

II. 2. HISTOPATHOLOGICAL STUDIES ON SHRIMP DISEASES

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A survey on the incidence of shrimp diseases was conducted in Tamilnadu and also at Nellore in Andhra Pradesh which included female brooder stock specimens collected from the Bay of Bengal near Chennai during the period December 1994 to February 1998. The objective of this study is to obtain information regarding prevalence of the diseases among shrimp in these regions.

MATERIALS AND METHODS

Shrimp tissue samples from both infected and apparently healthy animals were procured from farms culturing *Penaeus monodon* located on the east coast of Tamilnadu and Andhra Pradesh and wild-caught *P. monodon* and *P. indicus* from the Bay of Bengal. The samples were collected alive and transported over wet ice or fixed in Davidson's fixative (Bell and Lightner, 1988). Squash preparations and impression smears of hepatopancreas were also collected and stained with Giemsa stain. Tissue samples were processed for histopathological examination as per Bell and Lightner (1988). The tissue sections were stained with Meyer's Bennet haematoxylin and eosin-phloxine (Bell and Lightner, 1988), methyl green pyronin (Pappenheim, 1899 from Bancroft and Stevens, 1982), Feulgen's technique (Pearse, 1951), Lendrum's phloxine-tartrazine technique for inclusion bodies (Lendrum, 1947), Machiavello's stain for rickettsial inclusions (Culling 1974, from Bancroft and Stevens, 1982) and Gram's staining technique for bacteria (Lillie, 1965 from Bancroft and Stevens, 1982). Isolation and differentiation of *Vibrio sp.* of organisms were carried out as per Andrews (1992). Small pieces of these samples were also fixed in 3% glutaraldehyde, fixed in 1% osmium tetra oxide dehydrated and embedded. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under transmission electron microscope. The extraction of viral DNA from white spot disease affected frozen shrimps was carried out as described by Takahashi *et al.* (1996) with modifications. Japanese RV-PJ virus-specific oligonucleotide primers were used to amplify SEMBV specific DNA fragments in white spot disease affected shrimp. The shrimprobe^R in-situ hybridization diagnostic kit of diagnostics Inc. (USA) was also employed for diagnosing SEMBV. The diseases were identified and classified employing several different criteria including clinical signs, gross lesions, characteristic histopathological features and agent identification whenever necessary.

RESULTS

The diseases identified are dealt under viral diseases, bacterial diseases, protozoan diseases, diseases of multiple etiology and other miscellaneous lesions.

VIRAL DISEASES

Monodon baculo virus (MBV)

The MBV infection was recorded in 31 of the 48 submissions from Tamilnadu, one of six submissions from Nellore, Andhra Pradesh and three of 5 broodstock samples from Bay of Bengal.

Clinical signs and gross lesions

- ❖ Shrimps were apparently normal.
- ❖ Reduced feeding, stunted growth, suppressed preening activity
- ❖ Mild bluish discoloration of the body and epicommensal infestation with 10% mortality was also observed.

Histopathology

- ❖ Single to multiple spherical eosinophilic occlusion bodies were seen within the hypertrophied nuclei of the cells of hepatopancreas.
- ❖ The occlusion bodies ranged in diameter from 0.1 to 1.0 mm and appeared as rosettes comprising 3 to 8 bodies per nucleus.
- ❖ Vacuolar degeneration and necrosis of all types of hepatopancreatic cells except the F-cells were seen. Intertubular fibrosis was prominent in some, while hyperplasia of the hepatopancreatic cells was conspicuous in a few shrimps.
- ❖ Midgut epithelial cells revealed multiple spherical and eosinophilic occlusion bodies.
- ❖ These occlusions bodies could clearly be demonstrated and easily counted in impression smears. As many as 20 occlusions were seen in a single cell.

Systemic ectodermal and mesodermal baculovirus (SEMBV)

SEMBV infection was recorded in 18 of 48 submissions from Tamilnadu, 3 of 6 submissions from Nellore, Andhra Pradesh and 2 of 5 brood stock samples.

Clinical signs and gross lesion

- ❖ Frequent surfacing of the affected shrimps
- ❖ Lethargic and abnormal swimming behaviour
- ❖ Huddling at the dike
- ❖ Cessation of feeding
- ❖ Gray blue to bluish grey discolouration of the body
- ❖ White spots of about 0.8 to 1 mm on the cuticle, especially in the inner surface of cephalothorax and abdomen
- ❖ Cuticular deformities - Broken or withered antennae and damaged rostrum
- ❖ Opaque abdominal musculature
- ❖ Melanized gills
- ❖ Mortality 70 – 100% within three days after the onset of clinical signs

Histopathology

- ⊛ Subcutis of cephalothorax – focal necrosis
- ⊛ Subcuticular epithelium – Hyperplasia, hypertrophy of nuclei, chromatin margination containing eosinophilic to basophilic inclusion bodies
- ⊛ Gills – Degeneration, eosinophilic intranuclear inclusions in the pillar cells, haemocytic infiltrations
- ⊛ Intranuclear inclusion bodies - Eyestalk epithelium, posterior ganglion of the eye, stomach, midgut epithelium, myocardium and testis
- ⊛ Lymphoid cell necrosis and depletion. Intra nuclear inclusion also seen.
- ⊛ In general all the organs revealed necrosis, haemocytic infiltration and accumulation of haemolymph in the interstitium.
- ⊛ All the specimen which revealed SEMBV specific nuclear inclusions were stained positive for DNA (red) with Feulgen's technique, bluish green or blue with MGP technique and red with LPT staining.

Polymerase chain reaction (PCR)

PCR detected the presence of SEMBV genomic DNA (643 base pair fragments) from DNA extracted from infected tissues of the affected shrimps.

In situ hybridization:

In situ hybridization was positive in the cuticular epithelium of exoskeleton, eye stalk, heart, midgut epithelium, gills, and testis.

Transmission electron microscopy

TEM revealed ovoid, elongated or elliptoid virions measuring $300 \pm 10 \text{ nm} \times 100 \pm 5 \text{ nm}$. With a well developed tri laminar envelope.

Yellow head Virus (YHV)

The YHV infection was observed in shrimps above 46 days of age and also in brood stock specimens. Prevalence rate in Tamilnadu was 1.33% while in Andhra Pradesh it was 20.7%.

Clinical signs and gross lesions

- ✦ Mortality – 90 – 100%
- ✦ Light yellow discoloration of the dorsal cephalothorax
- ✦ Yellowing of the gills
- ✦ Rapid mortality within 3 days after the onset of the signs
- ✦ Occurrence with multiple infections - SEMBV, REO and MBV

Histopathology

- ✦ Fat droplets in the 'R' and 'B' cells of hepatopancreas
- ✦ Focal to diffuse necrosis of the parenchyma and stromal tissue
- ✦ Lymphoid organs – Necrosis of stromal matrix
- ✦ Stromal cells – Spherical refractile, basophilic, intracytoplasmic inclusion bodies
- ✦ Subcuticular epithelium – Basophilic intra cytoplasmic inclusions
- ✦ Gill – A few pillar cells revealed intra cytoplasmic inclusions.
- ✦ Cardiac muscle cells- Fatty degeneration
- ✦ Skeletal muscle cells – Necrosis
- ✦ Ovary – Basophilic intra cytoplasmic inclusions in the follicular cells
- ✦ None of the intracytoplasmic basophilic inclusion bodies stained positive with Feulgen's stain. However, these inclusions stained pink with MGP in gills, cuticular epithelium and lymphoid organs. They stained red with LPT, a non – specific inclusion body staining technique.

Transmission electron microscopy

Heart revealed rod shaped and filamentous, enveloped virus like particles of varying sizes and shapes enclosed within a vesicles. There were enveloped virion like particles each surrounded by a thick layer of electron dense material and all were enclosed in a vesicular membrane.

Infectious hypodermal and haematopoietic necrosis virus (IHHNV)

The prevalence rate of IHHNV was 7.97% in Tamilnadu. The disease occurred mostly in shrimps aged above 45 days. Multiinfection with other viruses nearly MBV and Reo-like virus and epicommensal infestation were commonly encountered.

Clinical signs and gross Lesions

- * Marked reduction in feed consumption
- * Rise slowly to the water surface and stay motionless for some time and then rolled over and sank to the bottom
- * Mortality 80 – 100%
- * White to buff coloured spots on the cuticle

Histopathology

- * Gill, cuticular epidermis and testis revealed necrosis and inflammation
- * Eosinophilic weakly Feulgen positive intranuclear Cowdry type A inclusion bodies within chromatin margined hypertrophied nuclei of gills, eye stalk, cuticular epithelium and ovary

Transmission electron microscopy

Sections from gills revealed intranuclear viral inclusions and the presence of condensed paracrystalline arrays of viral particles (22nm) reminiscent of IHHNV with isosahedral morphology in the cytoplasm.

Hepatopancreatic Parvo Virus (HPV)

HPV specific histological lesions were observed in the brood stock specimens of *P. monodon*. In these specimens evidence for infections with MBV, Reo-like virus, YHV and SEMBV were also seen along with gill fouling by the epicommensal, *Zoothamnium sp.*

Histopathology

- * Hepatopancreas – Necrosis and desquamation of tubular cells
- * Prominent basophilic intra nuclear inclusion bodies in the hypertrophied nuclei of 'R' cells
- * Inclusion bodies stained green with MGP, red with Macchiavello and LPT staining techniques.

Reo-like viruses

The Reo-like virus infections were found in 15 of the 48 submissions from Tamilnadu, 2 of the five brood stock specimen and 2 of the six submissions from Andhra Pradesh. It is found to occur in shrimps above 45 days of age.

Clinical signs and gross lesions

The Reo-like virus infections occurred along with viruses such as MBV, SEMBV, YHV or HPV as well as epicommensal organisms. Hence the clinical signs and gross lesions in those cases could not be attributed to Reo virus infections.

Histopathology

- ❖ Hepatopancreas – Degeneration and necrosis of tubular epithelial cells with accumulation of haemolymph in the interstium. 'F' and 'R' cells revealed intracytoplasmic eosinophilic inclusion bodies.
- ❖ Mid gut epithelium and testis – also exhibited similar inclusion bodies
- ❖ These inclusion bodies stained pyroninophilic with MGP technique and were Feulgen negative. They stained pink with LPT. Semithin sections from hepatopancreas stained with toluidine blue method revealed intracytoplasmic inclusion bodies.

Transmission electron microscopy

Hepatopancreatic cells showed aggregates of spherical viral particles measuring 62 ± 5 nm in size in the cytoplasm.

BACTERIAL DISEASES

Bacterial diseases that were recorded in the shrimps from Tamilnadu included vibriosis and Gram negative filamentous organisms in the hepatopancreas. Hepatopancreatic necrosis was observed with involvement of Gram negative bacteria in a specimen from Andhra Pradesh.

Vibriosis

The following species of the genus *Vibrio* were isolated from Tamilnadu specimens *Vibrio fluvialis*, *V. mediterranei*, *V. mimicus*, *V. alginolyticus*, *V. carchariae* and *V. parahemolyticus*.

Vibrio sp isolated from Andhra Pradesh were *V. alginolyticus* and *V. parahemolyticus*

On TCBS agar plate round yellow colonies of about 0.2 – 0.3 mm diameter with green centers (*V. alginolyticus*) and larger yellowish colonies (*V. parahemolyticus*)

Clinical signs and gross lesions

- Dull and sluggish movements
- Cloudiness or pale hepatopancreas with creamy white contents
- Cloudiness of muscle
- Black spots on either sides of carapace, above the gills on the exoskeleton

Histopathology

Extensive necrosis in the subepidermal layer of exoskeleton, muscles, eye, gills and hepatopancreas with multiple haemocytic nodules were seen. Invasion of Gram negative coccobacillary bacteria, necrosis, haemocytic infiltration, destruction of architecture, melanization and multiple haemocytic nodules with accumulation of haemolymph were noticed in these organs.

Transmission electron microscopy

Studies of the gills revealed rod shaped bacterial clumps in the cytoplasm of the pillar cells.

MISCELLANEOUS BACTERIAL INFECTIONS

In one submission of *P. monodon* from Tamilnadu the tubular epithelial cells of hepatopancreas showed the necrosis, membrane bound, long, Gram negative filamentous organisms

similar to *Leucothrix sp.* within the lumen and found attached to the free borders of the hepatopancreatic cells. These filamentous organisms were septate.

In another submission from Andhra Pradesh the hepatopancreas revealed necrosis of all types of cells except the 'F' cells. There was disintegration of the hepatopancreatic cells in the apical portion. Masses of Gram negative bacilli were seen in the necrotic areas. There were multifocal areas of melanization, focal areas of hyperplasia, scattered fibroplasias, atrophy and dilatation of tubules with haemocytic infiltration and accumulation of haemolymph.

PROTOZOAN FOULING DISEASE (PFD)

PFD due to the *Zoothamnium sp* occurred along with viral disease. In the 15 of 48 submissions from Tamilnadu, 3 of six submissions from Andhra Pradesh and 1 of five brooder stock specimens, gill fouling by *Zoothamnium sp* was identified. The infestation was found on the cuticular surface and stomach.

MULTIPLE INFECTIONS

Multiple infections with viruses and/or bacteria were observed in 22 out of 48 submissions from Tamilnadu, 3 of six submissions from Andhra Pradesh and 3 of five brooder stock specimens.

In *P. indicus specimen* from Tamilnadu, MBV, SEMBV, Reo-like virus infection, YHV and bacterial septicaemia with PFD were commonly encountered. Wild caught *P. monodon* female brood stock specimens from the Bay of Bengal revealed infection with MBV, SEMBV, Reo-like virus, YHV and HPV along with epicomensal infection without overt signs of any diseases. In commercial aqua culture farms culturing *P. monodon* in Nellore, Andhra Pradesh, MBV, SEMBV, Reo-like virus and YHV were detected. Bacterial septicaemia and infestation with PFD were also commonly observed. Multiple infections with viral and bacterial agents along with infestation by *Zoothamnium sp* were detected in most of the specimens examined.

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