

Figure 3-12b. Shell disease on the abdominal segment of *Penaeus monodon*

Figure 3-13. *Penaeus monodon* post larvae with necrotic pleopods. Necrotic area appears like cigarette butt

The disease manifests itself as brownish to black, single or multiple, eroded areas on the general body cuticle (Fig. 3-12a, 3-12b), appendages, and gills.

In larval and postlarval stages, the affected appendage shows a cigarette butt-like appearance (Fig. 3-13). Blister containing cyanotic gelatinous fluid may develop on the carapace and abdominal segment. The blister may extend to the underside of the ventro-lateral section of the carapace creating a bulge on the underside.

EFFECTS ON HOST:

Infection usually starts at sites of punctures or injuries caused by the telson or rostrum, in cracks on the abdominal segment from sudden flexure of the shrimp body, or from other damage caused by cannibalism. Another infection site is the cuticle colonized by a large number of bacteria. The bacteria produce extracellular lipases, proteases, and chitinases, which together erode the multiple-layered cuticle, resulting in the development of the disease. The progressive destruction of the cuticle also provides a route of entry for secondary pathogens like fungi or opportunistic bacteria. Such infections may become lethal because of osmotic imbalances, molting problems, secondary fungal infection and a generalized septicemia. The affected shrimp becomes susceptible to cannibalism or dies from stress and energy exhaustion. The disease is associated with trauma to the cuticle (e.g. heavy aeration), conditions that encourage a high number of bacteria in the culture water (e.g. poor hatchery hygiene, high organic loads or contaminated algae) and undefined nutritional and environmental stressors.

DIAGNOSIS:

Diseased penaeids are examined for appearance of multifocal melanized cuticular lesions on the cuticle or the general body surface, the appendages, or the gills. Diagnosis may also be made by bacteriological (isolation, purification and identification) and serological (slide agglutination) methods.

PREVENTION AND CONTROL:

- Maintain good water quality and use nutritionally adequate diets.
- Keep organic load of the water at low levels by removing sediments

which harbor high numbers of bacteria.

- Minimize handling and overcrowding and reduce other forms of stress.
- Avoid injuries to the exoskeleton of the shrimps to prevent the development of primary portals of entry.
- Induce molting, which eliminates the condition, but not when underlying tissues are damaged.

Internal or Systemic Infections

Luminous Bacterial Disease

CAUSATIVE AGENT:

Vibrio harveyi (Fig. 3-14) and *V. splendidus*

SPECIES AFFECTED:

Penaeus monodon, *P. merguiensis*, and *P. indicus*

STAGES AFFECTED:

Eggs, larvae, postlarvae, juveniles and adults

GROSS SIGNS:

Shrimps become weak and opaque-white. Affected shrimps often swim to the pond surface and edges. Heavily infected shrimps in tanks and ponds show a continuous greenish glow when observed in total darkness. When viewed under the microscope, the hemocoel and internal tissues appear densely packed with active bacteria.

EFFECTS ON HOST:

The hepatopancreas is the target organ of infection. Histopathology shows severe inflammation in and around hepatopancreatic tubules of the entire organ. In larger animals, melanized lesions are found in the proximal region of the hepatopancreas. These lesions affect the digestive function of the organ as the necrotic parts become non-functional. Total necrosis and dysfunction lead to death, while partial dysfunction causes slow growth as not all tubules function in digestion, absorption and storage. Systemic infections result in mortality of up to 100%.

Figure 3-14. Agar culture of the luminous bacteria *Vibrio harveyi*. Photo taken in total darkness

DIAGNOSIS:

The disease may be detected by bacteriological (isolation, purification and identification); histological (demonstration of rod-shaped bacteria in tissues of diseased shrimp); and serological [slide agglutination, fluorescent antibody technique (FAT) and enzyme linked immunosorbent assay (ELISA) using specific antibodies] methods.

PREVENTION AND CONTROL:

- Disinfect incoming water and use filtration equipment to prevent entry of luminous bacteria into the hatchery system.
- Use only previously disinfected water during spawning and rearing.
- Wash eggs.

- Siphon out sediments and debris from the tank bottom.
- Disinfect infected stock first before discarding.
- Wash and disinfect hatchery paraphernalia after each larval rearing period.
- Use microbially mature or aged seawater.
- Apply commercially available probiotics to maintain ecological balance within the system.
- Use immunoprophylaxis or vaccination.
- Monitor bacterial population and diversity in the intake and rearing waters of the shrimp pond.
- Apply commercially available probiotics.
- Use low salinity rearing water and reservoirs.
- Practice crop rotation.
- Install greenwater culture system and other system modifications.
- The disease may be prevented by rigorous water management.
- Apply antibiotics and other antibacterial substances only as the last resort.

Non-luminous Vibrios

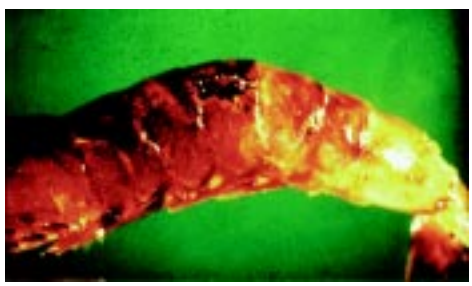

Figure 3-15. *Penaeus monodon* with melanized cuticular lesion on the body surface due to bacterial infection. Whitening of the necrotic muscle at the 6th abdominal segment can also be observed

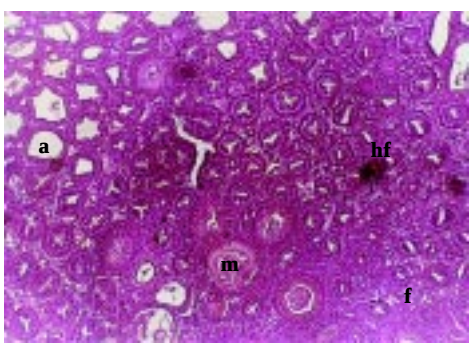

Figure 3-16. Hepatopancreas of *Penaeus monodon* infected with *Vibrio parahaemolyticus*. Note the presence of atrophied tubules (a) and melanized tubules (m). The intertubular spaces are infiltrated with hemocytes (hf) and connective tissues (f). (Hematoxylin and Eosin stain, 100x)

CAUSATIVE AGENT:

Vibrio parahaemolyticus, V. alginolyticus, V. anguillarum, V. vulnificus, V. damsela, V. fluvialis and *V. penaeicida.*

SPECIES AFFECTED:

Penaeus monodon, P. vannamei, P. japonicus

GROSS SIGNS:

Affected shrimp may show erratic or disoriented swimming alternating with periods of lethargy. There is loss of appetite. General signs of severe stress include opaqueness of abdominal muscle (Fig. 3-15), anorexia and expansion of chromatophores.

In larval and early postlarval shrimp, signs include melanization, necrosis of appendage tips and the presence of large numbers of swarming bacteria visible in the hemocoel of moribund or weak shrimp. Due to loss of appetite, fecal strands cannot be observed and gut is empty. Hepatopancreas of affected shrimp showed varying degrees of inflammation, hemocytic infiltration and fibriosis (Fig. 3-16).

EFFECTS ON HOST:

Mortality in some instances is nearly 100% of affected population. Majority of cases of vibriosis is secondary in nature, occurring as a result of other primary conditions, including other infectious diseases, nutritional diseases, extreme stress, wounds, etc.

DIAGNOSIS:

Infection may be detected by bacteriological (isolation, purification and identification), histological (demonstration of rod-shaped bacteria

in tissues of diseased shrimp), and serological (slide agglutination, FAT and ELISA using specific antibodies) methods; and by Polymerase Chain Reaction (PCR).

PREVENTION AND CONTROL:

- Maintain good water quality and use nutritionally adequate diets.
- Minimize handling and overcrowding; reduce effects of other forms of stress.
- Apply commercially available probiotics; use low salinity rearing water and reservoirs; practice crop rotation and install greenwater culture system and other system modifications.
- Perform immunoprophylaxis or vaccination.
- The disease can be treated with rigorous water management.
- Apply antibiotics and antibacterial substances only as a last resort.

SUMMARY

Fish and crustaceans that are not weakened by poor environmental conditions, or by other causes, such as parasitic infestation, nutritional deficiency, handling stress, or chemical intoxication, are more resistant to bacterial infections. This is due to the presence of a large amount of bactericidal substances in the blood, which helps overcome infections. So, the best precaution against the occurrence of bacterial infections is to provide the fish with optimum environmental conditions, adequate amounts of the right kinds of food and avoidance of stress, including overcrowding. Vaccination/ immunization and genetic manipulation (i.e., the development of specific pathogen resistant fry) are also some ways of preventing bacterial diseases. The use of antibiotics should always be an option of the last resort.

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