CHAPTER TWO

Viral diseases

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Outbreaks of viral disease in cultured fish and shrimps have been more frequently reported in the past two decades. Its significance cannot be ignored as their occurrences resulted in heavy mortalities. This chapter aims to provide basic information on viral disease problems involving economically important fishes, such as catfish (*Clarias* spp. and *Ictalurus* spp.) snakeheads (*Ophicephalus striatus*), carp (*Cyprinus* spp.), tilapia, milkfish (*Chanos chanos*), grouper (*Epinephelus* spp.), rabbitfish (*Siganus* spp.), sea bass (*Lates calcarifer*), mullet (*Mugil cephalus*) and penaeid shrimps.

CHARACTERISTICS OF VIRUS

Viruses are ultramicroscopic organisms with size ranges of 10 to 300 nanomicrons (nm). An electron microscope is required to visualize viruses. Because of their size, viruses are able to pass through filters of 0.5 micron pore size.

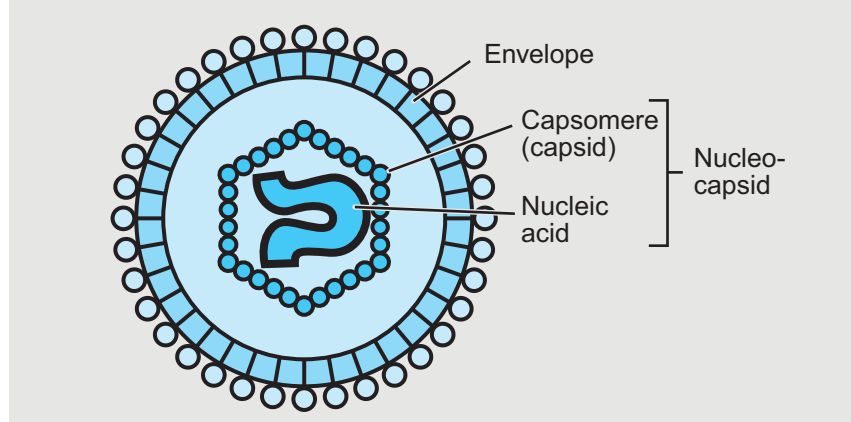

Figure 2-1. Structure of an enveloped virus

The basic structure of a virion consists of a capsid which encloses a nucleic acid genome (Fig. 2-1). The capsid is made up of identical protein subunits called capsomeres while the genome is either a ribonucleic acid (RNA) or a deoxyribonucleic acid (DNA). The combined viral components is called the nucleocapsid. This may have an envelop which is lipid in nature. Viruses that do not have an envelop are considered naked viruses.

Viruses have cubical/polyhedral, helical or complex morphology/symmetry. These microbes are obligately parasitic, multiplying only in its live host. In animal cells, the virus initially attaches on specific cell surface components called receptors. Subsequently, the whole virion penetrates the cell then makes use of the metabolic machinery and pathways of the living cell to make copies of its nucleic acid and synthesize protein subunits. Thereafter, these basic structural components are assembled to package the new viral particles. The new virions are usually released from the cell by lysis.

Cells infected by some virus develop inclusion/occlusion bodies within the infected cell as the replication cycle progresses. These are formed as a result of an accumulation of virions or viral components, although some inclusion bodies may not contain virions. These could be singular, multiple, intracytoplasmic or intranuclear and can be detected by histopathology using a compound microscope.

Aquatic viruses are transmitted from fish/shrimp to other fish/shrimp, from water to fish/shrimp or from reservoir to fish/shrimp by horizontal transmission. Disease transmission can also result from brooder to eggs/fry via vertical transmission. Known reservoirs of viral pathogens are farmed fish/crustacean, imported fish/crustacean, wild fish/crustacean, other aquatic animals/plants and survivors of viral epizooties.

Diagnosis of viral infections can be made by a combination of various methods such as signs of disease, detection of inclusion/occlusion bodies, electron microscopy (EM) or infection enhancement bioassay. Tissue culture techniques using established fish cell lines i.e. BB (Brown Bullhead), BF2 (Bluegill Fin), CCO (Channel Catfish Ovary), CFS (Catfish Spleen), CHSE (Chinook Salmon Embryo), EPC (Epithelioma Papulosum Cyprini), FHM (Fathead Minnow), GCK-84 (Grass Carp Kidney), GCG (Grass Carp Gonad) and GCF (Grass Carp Fin), RTG-2 (Raibow Trout Gonad), SBK-2 (Sea Bass Kidney), SHS (Snakehead Spleen), SSN-1 (Striped Snakehead Fry). To date, however, no continuous cell line from shrimp has been established.

Filtrates prepared from homogenized tissues of virus infected fish when inoculated onto monolayers of susceptible cells will result in lysis/destruction of the cells known as cytopathic effect (CPE) . Serial dilution of the filtrate will provide information on the estimated titer of the virus expressed as tissue culture infection dose (TCID). Alternatively, the focal site of CPE induced by each virion will cause the development of a clear zone called plaque. In such plaque assay, the number of plaques formed indicates the estimated viral particles in a given sample.

Other techniques based on the principle of serology are also applied in the diagnosis of viral infections. The more commonly used tests include neutralization index (NI) determination, Western Blot, Enzyme-linked Immunosorbent Assay (ELISA), Fluorescent Antibody Technique (FAT) and Indirect Fluorescent Antibody Technique (IFAT). Recent molecular biology techniques such as Polymerase Chain Reaction (PCR), Reverse Transciptase-Polymerase chain reaction (RT-PCR), DNA Probe have been developed and is currently widely applied for diagnosis of viral infections in shrimp.

In establishing the pathogenicity of a virus, provisions embodied in the River's Postulates are followed:

- The virus must be present in the host cells, blood, or body fluids showing specific lesions at the time of the disease.
- Filtrates of infectious material, blood or tissue, shown not to contain bacteria or other visible cultivable pathogen in inanimate media, must produce the disease or specific antibody in appropriate animals.
- Similar filtrates from animals or plants must transmit the disease.

It is only when these provisions are complied with that a virus can be considered pathogenic to fish or shrimp.

MAJOR VIRAL INFECTIONS IN FISH

Epizootic Ulcerative Syndrome (EUS)

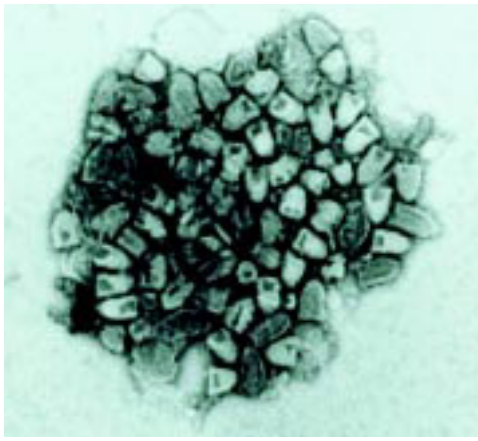

Figure 2-2. Electron micrograph of rhabdovirus isolated from EUS-affected snakehead *(Ophicephalus striatus)*

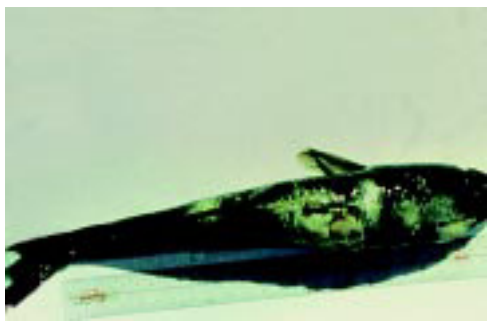

Figure 2-3. Snakehead *(O. striatus)* with EUS lesion

CAUSATIVE AGENT:

This is a complex disease attributed to a combination of rhabdovirus (65x175 nm) (Fig. 2-2), the bacterium *Aeromonas hydrophila*, and the fungus *Aphanomyces invadans*.

SPECIES AFFECTED:

A wide range of cultured and wild fish species are affected namely: snakehead (*Ophicephalus striatus*), catfish (*Clarias* sp.), climbing perch (*Anabas* spp.), spiny eel (*Mastamcembelus armatus*), gourami (*Trichogaster* spp.), barbs (*Puntius* spp.), serpent fish (*Channa micropeltes*), sand goby (*Oxyeleotris marmoratus*), barbs, three-spot gourami (*T. trichopterus*), striped croaking gourami (*Trichopsis vittatus*), siamese fighting fish (*Betta splendens*), wrestling half-beak (*Dermogenus pustillus*), swamp eels (*Fluta alba*). EUS has occurred in Australia, Malaysia, Indonesia, Thailand, Myanmar, Kampuchea, Lao PDR, Philippines, Sri Lanka, Bangladesh, Nepal, Bhutan, Singapore, Vietnam and Pakistan.

GROSS SIGNS:

EUS lesions are characterized by severe, ulcerative, dermal necrosis with extensive erosion/sloughing of the underlying musculature (Fig. 2-3). The necrotic muscular tissue emits a foul odor. Fish manifest frank ulcers consisting of eroded dermal layer, exposing the underlying musculature, which may be hemorrhagic. Fish with less severe lesions exhibit scale loss with erosion of the skin surface with or without hemorrhagic signs.

EFFECT ON HOST:

The histopathological profile of the disease consists of a chronic, necrotic, and mycotic granulomatous inflammatory response.

Outbreaks are observed annually between November to February

when the water temperature is at its lowest. EUS is transmitted by cohabitation with diseased fish or exposure to contaminated waters. The virus replicates at 15 ~ 25°C in 2-3 days to 10^7 $TCID_{50}$/ml in SHS cells and in SSN-1 cells. The virus experimentally induced dermal lesions and mass mortalities of snakehead fry and fingerlings.

Diagnosis:

Signs of disease and isolation/identification of the associated virus, *Aeromonas hydrophila/Aphanomyces* sp., histopathology and electron microscopy.

Channel Catfish Virus Disease (CCVD)

Causative agent:

Herpesvirus ictaluri (90 to 100 nm)

Species affected:

Channel catfish (*Ictalurus punctatus*)

Gross signs:

Acute infection of cultured channel catfish fry and fingerlings less than 10 cm long, juveniles and adults occurs following waterborne exposure to CCV. Clinical signs are abdominal distension (Fig. 2-4), exophthalmia, pale or hemorrhagic gills, petecchial hemorrhage at the base of the fins and throughout the skin. In 20 to 50% of the epizootics, affected fish swim in head-high or hanging position at the surface.

Effect on host:

Severe mortality of sometimes nearly 100% occur among young-of-the-year *Ictalurus punctatus* at water temperatures of 25°C or higher within 7 to 10 days. It causes moderate mortalities at 21 to 24°C and almost no mortalities below 18°C. Secondary invasion of external lesions *by Flavobacterium columnares*, *Aeromonas hydrophila* or aquatic phycomycetes may develop. CCVD develops into a hemorrhagic viremia after initially replicating in the kidney and then in the spleen. Thereafter, the virus is transported to the intestine, liver, heart, and brain. Necrosis of the renal hematopoietic tissue and tubules; edema, necrosis and congestion of the liver; intestinal edema; congestion and hemorrhage in the spleen are characteristic histopathological findings. Skeletal muscle hemorrhage among experimentally infected fish has been observed.

Figure 2-4. Channel catfish (*Ictalurus punctatus*) infected with channel catfish virus (CCV). Note the swollen abdomen

Survivors of experimental CCVD averaged only two-thirds the length and one-seventh the weight of control fish 6 months after a standardized feeding regime. The portal of entry for CCV from water is through the gills and the gut. The virus can be isolated from the kidney of fish with active infections during an epizootic using CCO or BB cells, which develop CPE 24 to 48 h post exposure. Optimum replication was observed at 25 to 30°C. The virus is readily transmitted from fish to fish during an epizootic. In natural and experimental infections, exposed fry die within 3 days of exposure and within 7 to 10 days after the first mortality. The virus also persists in apparently healthy adult broodfish.

Diagnosis:

Electron microscopy (EM), serum neutralization tests, indirect fluorescent antibody technique, nested polymerase chain reaction (PCR) and by a channel catfish virus probe.

Grass Carp Hemorrhagic Disease

Causative agent:

Aquareovirus (60 to 80 nm)

Species affected:

Grass carp (*Ctenopharyngodon idellus*), black carp (*Mylopharyngodon piceus*), topmouth gudgeon (*Pseudorasbora parva*), silver carp (*Hypophthalmichthys molitrix)*, Chinese minnow (*Hemiculter bleekeri*) and rare minnow (*Gobiocypris rarus*)

Gross signs:

Clinical signs include exophthalmia, hemorrhagic or pale gills and hemorrhagic fin bases or gill covers.

Effect on host:

This disease was first observed in China more than 20 years ago. Outbreaks occured in Southern China during the summer at temperatures of 24-30°C. Acute infections cause significant mortalities of up to 80% among fingerlings and sometimes among yearlings. Internally, hemorrhages occur in the musculature, oral cavity, intestinal tract, liver, spleen and kidneys. Naturally and experimentally infected fish manifest reduced erythrocytes, plasma protein, calcium and urea nitrogen. Serum potassium elevated. Signs of disease and mortality are observed within 1 to 2 weeks of exposure of fish in water at temperatures of 25°C or higher. Experimental vaccination induced 80% level of immunity by day 4 at temperatures above 20°C.

Diagnosis:

The virus can be propagated in cell cultures of GCK-84, GCG and GCF yielding titers as high as 10^8 to 10^9 $TCID_{50}$ per ml. In vitro replication is considered optimum between 28 and 30°C inducing CPE in 3 to 4 days post inoculation. Reverse transcription-polymerase chain reaction (RT-PCR) and electron microscopy are also used for detecting the virus.

Spinning Tilapia (ST) Syndrome

Causative agent:

Iridovirus (110-140 nm)

Species affected:

Oreochromis mossambicus, O. aureus, O. niloticus, and *Sarotherodon galilaeus*

Gross signs:

Affected tilapia fry and fingerlings swim in a spiral pattern, sink to the bottom then rise and hang at a 45 degrees angle just under the water surface, gasping for air. They do not feed and are darker in color.

Effect on host:

Tilapia manifesting the spinning syndrome die within 24 h of onset. Up to 100% mortality in affected population of Tilapia fry has been reported. Histopathologically, the renal tubules are shrunken, hemorrhaging and infiltrated with eosinophilic granular cells. In addition, focal myolysis develops in the muscles.

Diagnosis:

The disease is detected by signs of the disease, EM, isolation/identification of the virus.

Viral Nervous Necrosis (VNN)

Causative agent:

Nodavirus (20-25 nm)

Species affected:

Grouper (*Epinephelus* spp.), sea bass (*Lates* spp.) (Fig. 2-5), barfin flounder *(Verasper moseri)*, European bass *(Dicentrarchus labrax)*, parrotfish *(Oplegnathus fasciatus)*, striped jack *(Pseudocaranx dentex)*, turbot *(Scophthalmus maximus)*, Japanese flounder *(Paralicthys olivaceus)*, barfin flounder *(Verasper moseri)*, red sea bream *(Pagrus major)*, sea bream *(Sparus aurata)*, shi drum *(Umbrina cirrosa)*, cod *(Gadus macrocephalus)*, Atlantic halibut *(Hippoglossus hippoglossus)*, purplish amberjack *(Seriola dumerili)*, and tiger puff *(Takifugu rubripes)* in Thailand, Japan, Taiwan, Singapore, Tahiti, Greece, Australia and Europe.

Gross signs:

Affected larvae and juveniles show lethargy, pale color, loss of appetite, thinness, loss of equilibrium and corkscrew swimming. Some fish sink to the bottom then float to the surface again.

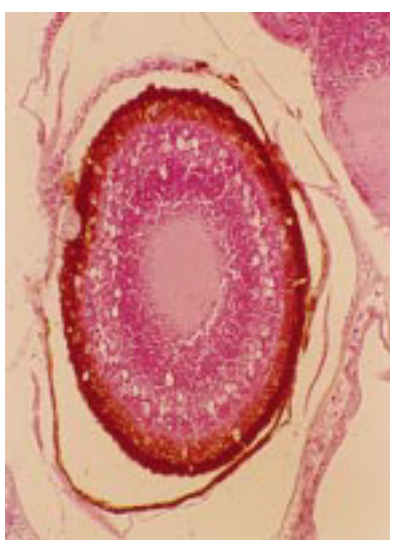

Figure 2-5. Seabass larva: eye with vacuolation (Hematoxylin and Eosin, 40x)

Effect on host:

This viral infection is also known as Paralytic Syndrome, Viral Encephalopathy and Retinopathy, Spinning Grouper Disease, Piscine Neuropathy or Fish Encephalitis. The disease is more severe in less than 20 days old larvae. Diseased fish had pale livers, empty digestive tracts, the intestines filled with greenish to brownish fluid and the spleens are red-spotted. The virus replicates in the eye, the brain, and the distal spinal cord of affected fish causing vacuolating encephalopathy and retinopathy. It also multiplies in the gonad, liver, kidney, stomach and intestine. This disease caused 50-95% mortalities among fish larvae and juveniles at 26-30°C in Taiwan and Thailand. It can be transmitted from diseased to healthy fish within 4 days of contact. The virus is more virulent at 28°C than at 16°C. Fish broodstocks can be virus reservoirs.

Diagnosis:

Histopathology showing vacuolations in the nerve cells of the eye retina and the brain of affected fish is diagnostic for this disease. The virus can be isolated in SSN-1 and barramundi cell lines on which it induces CPE. The virus can be identified by EM, PCR, RT-PCR, ELISA, FAT and by *in-situ* hybridization assay.

Lymphocystis Disease

CAUSATIVE AGENT:

Iridovirus (130-330 nm)

SPECIES AFFECTED:

Lates sp. and *Siganus* sp.

GROSS SIGNS:

Infected fish have clusters of pear-like nodules up to 5 mm in diameter that develop on the skin, gills or fins resulting from an enlargement of tissue cells.

EFFECT ON HOST:

The disease is rarely fatal in older fish. It can be transmitted by cohabitation and exposure to contaminated water.

DIAGNOSIS:

Signs of the disease, histopathology and EM

Grouper Iridovirus of Taiwan Disease (TGIV)

CAUSATIVE AGENT:

Iridovirus (220-240 nm)

SPECIES AFFECTED:

Grouper, *Epinephelus* sp.

GROSS SIGNS:

Diseased fish swim in circles and are anemic. Fish lose appetite then become underweight and lethargic. The spleen of affected fish has abnormal hypertrophied cells containing numerous icosahedral virions.

EFFECT ON HOST:

This virus has antigenic similarities with the red seabream iridovirus isolated in Japan, the epizootic, haematopoietic necrosis virus and the iridovirus isolated from sheatfish and the grouper iridovirus isolated in Thailand.

The disease affects farmed groupers, 5-8 cm in total length at 25-28°C. Acute disease causes up to 60% mortality. Experimentally infected fish reach a cumulative mortality of 100% in 11 days without other clinical signs.

DIAGNOSIS:

Signs of the disease; EM

Sleepy Grouper Disease (SGD)

CAUSATIVE AGENT:

Iridovirus (130-160 nm)

SPECIES AFFECTED:

Epinephelus tauvina

GROSS SIGNS:

Affected fish exhibited extreme lethargy and low appetite. Affected fish swim alone or hung at the water surface or remain at the bottom.

EFFECT ON HOST:

It was first reported among farmed groupers, 100-200 g and 2-4 kg in size, in 10 of 33 farms in Singapore and Malaysia. Acute disease causes up to 50% mortality mostly occurring during the night or in the early hours of the morning. Gradual mortalities follow after fish become sluggish over 3-5 days, after which affected fish lie at the net or tank bottom exhibiting weak fin movements. Some terminally ill fish display gill pallor, rapid opercular movements and frantic dashing to the water surface to gulp air. Acute mass mortalities may occur 12-24 h after handling or excessive feeding. Internal pathology consists of enlargement of the spleen or occasional enlargement of the anterior kidney and heart inflammation. The virus was detected in the spleen, heart and kidney of infected fish. Experimentally exposed fish develop signs of SGD and die in 3-4 days. The virus was introduced into a farm with some imported fish and subsequently spread to neighboring farms.

MAJOR VIRAL INFECTIONS IN PENAEID SHRIMPS

White Spot Syndrome Virus (WSSV) Disease

CAUSATIVE AGENT:

Baculovirus (100-140 x 270-420 nm)

SPECIES AFFECTED:

All stages of shrimps like *Penaeus monodon, P. chinensis, P. indicus, P. penicillatus, P. japonicus, Metapenaeus ensis, P. aztecus, P. duorarum, P. merguiensis, P. semisulcatus, P. stylirostris, P. vannamei, P. curvirostris, P. setiferus,* and also other crustaceans such as *Scylla serrata, Charybdis feriatus, Helice tridens, Calappa lophos, Portunus pelagicus, P. sanguinolentus, Acetes* sp., *Palaemon* sp., *Exopalaemon orientalis, Panulirus sp. Macrobrachium rosenbergii, Procambarus clarkii, Orconectes punctimanus, Artemia*

GROSS SIGNS:

Figure 2-6. *Penaeus monodon* affected with the white spot syndrome virus (WSSV)

Typical signs of disease is the presence of distinct white cuticular spots (Fig. 2-6) (0.5-3 mm in diameter) most apparent at the exoskeleton and epidermis of diseased shrimp about 2 days after onset. The white spots start at the carapace and 5th and 6th abdominal segments that later affect the entire body shell. The moribund shrimp display red discoloration and have loose cuticle. Affected shrimps manifest surface swimming and gathering at pond dikes with broken antennae.

EFFECTS ON HOST:

This disease has been reported with the following names: White spot baculovirus (WSBV), White spot virus (WSV), Systemic ectodermal and mesodermal baculo-like virus (SEMBV), Chinese baculovirus (CBV), Hypodermal and hematopoietic necrosis baculo-like virus (HHNBV), Rod-shaped virus of *Penaeus japonicus* (RV-PJ), Penaeid acute viremia (PAV), Penaeid rod-shaped Dovavirus (PRDV). Reduc-

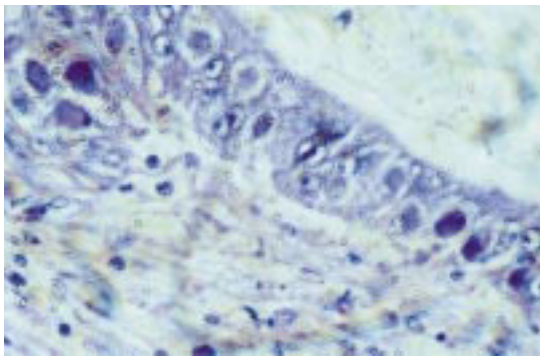

Figure 2-7. Histological section of the stomach of a juvenile *P. monodon* with WSSV intranuclear inclusion bodies (Hematoxylin and Eosin stain, 400x)

tion in food consumption and empty gut develops followed by a rapid onset of the disease and high mortalities of up to 100% in 3 to 10 days. This disease affects a wide host range of crustaceans and targets various tissues (pleopods, gills, hemolymph, stomach, abdominal muscle, gonads, midgut, heart, periopods, lymphoid organ, integument, nervous tissue and the hepatopancreas) resulting in massive systemic pathology. Shrimps, 4-15 g, are particularly susceptible but the disease may occur from mysis to broodstock. Pre-moulting shrimps are usually affected. *Penaeus indicus* suffers earlier and greater losses compared to *P. monodon*. Crabs, krill and other shrimps are viral reservoirs. Pandemic epizootics have occurred in extensive, semi-intensive and intensive culture systems regardless of water quality and salinities.

DIAGNOSIS:

Clinical signs are diagnostic for this disease. However, recent reports indicate that some bacteria may induce similar signs, hence confirmation with other diagnostic tests should be done. Demonstration of the presence of hypertrophied nuclei in stained squashes, smears of epithelial and connective tissues of the gills or stomach of affected shrimp. Histological sections show widespread cellular degeneration and severe nuclear hypertrophy, chromatin margination and eosinophilic intranuclear inclusions in the subcuticular epithelium of the shell, gill, stomach, connective tissues, hematopoietic tissues, lymphoid organ, antennal gland and nervous tissues (Fig. 2-7). Electron microscopy, PCR, DNA probe, Western Blot, and infection bioassay are confirmatony diagnostic tests.

Yellow Head Virus (YHV) Disease

CAUSATIVE AGENT:

Rhabdovirus (40-50 x 150-170 nm)

SPECIES AFFECTED:

Subadults and broodstock of *P. monodon, P. aztecus, P. duorarum, P. merguiensis, P. setiferus, Palaemon styliferus, Acetes* spp.

GROSS SIGNS:

Infected shrimps show light yellowish, swollen cephalothorax. The gills appear whitish, yellowish or brown.

EFFECT ON HOST:

Before the appearance of clinical signs of disease, the shrimps develop an abnormally high feed intake and rapid growth. Thereafter, there is marked reduction in food consumption prior to cessation of feeding and the onset of rapidly accelerating mortality. Moribund shrimps swim slowly near the surface at the edge of the pond. Acute epizooties occur in juvenile to sub-adult shrimps about 20 days post stocking especially during the 50-70 days grow-out culture period. The occurrence of this disease may be associated with unstable phytoplankton bloom, bad pond bottom, high stocking density or exposure to pesticides.

Systemic infection is associated with virus assembled in the cytoplasm of ectodermal and mesodermal cells (gills, lymphoid organ, hemocytes and connec-

tive tissues). Massive necrosis is attributed to cytoplasmic replication of the virus. The virus can cause a total crop loss within 3 to 5 days of onset of clinical signs with incubation period of 7-10 days. The virus in water remain infective up to 72 h. Shrimp reservoirs include *Palaemon styliferus*. About 4% broodstock are infected. In the Philippines, a recent sampling of 250 shrimps reported positive for YHV in 16% of specimens.

Diagnosis:

Signs of disease and phase contrast microscopy of fresh hemolymph stained with Wright/Giemsa stain. Histopathological analyses show the presence of basophilic usually spherical, perinuclear cytoplasmic inclusions in the hemocytes, lymphoid organ, hematopoietic tissues, pillar and epithelial cells in the gills, spongy connective tissue cells in the subcutis, muscle, gut, antennal gland, gonads, nerve tracts, ganglia and other cells of ectodermal and mesodermal origin. Electron microscopy, Western blot, RT-PCR, and infection bioassay are confirmatory diagnostic tests.

Monodon Baculovirus (MBV) Disease

Causative agent:

P. monodon-type baculovirus (75 x 300 nm)

Species affected:

The giant tiger prawn *Penaeus monodon*, and other penaeid shrimps like *P. merguiensis, P. vannamei, P. esculentus, P. semisulcatus, P. penicillatus, P. plebejus, P. kerathurus*.

Gross signs:

Affected shrimps exhibit pale-bluish-gray to dark blue-black coloration, sluggish and inactive swimming movements, loss of appetite and retarded growth. An increased growth of benthic diatoms and filamentous bacteria may cause fouling on the exoskeleton/gills. Infected pond-reared shrimps at 45 days of culture (DOC) stocked at 4 to 100 per m^2 manifested slow growth rates and pale yellow to reddish brown hepatopancreas.

Eeffect on host:

This is among the first viral infections diagnosed in mysis, postlarvae, juveniles and adults of the giant tiger prawn, *Penaeus monodon*. The virus causes destruction of the hepatopancreas and lining of the digestive tract. Spherical, eosinophilic occlusion bodies fill up enlarged nuclei of hepatopancreatic cells and are discharged into the lumen after cells have been destroyed. This may be followed by necrosis with secondary bacterial infection. PL-3 is the earliest stage found infected with MBV. However, experimental waterborne inoculation of MBV to mysis-2 (M-2), postlarvae-3 (PL-3), PL-6, PL-9 and PL-11 resulted in MBV infections within 12 days post-inoculation. The incidence rate of MBV was reported at 20-100%. Cumulative mortality of 70% was observed among *P. monodon* juveniles cultured in raceways and tanks. It is associated with a high incidence of bacterial infections expressed as localized "shell disease." In addition, significant mortalities can occur during stress and crowding.

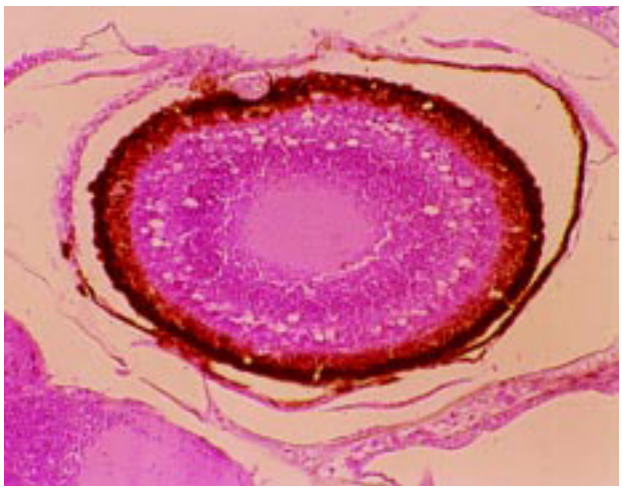

Figure 2-8. Squash preparation of the *Penaeus monodon* hepatopancreas showing normal cells and occlusion bodies of Monodon Baculovirus (MBV) (arrow) (Malachite green stain, 400x)

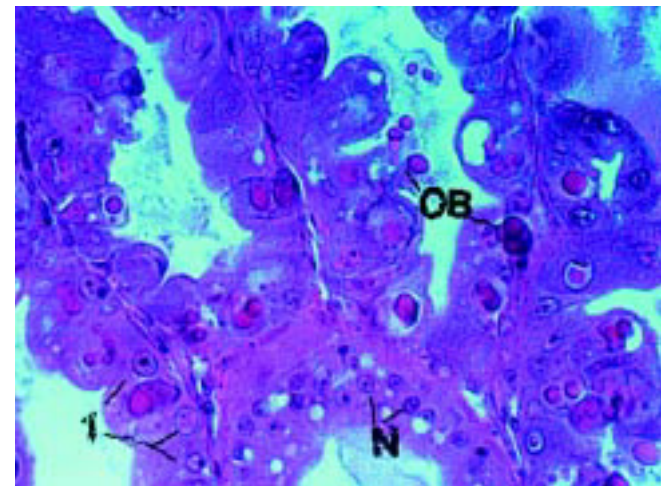

Figure 2-9. Histological section of the *Penaeus monodon* hepatopancreas with occlusion bodies (OB) of Monodon Baculovirus (MBV) (Hematoxylin and Eosin, 400x)

DIAGNOSIS:

Demonstration of occlusion bodies in wet mounts of feces, midgut or hepatopancreas stained with malachite green (Fig. 2-8). Histological sections show the presence of eosinophilic, multiple occlusion bodies within the hypertrophied nuclei of the hepatopancreatic tissues with the following development (Fig. 2-9). Other diagnostic tests are DNA probe and PCR.

Stages of Cytopathology

Stage 0 Cell infected by MBV but cytopathic changes not yet apparent

Stage 1 Slight hypertrophy of the nucleus, chromatin margination, peripheral migration of nucleolus. Viral replication is initiated.

Stage 2 Increased nuclear hypertrophy, proliferation of the virus and development of eosinophilic occlusion bodies.

Stage 3 Hypertrophied nucleus up to twice the normal diameter and six times the normal volume. One or more occlusion bodies and abundant virions are present

Infectious Hypodermal and Hematopoietic Virus (IHHNV) Disease

CAUSATIVE AGENT:

Parvovirus (20-22 nm)

SPECIES AFFECTED:

Postlarvae, juveniles and adults of *P. monodon, P. stylirostris, P. vannamei, P. semisulcatus, P. schmitti, P. setiferus, P. aztecus, P. duorarum, P. californiensis* and *P. japonicus*.

GROSS SIGNS:

Shrimps show erratic swimming behavior, rising slowly to the water surface, hanging and rolling over until the ventral side is up. Eventually, the animal

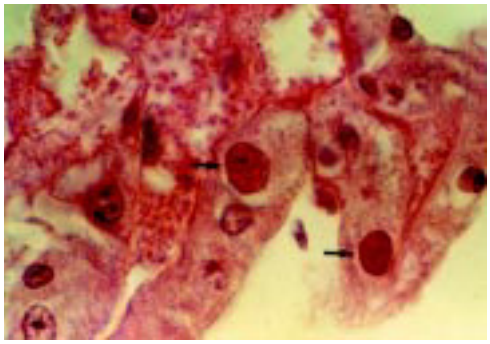

Figure 2-10. Histological section of *P. monodon* and antennal gland showing intranuclear inclusion bodies of IHHNV (arrows) (Hematoxylin and Eosin, 1000x)

sinks to the bottom. Shrimps would eventually right themselves up, become weak and lose their appetite for food. They repeat the process of rising to the surface and sinking until they die usually within 4-12 h. The shrimps manifests decreased preening and delayed molting. Acutely affected shrimps develop white opaque abdominal muscles, bluish to distinctly blue cuticular color often with mottled buff to tan pigment patches in the cuticular hypodermis and very soft cuticles.

Effect on host:

They have poor resistance to stress. Mortality rates of above 90% were observed among penaeid juveniles in intensive culture systems. The virus infects cells of the ectodermal tissues (epidermis, hypodermal epithelium of foregut and hindgut, nerve cord and nerve ganglia) and mesodermal tissues (hematopoietic organs, antennal gland, connective and striated muscles, heart, gonad, mandibular organ, hemocytes). It induces the development of eosinophilic inclusion bodies in the cytoplasm of affected cells during the early acute stage of the disease, followed by necrosis and inflammation of target tissues. The presence of the virus can cause death of the cells of the cuticle, blood-forming tissues and connective tissues of the shrimp that leads to abnormal metabolism and eventually mortalities. Inclusion bodies are common early in acute infections, later decreasing in number followed by necrosis and inflammation of target tissues.

The disease can be experimentally induced in *P. setiferus, M. japonicus, P. aztecus* and *P. duorarum*. It has been linked to the Runt deformity syndrome in *L. vannamei*. Secondary bacterial infection may occur. Some survivors of epizootics may carry the virus for life.

Diagnosis:

Histological demonstration of eosinophilic intranuclear inclusion bodies (Fig. 2-10) in the hepatopancreas by H & E staining. Electron microscopy, PCR, DNA probe, infection bioassay are other diagnostic tests.

Hepatopancreatic Parvo-like Virus (HPV) Disease

Causative agent:

Parvovirus (22-24 nm)

Species affected:

Postlarvae, juveniles and adults of *P. monodon, P. merguiensis, P. vannamei, P. esculentus, P. semisulcatus, P. penicillatus, P. indicus, P. chinensis*.

Gross signs:

Affected shrimps develop loss of appetite and retarded growth. Benthic diatoms, protozoans such as *Zoothamnium* sp., and filamentous bacteria may cause fouling on the exoskeleton of infected shrimps. Occasionally, white opaque areas on the tail/abdominal muscles are observed.

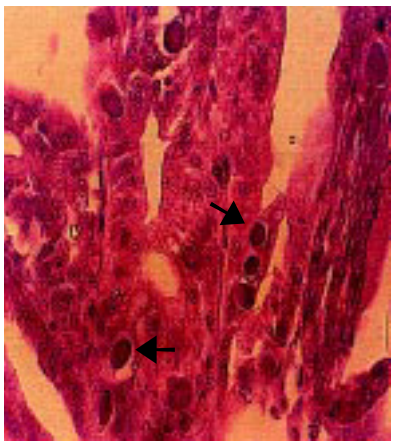

Figure 2-11. Histological section of the hepatopancreas of *Penaeus monodon* infected with the Hepatopancreatic Parvo-like virus (HPV). Arrows point to occlusion body (Hematoxylin and Eosin, 400x)

EFFECT ON HOST:

Postlarvae (PL-1–PL-19) from three hatcheries in Iloilo showed prevalence rates of 7.8 to 26.4%. Mortalities among *P. merguiensis* may reach as high 50% within 4-8 weeks of disease onset. The virus cause hypertrophy of the hepatopancreatic nucleus with lateral displacement and compression of the nucleolus and chromatin margination with development of a prominent basophilic occlusion body. This leads to cell death and shrinkage of the hepatopancreas. Damage of this organ can result in abnormal metabolism and eventually death.

DIAGNOSIS:

Signs of disease and histological demonstration of single prominent basophilic (H&E stain) intranuclear occlusion body in the hypertrophied nucleus of the hepatopancreatic cell (Fig. 2-11) are diagnostic. Electron microscopy, DNA Probe, PCR, infection bioassay are confirmatory tests.

PREVENTION OF VIRAL INFECTIONS

There are no treatments for viral infections in fish or shrimps. Hence, preventive measures must be adapted to keep the viral pathogens away. The basic consideration in preventing the occurrence of viral diseases is avoidance, and the use of virus-free fry for stocking in ponds is highly recommended. It should also be borne in mind that semi-intensive and intensive culture systems promote conditions conducive for disease development. As such, reduction of stress and the application of good husbandry or efficient technology may deter the occurrence of disease. Details on the general methods of disease prevention are discussed in Chapters 9 and 10.

In addition, specific precautions of egg washing with ozone-disinfected seawater; using fine screens for inlet water and adherence to strict hygiene; stress test of shrimp postlarvae (PLs) with 100 ppm formalin for 30 min with aeration and stocking only tolerant PLs; the use of only dry commercial feeds and pasteurized fresh feed at 60°C for 15 min before use; and feeding with feeds containing 100 ppm phosphated ascorbic acid (MAP) for 92 days were reported effective for viral infections. For WSSV management, the effective use of immunostimulants mixed with feed such as peptidoglycan (PG) at 0.2 mg/kg body weight/day for 2-3 months or Fucoidan, an extract from *Cladosiphon okamurans* at 50-100 mg/kg shrimp/day for 15 days, were reported to increase survival of WSSV-exposed shrimps.

It is also important that surveillance for early signs of disease and stressful factors become essential components of farm/hatchery management in order to monitor the health status of fish stocks, to assess adequacy of rearing procedures and to prevent introduction of pathogens. Early detection of a disease

outbreak will reduce mortalities and prevent a catastrophic spread of the virus. Finally, since virus can remain viable outside the living host for at least 72 hr, water change should be contemplated only at least 5 days after effluents from infected ponds in the area have been discharged.

SUMMARY

Outbreaks of viral infections can cause massive mortalities among cultured fishes or shrimps. Water temperature and age of the fish or shrimps are significant factors that influence the development of viral infections. Most fish viral infections occur at low water temperatures, hence, very few viral infections among fishes in warm water culture systems are reported. In addition, most viral infections occur among fry or fingerlings often causing severe mortalities, while older fish or shrimp develop resistance or are hardly affected. Stress from handling, poor water quality, high stocking density and poor nutrition also affect the severity of viral infections.

Finally, aquaculturists should beware in importing non-indigenous fish or shrimps into the country as these are potential carriers of viral pathogens.

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