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# Histopathological and bacteriological study of white spot syndrome of *Penaeus monodon* along the west coast of India

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**Abstract**

White spot syndrome (WSS) is a serious viral disease of shrimp causing severe mortalities in several parts of Asia. This paper describes the histopathology of the syndrome which occurred on the west coast of India. The affected shrimp showed white spots on the carapace and post abdominal segments and histopathologically, hypertrophied nuclei with eosinophilic to basophilic intranuclear inclusion bodies were detected in the epithelial cells of the stomach. Moderate to heavy septicaemia was noticed in moribund shrimp. © 1997 Published by Elsevier Science B.V.

*Keywords:* White spot syndrome; *Penaeus monodon*; Shrimp; Mortality

## 1. Introduction

White spot syndrome is a recently recognised viral disease of shrimp which causes severe shrimp mortalities in several parts of Asia. The causative agent has been given different names, e.g. rod shaped nuclear virus of *Penaeus japonicus* (RV-PJ), systemic ectodermal and mesodermal baculovirus (SEMBV), *Penaeus monodon* non occluded baculovirus II (PmNOBIII), and has been observed in *Penaeus chinensis*, *Penaeus japonicus* and *Penaeus monodon* (Inouye et al., 1994; Momoyama et al., 1994; Nakano et al., 1994; Takahashi et al., 1994; Chou et al., 1995; Wang et al., 1995; Wongteerasupaya et al., 1995). The disease is characterised by rapid reduction in food consumption, loose cuticle and appearance of white spots of 0.5–2.0 mm diameter predominantly on

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the carapace. The mortality rate has been reported to be very high, reaching 100% within 3–10 days of the onset of clinical signs (Inouye et al., 1994; Nakano et al., 1994; Anonymous, 1995; Chou et al., 1995; Wang et al., 1995).

During 1994–1995, white spot disease caused severe mortalities of cultured shrimp *P. monodon* and *Penaeus indicus* along the east coast of India (Anonymous, 1995). All age groups and sizes were affected in all kinds of systems (extensive, semi-intensive and intensive) and even in areas where the pond environment was apparently good. There has been no documentation of the disease from India in the scientific literature. A perusal of the literature indicated that there is very little information on the natural outbreak of the disease (Inouye et al., 1994; Nakano et al., 1994; Chou et al., 1995). In this report, we describe the outbreak of white spot disease syndrome on the west coast of India.

## 2. Materials and methods

Several shrimp farmers growing *P. monodon* in the Kumta area of North Canara District, Karnataka State, on the west coast of India noticed shrimp mortalities during July 1995. At this time, the monsoon was at its peak and salinities were nearing 0 ppt. The ambient temperature was in the range 20–23°C. All the farms were drawing water from the Aghanashini estuary. The affected farms ranged in size from 0.6 to 1.0 ha and were of semi-intensive type (stocking density 15–20 $m^{-2}$). Initially, mortalities were low (10–15 per pond $day^{-1}$) but increased rapidly in 3–5 days. Shrimp size ranged from 5 to 20 g. Affected shrimp showed white spots ranging in size from 1 to 3 mm on the carapace and post-abdominal segments (Fig. 1).

For histopathological studies, live shrimps were fixed in Davidson's fixative following the protocol described by Bell and Lightner (1988). At least five shrimp were examined from each of the six farms included in this study. Histological sections were stained with haematoxylin–eosin.

For bacteriological studies, haemolymph was drawn aseptically from the haemocoel

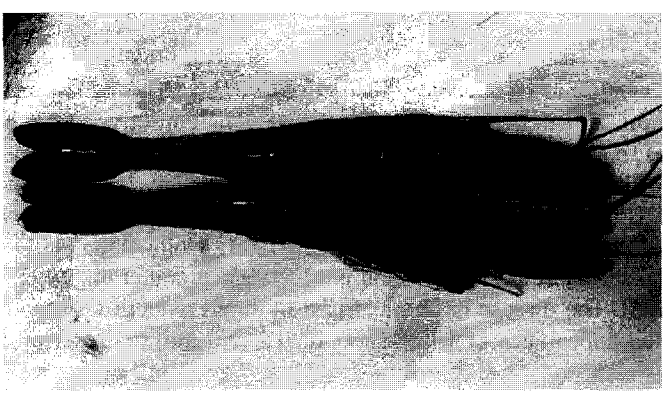

Fig. 1. Photograph of shrimps (*P. monodon*) with white spots (indicated by arrows).

of clinically symptomatic and asymptomatic shrimps. Serial ten-fold dilutions of haemolymph were spread on tryptic soy agar containing 1% NaCl (TSAS). The plates were incubated at 28°C and colonies counted at 48 h. Ten colonies from each sample were picked up for identification of bacteria. The scheme of West and Colwell (1984) was used for identification. *Vibrio cholerae* isolated were serotyped using polyclonal O1 antiserum obtained from Central Research Institute, Kasauli, India. *V. cholerae* and *Vibrio mimicus* isolates were tested for enterotoxin production using reverse passive latex agglutination test (Vet RPLA kit, Oxoid, UK).

## 3. Results and discussion

White spot syndrome (WSS) is dependent on the presence of hypertrophied or vacuolated nuclei and eosinophilic to basophilic intranuclear inclusion bodies in ectodermal and mesodermal tissue such as cuticular epithelial cells or connective tissue of gills or stomach, antennal gland, haematopoietic tissue and nervous tissue (Momoyama et al., 1994; Wongteerasupaya et al., 1995). Histopathological examination of affected shrimp from the west coast of India revealed pathological changes typical of WSS. As shown in Fig. 2, epithelial cells of stomach showed hypertrophied nuclei with eosinophilic to basophilic inclusions. In these nuclei, the clear zone between the eosinophilic centre and marginated chromatin was no longer present. In some of the affected cells, the nucleus had completely disintegrated, leaving vacant areas (arrow heads, Fig. 2). Similar pathology has been described in the case of SEMBV infection of *P. monodon* (Wongteerasupaya et al., 1995).

The histopathological evidence presented above suggests that the shrimp samples examined from the west coast of India had WSS. This disease was noticed for the first time along the west coast of India in July 1995, although shrimp culture activity has been prevalent in this region for several years. It is possible that the source of infection

Fig. 2. Section of stomach stained with haematoxylin–eosin (HE). Arrows indicate abnormal nuclei surrounded by vacant cytoplasm (bar, 10 μm). Arrow heads indicate late pathological changes where nuclei have disintegrated, leaving vacant areas.

for the outbreak was the brood stock or shrimp seeds brought from the east coast where the disease was noticed at the end of 1994 (Anonymous, 1995). The source of seeds was different in various farms. Some farms had stocked post larvae (PL) from the east coast while others had used PL obtained from brood stock caught along the east coast of India. At present, there are no methods available for the detection of asymptomatic infections and therefore it is not possible to say whether the virus came to the west coast with brood stock. It is possible that cross contamination of ponds occurred since the outflow and inflow of farms were close to each other.

It is possible that the outbreak of WSS is triggered by environmental conditions. In the east coast of India, the epizootic occurred immediately following cyclone and rains (Anonymous, 1995). Even along the west coast, the outbreak occurred when the monsoon was at its peak, salinity near 0 ppt, with heavy surface run-off and turbidity in natural waters. These observations support the view that environmental factors are important in triggering an epizootic of WSS.

The results of this study and other reports (Anonymous, 1995) suggest that WSS is spreading to new geographical areas. Though histopathologically the disease syndrome is similar to what has been reported for *P. japonicus*, *P. monodon* and *P. chinensis* (Momoyama et al., 1994; Wongteerasupaya et al., 1995), without studies on viral DNA it is not possible to say whether the same virus has been spreading. It is also not known whether the virus existed in a dormant state in *P. monodon* from the Andaman Sea, which are being used by hatcheries as brood stock, or whether it came with the larvae imported by some farmers in the east coast. Development of DNA probe and studies on apparently healthy brood stock and larvae would be necessary to answer these questions.

The bacteriological aspect of shrimp affected by WSS has not been reported in the literature. No bacteria could be detected in the haemolymph of clinically asymptomatic shrimp. Shrimp with WSS, however, had varying degrees of septicaemia (Table 1). Bacterial counts ranged from $4 \times 10^1$ to $4.2 \times 10^5$ cfu $ml^{-1}$. However, different *Vibrio* species were encountered, suggesting the secondary nature of bacterial infection. Non O1 *V. cholerae*, *V. mimicus*, *Vibrio harveyi* and *Vibrio alginolyticus* could be detected in the shrimp haemolymph. *V. cholerae* non O1 and *V. mimicus* isolates were found to

Table 1
Bacteriological profile of shrimp affected by white spot syndrome

| Source | Bacterial count (cfu $ml^{-1}$) | Bacterial species |
|---|---|---|
| Pond 1 | $3.1 \times 10^4$ | *V. cholerae* |
| | | *V. mimicus* |
| Pond 2 | $4.2 \times 10^5$ | *V. cholerae* |
| | | *V. mimicus* |
| Pond 3 | $2.0 \times 10^3$ | *V. cholerae* |
| | | *V. mimicus* |
| Pond 4 | $8.0 \times 10^1$ | *V. harveyi* |
| Pond 5 | $4.0 \times 10^1$ | *V. harveyi* |
| Pond 6 | $2.0 \times 10^3$ | *V. harveyi* |
| | | *V. alginolyticus* |

be negative for enterotoxin production when tested using Vet RPLA kit (Oxoid, UK) and therefore septicaemic shrimp are not a public health hazard. The results suggest that shrimp affected by WSS became susceptible to invasion by environmental *Vibrio* species.

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