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RESEARCH ARTICLE

Cytopathic Effects of Systemic Ectodermal and Mesodermal Baculovirus in Different Tissues of *Penaeus monodon* (Fabricius)

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Abstract

The White spot disease caused by the Systemic ectodermal and mesodermal baculovirus (SEMBV) in *Penaeus monodon* farms leads to mass mortality. The prawns exhibited clinical signs such as loss of appetite, lethargy and a loose cuticle with white spots on the inner surface of the carapace and exoskeleton. The tissues hepatopancreas, muscle, gills, nerve cord, gut, muscle epidermis and gill epidermis were removed and prepared for the histological studies by the familiar microtechnique method. Marked differences were noticed between the tissues of normal and diseased prawns. The most characteristic pathological changes were the presence of prominent eosinophilic and basophilic inclusion bodies in hypertrophied nucleus in all the tissues and the absence of occlusion bodies.

Keywords: *Penaeus monodon*, cytopathic effects, SEMBV, microtechnique method, occlusion bodies.

Introduction

In fishes, viruses are best known as suspected or known etiological agents of several neoplastic, hyperplastic and hypertrophic diseases. Recently viruses have also been reported with increasing frequency in crustaceans. Among crustacean infecting viruses, *Baculovirus penaei*, which causes mortalities in Gulf of Mexico pink shrimp is probably the best known and represents the dominant viral group, although knowledge of other viruses has also been recognized in shrimp culture systems. The first viral disease was described by Vago (1996), since then; a bewildering array of viruses has been described in species such as the blue crab (Johnson, 1983) and shrimp (Sano *et al.,* 1981; Lightner, 1983). Some of the viruses have been associated with disease and mortality in captivity or in capture. A decrease in survival and/or a reduction in reproductive potential are probably the most significant effects of these viral infections on prawn population. Much of the effort in virus research therefore was concentrated on methods for identification of viral organisms and their geographical location. Lightner and Redman (1992) devoted most of their review of shrimp viral disease on diagnostic procedures. The white spot syndrome baculovirus complex has been reported from the regions like China, Japan, Korea, India, Texas, Taiwan etc. The interregional transport of stocks was attributed for the virus distribution and for its epidemic proportion of infection in the different regions mentioned (Lightner, 1996). This virus belongs to family Baculoviridae, subfamily Nudiabculovirinae as PmNoBll, but for convenience, it is informally named as SEMBV. As the diagnostic monitoring of the shrimp diseases are mostly based on clinical signs and histopathology, in the present study, the histopathological studies on different tissues of the viral infected prawns were made to attribute the specific effects of virus over the tissue pathology.

Materials and methods

The prawns infected with systemic ectodermal and mesodermal baculovirus exhibiting the clinical sings were collected from the commercial black tiger prawn farms around Chennai and transported to the laboratory. The various clinical sings of viral infection include the loss of appetite, lethargy, presence of white spots on the carapace and exoskeleton. The different tissues of the *Penaeus monodon* such as the hepatopancreas, gills, muscle, gut, nerve cord, muscle epithelium and gill epithelium were dissected and fixed in 5% formalin. Tissues from normal uninfected prawns represented the control. The tissues were subjected to histological sections by the established microtechnique method using hematoxylin and eosin stains. The sections were taken at 7 μ thicknesses and studied under light microscope for pathogenesis.

Results

In the body, cuticular epithelium prominent hypertrophied intranuclear inclusion body was identified in the infected prawns (Fig. 2). The above inclusion body was found to be more in numbers in gill epithelium. The inclusion body resembled the cow dry type A intranuclear inclusion body (Fig. 4).

Fig. 1. Section of body epithelium of *P. monodon* (control) at 7 μ thickness (160x).

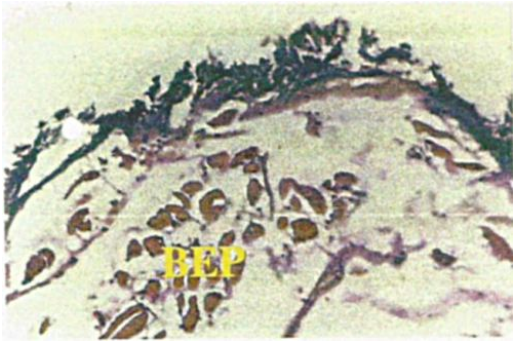


Fig. 2. Section of body epithelium of *P. monodon* (infected) at 7 μ thickness (160x).

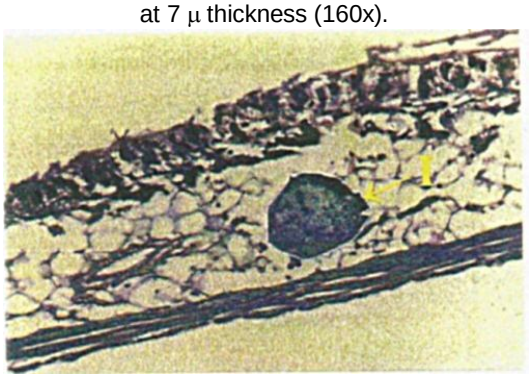


Fig. 3. Section of gill epithelium of *P. mondon* (control) at 7 μ thickness (160x).

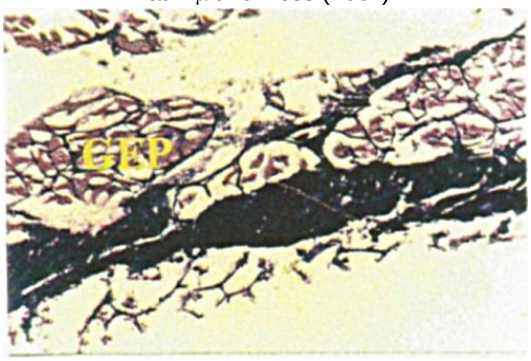
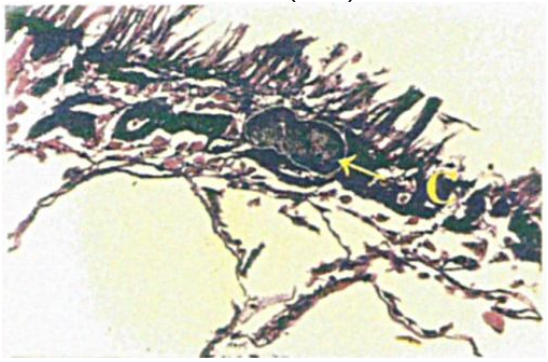


Fig. 4. Section of gill epithelium of *P. mondon* (infected) at 7 μ thickness (160x).



C →cowdry type A inclusion body

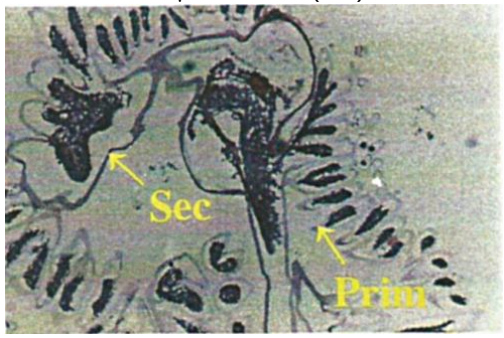
Fig. 5. Section of body musculature of *P. monodon* (infected) at 7 μ thickness (160x).

S→striated muscle fibres

Fig. 6. Section of body musculature of *P. monodon* (infected) at 7 μ thickness (160x).

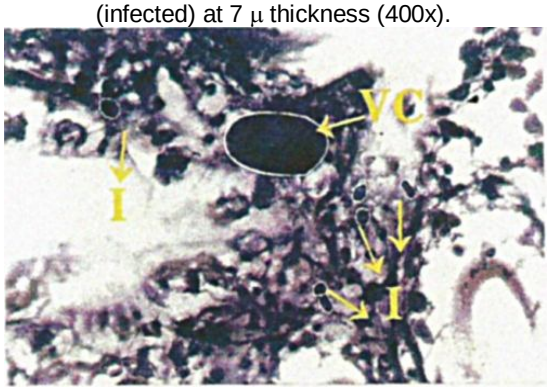
S→striated muscle fibres

Fig. 7. Section of gill of *P. mondon* (control) at 7 μ thickness (63x).



Prime → Prim → primary gill filament, Sec → secondary gill filament

Fig. 8. Section of secondary gill filament of *P. monodon* (infected) at 7 μ thickness (400x).



I→inclusion body, Vc→vibrio colony.

Fig. 9. Section of alimentary canal of *P. mondon* (control) at 7 μ thickness (25x).

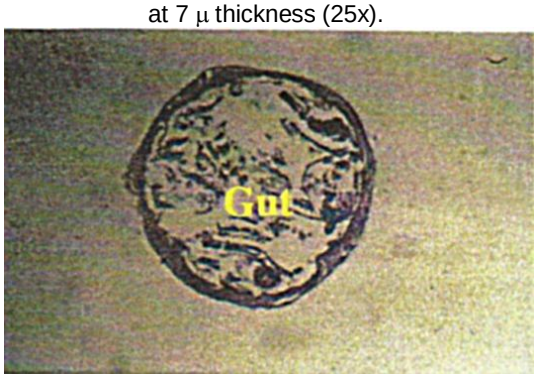
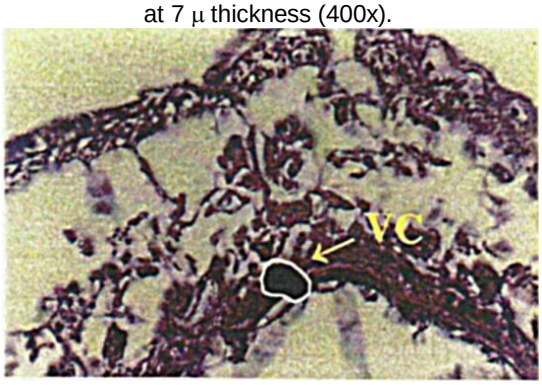


Fig. 10. Section of alimentary canal of *P. monodon* (infected) at 7 μ thickness (400x).

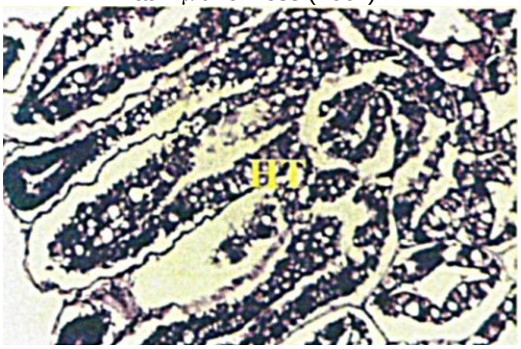


Vc→ vibrio colony

Fig. 11.Section of Hepatopancreas of *P. monodon* (control) at 7 μ thickness (160x).

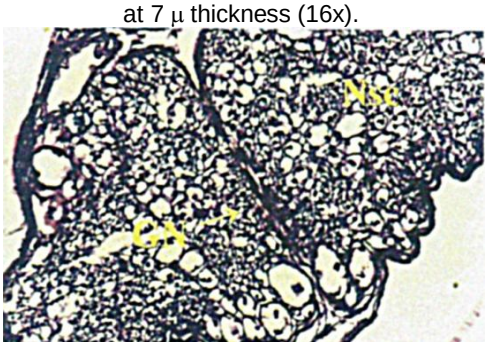
HT→hepatopancreatic tubules.

Fig. 12. Section of hepatopancreas of *P. monodon* (infected) at 7 μ thickness (160x).



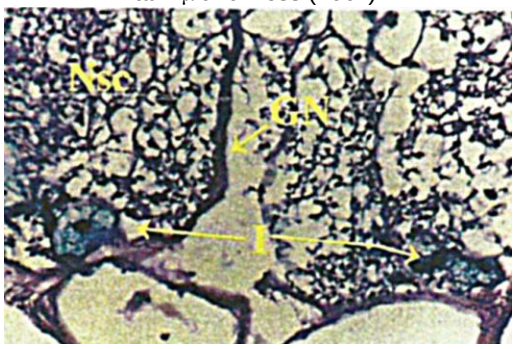
HT→hepatonpancreatic tubules.

Fig. 13. Section of Ventral nerve cord of *P. monodon* (infected) at 7 μ thickness (16x).



GN → giant nerve fibre, Nsc→ neurosecretory cells

Fig. 14. Section of ventral nerve cord of *P. monodon* (infected) at 7 μ thickness (400x)



GN → giant nerve fibre, Nsc→neurosecretory cells, I→inclusion body

The normal body muscle section revealed the presence of longitudinal striated muscle fibres arrangement (Fig. 5), but in the infected ones, they discernible in irregular pattern (Fig. 6). In the gill sections, cytopathological changes were found to be significant with several small inclusion bodies in the secondary gill filaments and the necrosis of the primary gill filaments at the apical end along with presence of bacterial colony (Fig. 8). In case of infected alimentary canal, the gut was empty compared to the normal, but a colony of bacteria remain on the layer of the gut (Fig. 10). In the infected hepatopancreas, the inclusion bodies were absent and destruction of the hepatopancreatic tubules was obvious (Fig. 12). In the infected ventral nerve cord, large inclusion bodies are present on both the sides of the giant nerve fibres and also among the neurosecretory cells. The occlusion bodies were specifically absent in all the above tissues of infected prawns.

Discussion

Viruses may be present in shellfish as virulent pathogens, as latent infections or as harmless contaminants. Recognition of those viruses which may be present in the shrimp can also be important to understand possible disease transmission in other marine populations. Some viruses which probably exist in latent form in wild populations have emerged as pathogenic forms in cultured environments, often as a consequence of stressors such as high temperatures,

overcrowding, inadequate nutrition or poor water quality (Sindermann, 1990). Couch (1974) while demonstrating shrimp's mortality in overcrowded holding tanks, revealed the presence of rod shaped to somewhat elliptical, non-occluded virions of about 70-150 nm in width and about 275-380 nm in length in the intranuclear inclusion bodies of infected cells of the target tissues. The presence of white spots or patches measuring 0.5 to 2 mm in diameter on the inner surface of both the carapace and the exoskeleton suggests that, this may be due to infection by SEMBV. This is supported by Takahashi (1994), who observed the non-occluded SEMBV under the transmission electron microscope (TEM) in the diseased shrimps accompanied by the white patches on the inner surface of the carapace. A single large cytoplasmic intranculear inclusion body present in the connective tissue of the body epithelium appears to be eosinophilic halo centronuclear inclusion. This suggests that the infection is of initial stage in this region. In the gill epithelium, the two inclusion bodies present match closely the characteristic of the Type cow dry A intranuclear inclusion body as described by Lightner *et al.* (1987). As there is more than one inclusion body in this region, it reveals that the infection may be in advanced stage. The symptoms in the body muscle suggest that this pathogen affected the agility of prawns as revealed by the lethargic movement, one of the clinical signs of the diseased prawns. In gills, the basophilic nature of small inclusion bodies among the hemocytes and the necrosis of the primary gill lamellae accompanied with the secondary infection by the bacterial pathogens which might be of vibro species infer that the infection is in advanced state in this particular region. It also suggests that the infection may be through the gills from the infected to the normal ones by the surrounding water that flows through the gills for respiration. The inclusion bodies are absent in the alimentary canal. But a larger colony of the cells in the gut wall noticed reveals that there might be secondary infection of bacterial pathogens like vibrios. This could be possible as Chanratchakool *et al*. (1995) noticed both the viral and bacterial species in *P. monodon* with White patch disease. The inclusion bodies are absent in the hepatopancreas but the hepatopancreatic tubules showed irregular shapes suggesting the disturbance in the nutrition of the animal. In the ventral nerve cord, the eosinophilic inclusion bodies present on both sides of the giant nerve fires and neurosecretory cells infer that the infection in this region is of earlier stage. This is supported by Wongteersupaya *et al.* (1995) who experimentally infected *P. monodon* with the Yellow head virus (YHV), but yielded non-occluded systemic baculovirus and notice eosinophilic cow dry type A inclusion in hypertrophied nucler with marginated chromatin which became lightly basosphilic in later stages of infection. The absence of occlusion bodies in almost all the above tissues suggests that the inclusion bodies are of non-occuluded type as opined by Lightner (1985).

In sum, the above results reveal that SEMBV infects almost all vital organs of the prawns thereby affecting all the physiological activities of the animal. The various pathogenic effects include:

i. The destruction of the body muscle and the obstruction of the movement.
ii. Loss of appetite due to disruption of gut layers and hepatopancreatic cells.
iii. Secondary infection of some tissues by other microbial pathogens.
iv. Disruption of normal gill architecture affecting respiration.
v. The presence of eosinophilic inclusion bodies in the neurosecretoy cells of the ventral nerve cord attribution dysfunction of neurotransmitters and derangement in neurosecretion.

The above may result in failure of molting and growth in the infected individuals. The infected prawns act as carries of the viruses to pass it on to their offspring generation. The result of the histopathological analyses in infected prawn represent as an indicator profile to take up the prophylactic measures. The various prophylactic measures include the selection of healthy and quality brood prawns and stocking material, maintenance and management of water quality, management of pond environment, use of balanced feed and appropriate feeding strategy. As there is no direct treatment for viral infection, experimental vaccines may effective in protecting the culture species from the viral infections. Chemolaxies using antibiotics for viral diseases are now widely employed for preventing diseases. Besides, immunprophylaxis of prawns against infectiontious diseases may promote productivity.

Acknowledgements

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