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Histopathology of Y-organ in Indian white shrimp *Fenneropenaeus indicus*, experimentally infected with white spot syndrome virus

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Abstract

Histopathology of Y-organ (moulting gland) in Indian white shrimp *Fenneropenaeus indicus* was studied after challenging the shrimp with virulent white spot syndrome virus (WSSV). Histopathological investigation on the Y-organ collected from moribund shrimps revealed the presence of intranuclear basophilic inclusions, characteristic of WSSV. More than 70% of the Y-organ cells were infected, suggesting the degenerated state of the organ. The cellular integrity of the Y-organ was completely destroyed by the WSSV. Further, Y-organ tissue samples collected from all the 16 experimental shrimp were positive by one-step polymerase chain reaction (PCR), confirming severe WSSV infection. The infected Y-organ in *F. indicus* with majority of the organ cells observed in the lysed condition suggests a physiological dysfunction of the organ. In uninfected and healthy controls, the lobulated Y-organ showed closely packed normal cells with prominent nuclei and sparse cytoplasm. Physiological implication of a degenerated Y-organ in the moulting and reproduction of the penaeid shrimp is discussed.

Keywords: WSSV; Ectoderm; Y-organ; Moulting gland; Histopathology; Pathophysiology; *Fenneropenaeus indicus*

1. Introduction

White spot disease (WSD), caused by white spot syndrome virus (WSSV), has become the major deterrent in the growth and sustainability of shrimp aquaculture across

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the globe (Lightner, 1996). The WSSV affects all known species of cultivated shrimps in the eastern and western hemisphere, and it is the most virulent virus hitherto reported from the farmed shrimps (Flegel and Alday-Sanz, 1998; Van Hulten et al., 2001). WSSV causes heavy crop loss and it continues to seriously affect most of the cultured penaeids throughout the shrimp farming systems in the world. The wide host range of this virus includes many aquatic crustaceans and even aquatic insects (Lo et al., 1997; Wang et al., 1998; Rajendran et al., 1999; Hossain et al., 2001). This rod-shaped, non-occluded virus affects all the tissues of ectodermal and mesodermal origin (Lightner, 1996). Pathogenesis of WSSV has been shown in different tissues of the shrimp such as epidermal tissue, gill, pleopod, pereopod, gut, heart, lymphoid organ, antennal gland, nervous tissue, ovary, testes, spermatophore, eyestalk and haemolymph (Chang et al., 1996; Lightner, 1996; Lo et al., 1997). However, pathogenesis of WSSV on the vital endocrine glands such as Y-organ, which plays major role in the growth physiology of shrimp is completely lacking.

The Y-organ, termed as the moulting gland or ecdysal gland, consists of paired organs of ectodermal origin in the penaeid shrimp's head region (Lachaise et al., 1993). The Y-organ plays a crucial role in the moulting and growth of crustaceans (Spindler and O'Connor, 1980; Chang et al., 1993; Chang, 1995; Huberman, 2000). The moulting hormone secreted by Y-organ mediates several aspects of crustacean growth and reproduction (Quackenbush, 1986; Chang et al., 1993). The structure and role of Y-organ in the moulting and growth of penaeids have been reported in *Marsupenaeus japonicus* (Bourguet et al., 1977) and in *Fenneropenaeus indicus* (Vijayan et al., 1993). Moulting, being the indirect version of growth, the structural integrity of the Y-organ, which regulates the moulting process through the moulting hormone, is vital for the successful moulting and growth in penaeids. Because the Y-organ is ectodermal in origin (Spindler and O'Connor, 1980; Lachaise et al., 1993), it is logical to assume its susceptibility to WSSV infection. As this endocrine gland plays a major role in the moulting and growth of shrimps, we investigated the histopathology of Y-organ with respect to WSSV infection in Indian white shrimp *F. indicus* and to determine the physiological implications of WSSV infected Y-organ in shrimp.

2. Materials and methods

2.1. Experimental animals

Sub-adults of *F. indicus*, 80–120 mm TL, were obtained from grow-out ponds or sea in and around Chennai, India. Shrimps were maintained in 1000-l fibreglass tanks with filtered and aerated seawater (salinity 20–25 ppt; temperature 28–32 °C; pH 8.0–8.2). Animals were fed with formulated shrimp feed (30% protein) at 5% body weight, once a day. All experimental animals were observed for 1 week in a clean laboratory environment for clinical signs of WSSV. Further, a representative sample of these animals was subjected to nested PCR, using a WSSV-nested PCR kit (Bangalore Genei, India). Only animals shown to be healthy were used for the experiment.

2.2. Experimental infection

White spot disease affected moribund *Penaeus monodon* were collected during an emergency harvest resulting from a WSD outbreak at two shrimp farms located in Nellore, Andhra Pradesh (India). WSSV infected tissues viz., gill, epidermal layer and stomach, were removed and kept at −70 °C for experimental use. About 2 g tissues in total was homogenized in sterile marine phosphate-buffered saline (PBS) and centrifuged at 1600 × *g* for 15 min at 4 °C. The supernatant fluid was then passed through a 450-nm pore size syringe filter. This virus containing supernatant fluid was diluted to 1 part filtrate to 10 parts PBS, and stored at −70 °C for infectivity studies.

The filtrates containing virus were injected intramuscularly into the second abdominal segment of the experimental shrimp (*F. indicus*), each shrimp received 0.1 ml inoculum. Thirty-two shrimps exposed for WSSV were held in four fibreglass tanks, each containing eight shrimps in 80 l of seawater (salinity 20–25 ppt; temperature 28–32 °C; pH 8.0–8.2). The control groups comprising three tanks with eight shrimps each were kept isolated from the experimental sets, wherein eight shrimps in one of the tanks were injected with PBS, and the other two tanks with eight shrimps each were unexposed. Maintenance and feeding in the control sets were similar to that of experimental sets. All the experimental and control animals were examined daily for morbidity and mortality. The moribund shrimps were removed and tissues were collected for histological studies and PCR. The experiment was terminated on the seventh day of post injection (p.i.).

2.3. Histopathology and PCR

For histological studies, Y-organ tissues were collected from 16 moribund shrimps from the infected groups, and 12 (four from each tank) control shrimps, according to Vijayan et al. (1993). The Y-organs along with the branchiostegite were bilaterally removed from the region between the mandibular and posterior dorsoventral muscle of the prebranchial chamber of the shrimp (Fig. 1). From the paired Y-organs, one of the Y-organs was immediately fixed in Davidson's fixative for histology, and the other one was fixed in 95% ethanol for PCR. For histology, routine procedures were followed for preparation, sectioning, and staining with haematoxylin and eosin (Bell and Lightner, 1988). Y-organs from the experimental and control shrimps were examined histologically for WSSV-specific manifestations following the routine diagnostic protocol of Lightner (1996). The severity of the infection was graded as low (<30%), medium (30–70%) and high (>70%) based on the percentage of WSSV positive cells in a selected field of 40 ×, in the reference tissues. Sixteen Y-organs collected from moribund shrimps from the WSSV-challenged group were examined for grading.

PCR on 16 Y-organ tissues collected from moribund shrimps and 12 from dead shrimps were conducted using a commercial PCR kit (Bangalore Genei, India). An alkaline lysis buffer supplied with the kit was used to prepare template DNA from the tissues containing Y-organ (<20 mg). The extraction followed the protocol of Vijayan et al. (1998). The PCR kit comprised an outer primer pair and an inner primer pair. The first and second step

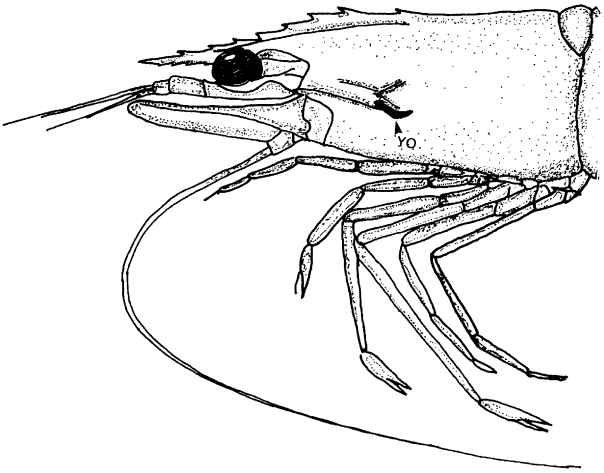

Fig. 1. Schematic representation of Y-organ of the *F. indicus*, YO: Y-organ.

of amplification using the kit was expected to amplify WSSV DNA fragment of 650 and 300 bp, respectively.

3. Results and discussion

Eosinophilic to basophilic intranuclear inclusion bodies in the hypertrophied nuclei of infected cells are the typical histopathological diagnostic feature of WSSV (Lightner, 1996). The nuclear hypertrophy and the inclusion bodies are due to the development and accumulation of developing virions within the nucleus. In the initial stages of infection, WSSV inclusion is eosinophilic in nature, which becomes basophilic as the infection advances (Lightner, 1996). Histopathological findings in this study clearly indicate that, the Y-organ cells were highly susceptible to WSSV infection (Figs. 2–5). More than 70% of the Y-organ cells in the 16 moribund shrimp showed WSSV-characteristic cytopathological changes such as, hypertrophic cells, lysed and necrotic cells, cells with hypertrophy and intranuclear basophilic inclusions (Figs. 4 and 5), exhibiting a very high level of WSSV infection (Table 1). In the uninfected controls, a lobulated Y-organ with closely packed Y-organ cells with sparse cytoplasm and prominent nuclei and chromatin granules were observed (Figs. 6 and 7). This is in agreement with the penaeid Y-organ histology in healthy shrimps, reported by the earlier workers (Bourguet et al., 1977; Vijayan et al., 1993). Infected Y-organ tissue appeared degenerated with necrotic, atrophied and hypertrophied cells with basophilic inclusions of WSSV (Fig. 3). The cellular integrity of the Y-organ was found to be destroyed completely by WSSV infection (Figs. 3–5). Cytopathological studies done by Chang et al. (1996) in tissues of *P. monodon* have showed that the cuticular epidermis was initially infected by WSSV at 16 h p.i. and that the degree of infection became acute after 40 h p.i. Similar observations were also made by Wang et al. (1997) in the WSSV infected cuticular epidermis of *M. japonicus.* These studies (Chang et

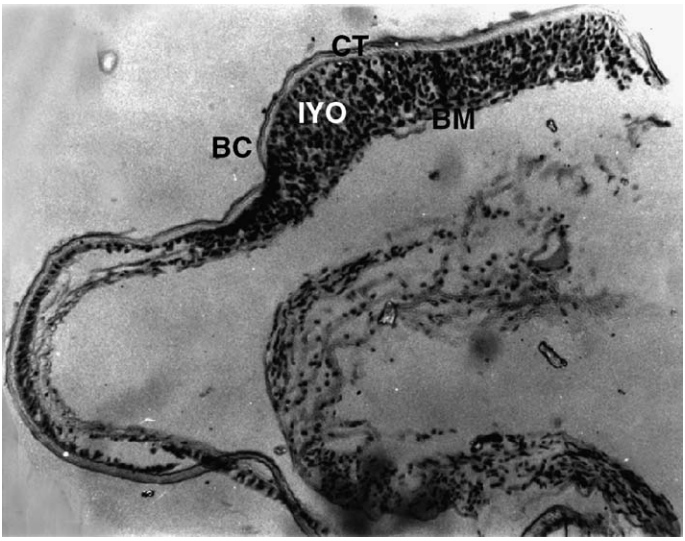

Fig. 2. Y-organ infected with WSSV in *F. indicus*, BC: branchial chamber; BM: basal membrane; CT: cuticle; IYO: infected Y-organ (× 100).

al., 1996; Wang et al., 1997) support our observation that ectodermal-Y-organ (moulting gland) in moribund *F. indicus*, infected with WSSV, was completely degenerated. PCR analysis on the Y-organ tissue from the moribund shrimps showed that all the 16 Y-organs collected from the moribund shrimps were first-step PCR positive (Fig. 8), confirming the histological observations of Y-organ cells from the moribund shrimps. This indicates a higher level of WSSV infection in the moulting glands of shrimps. Among the dead shrimps, 11 out of 12 were first-step PCR positive. This observation suggests that, similar

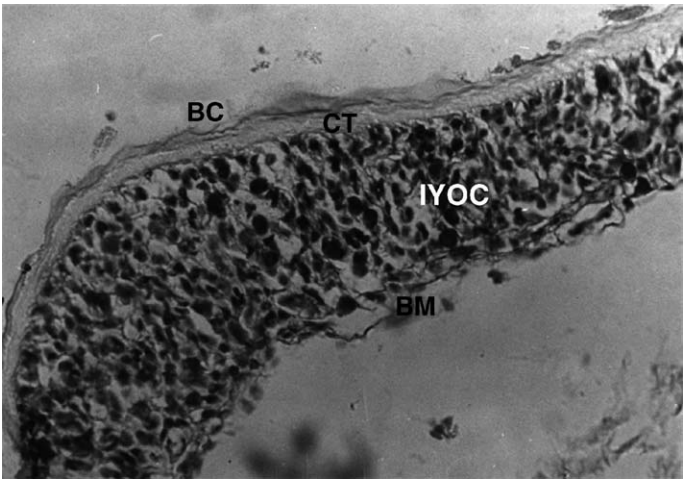

Fig. 3. Close-up of Y-organ infected with WSSV in *F. indicus*, IYOC: infected Y-organ cells (× 200).

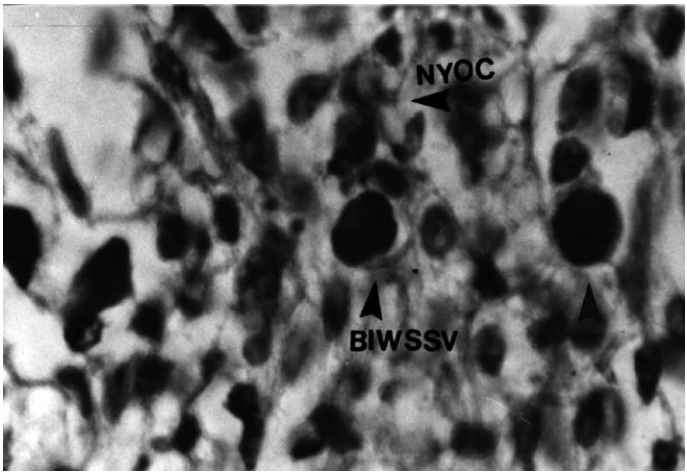

Fig. 4. Necrotic Y-organ with basophilic intranuclear inclusions of WSSV, arrows indicate basophilic inclusion bodies (BIWSSV). NYOC: necrotic Y-organ cells (× 1000).

to other ectodermal tissues in penaeid shrimps, the paired Y-organ is one of the main targets of WSSV replication. The death of one shrimp recorded on day 1, which gave PCR-negative result, might be due to stress.

Our study of pathophysiological impact of WSSV infection on endocrine organs such as the Y-organ has clearly shown that the structural integrity of the Y-organ in penaeid shrimp, *F. indicus*, was destroyed by the invading WSSV virions. Earlier, Chassard-Bouchaud and Hubert (1976) reported partial Y-gland degeneration in decapod crustacean (*Carcinus* sp.), due to parasitism by *Sacculina*. The malfunctioning of the Y-organ could

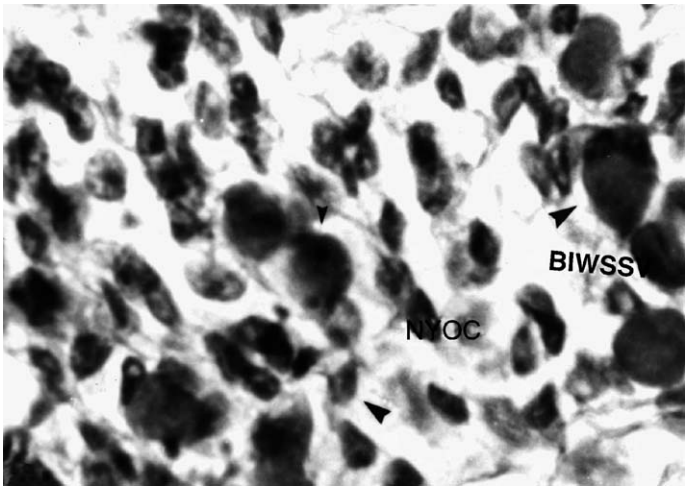

Fig. 5. Necrotic and degenerated Y-organ due to WSSV infection (× 1000).

Table 1
F. indicus challenged with WSSV, and infection levels of Y-organ using histology and PCR

	Days (post injection)								Animals with WSSV-specific histological characteristics (%)	WSSV-infected cells in the Y-organ (%)	PCR on Y-organ (%)
	0	1	2	3	4	5	6	7			
Treatment (n = 32)											
Mortality	1	0	1	3	2	2	3	–	92	–	92
Moribund	0	1	2	1	4	3	2	3	94	>70	100
Control (n = 24)											
Mortality	1	0	0	0	0	0	0	0	–	–	–
Moribund	0	0	0	0	0	0	0	0	–	–	–

affect the secretion of the Y-organ hormones, which regulates the moulting and reproduction in shrimp. The infected Y-organ of *F. indicus* with the majority of the organ cells in the lysed condition suggests a physiological dysfunction of the moulting gland. Vijayan et al. (1993) reported that the removal of the Y-organ in *F. indicus* caused delayed and extended moulting resulting in poor growth. Thus, one of the reasons for the reduced production from the WSSV-infested shrimp farms (unpublished data) could be attributed to the malfunctioning of the moulting process resulting from an infected and degenerated Y-organ. Further, it is a common observation in the post-WSSV scenario that the *P. monodon* and *F. indicus* wild broodstock often failed to respond to the popular eyestalk ablation technique practiced in shrimp hatcheries for induced maturation (Surendran, V., Nellore Hatcheries, Nellore, India, personal communication). The hormone produced by the Y-organs is believed to have a gonadotropic effect (Subramoniam, 2000; Chang et al., 2001). Therefore, the failure of the eye-ablation technique could be due to the degeneration of the Y-organ as a result of WSSV infection. Our study is the first report on the

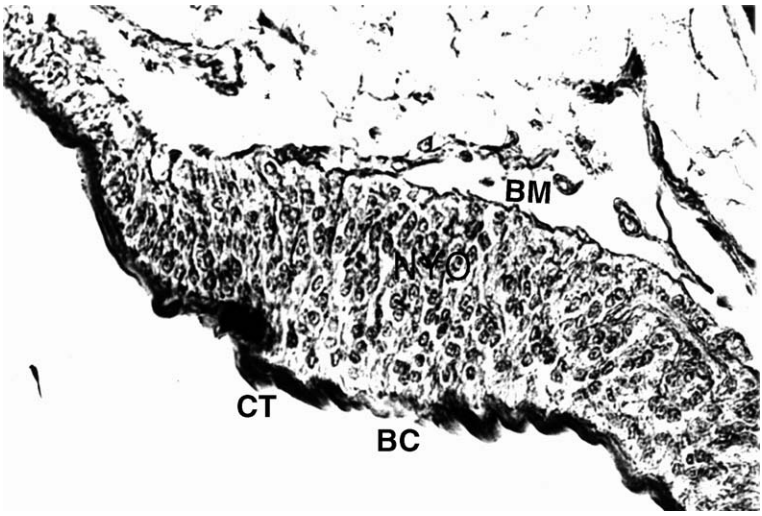

Fig. 6. Y-organ from healthy shrimp *F. indicus*. NYO: normal Y-organ (× 200).

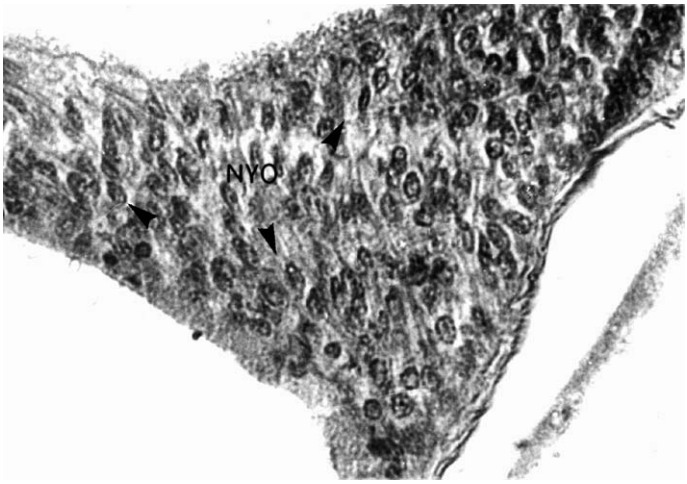

Fig. 7. Close-up of Y-organ from healthy shrimp *F. indicus*. Arrows indicate healthy Y-organ cells (× 400).

pathogenesis of WSSV on a vital endocrine gland, examining the pathophysiological implications on the cultured penaeid shrimp. More detailed studies are necessary to gain a more complete understanding of pathophysiology resulting from WSSV infection in cultured shrimps, with reference to endocrine and neuroendocrine glands and their hormonal control of moulting and reproduction. We need to know the nature of damage

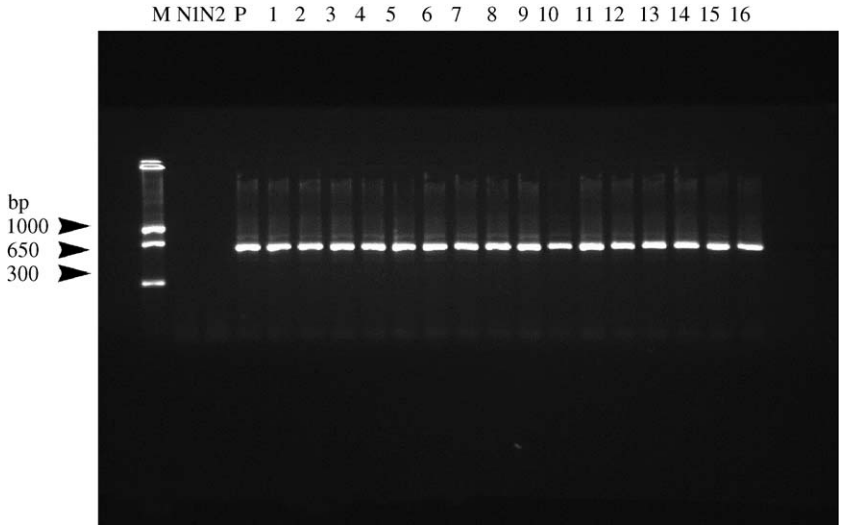

Fig. 8. PCR diagnosis of WSSV-infected Y-organ tissue from experimentally infected *F. indicus*. Lanes: M, DNA marker; N1, negative control (distilled water); N2, negative control tissue; P, positive control; 1–16, WSSV-infected Y-organ tissue.

this virus is causing in the vital endocrine organs of the shrimp to formulate rational disease management programs in shrimp farming.

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