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Note

Epizootology and pathology of bacterial infections in cultured shrimp *Penaeus monodon* Fabricius 1798 in West Bengal, India

T. J. ABRAHAM, D. SASMAL, G. DASH, T. S. NAGESH, S. K. DAS, S. K. MUKHOPADHAYAY* AND S. GANGULY

Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata - 700 094, West Bengal, India
**Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata - 700 037, West Bengal, India*
e-mail: skmpath@gmail.com

ABSTRACT

A severe epizootic of bacterial diseases affected a number of commercial penaeid farms in Contai, Soula, Balisai and Digha regions of East Midnapur District, West Bengal, India in 2004. Histological and scanning electron microscopy (SEM) examination of affected *Penaeus monodon* showed massive infections in the hepatopancreas and partly in gills, pleopods and on shell. The affected shrimps had symptoms of vibriosis, gill disease, shell disease, red discolouration due to hepatopancreatitis *etc.* Shrimps with bacterial infection showed significant host inflammatory response in the form of hemocytic infiltration, granulomatous reaction and nodule formation. Histopathological observations revealed necrosis of hepato-pancreatic cells, thickened basal lamina and subsequent granulomatous encapsulation of the invaded tubules. Scanning electron microscopic (SEM) studies on shell diseased shrimp epithelial cells revealed signs of pathology due to bacterial infection. The normal SEM architecture of shell was totally lost and the shell had irregular indentations due to degenerative process. A large number of bacteria, which remain attached to the granular core of damaged epithelium of diseased shrimps were visible.

Keywords: Bacterial disease, Hepatopancreatitis, *Penaeus monodon,* Red discolouration, Vibriosis, West Bengal

Bacterial diseases are considered as major cause of mortality in shrimps (Lightner, 1993). Bacterial infections in shrimps may take three forms: localised pits in the cuticle called bacterial shell disease, localised infections of the gut or hepatopancreas or localised infections from puncture wounds, limb loss *etc*. and generalised septicemia. *Vibrio damsela* and *Vibrio harveyi* have been reported to cause red gill disease in shrimps (Limsuwan, 1993). Red disease or red discolouration (RD) was first noted in *Penaeus monodon* cultured in Taiwan (Liao *et al*., 1977). Limsuwan (1993) reported red disease due to Gram negative bacteria, although the type of causative bacteria was not always the same. Bacteria such as *V. harveyi*, *V. parahaemolyticus, V. fluvialis* and unclassified *Vibrio* spp. have been isolated from diseased shrimp (Alapide-Tendencia and Dureza, 1997; Karunasagar *et al*., 2007). A form of bacterial necrotising hepatopancreatitis was first described in penaeid shrimp cultured in Texas, where it caused serious losses in almost every summer, since 1985. This was caused by the pleomorphic intracellular Gram negative bacterium in the hepatopancreas of juvenile *Litopenaeus vannamei*, as revealed by histopathological and transmission electron microscopic examinations (Lightner and Redman, 1994). Shell disease in the form of erosion/ ulceration of the cuticle and is attributed to the activities of lipase, protease and chitinase enzymes produced by several species of bacteria (Lightner, 1993).

In West Bengal, the development in coastal aquaculture is centered on shrimp farming throughout the 158 km coastline of the state. The dominant species under shrimp culture is *P. monodon* due to its high unit value and ever-expanding export demand. Three districts of West Bengal *viz*., East Midnapore, North 24 Parganas and South 24 Parganas have brackishwater area of about 2,10,000 ha. suitable for coastal farming. The scientific culture of shrimps started in West Bengal during late 1980's and more than 39,000 ha area was brought under culture. There are three different types of shrimp farming *viz*., extensive, modified extensive and semi-intensive, based on inputs used and management practices followed. In 2004, the shrimp farmers of East Midnapore District, West Bengal noticed significant mortalities of *P. monodon*, which occurred between 45 and 70 days of stocking (Abraham and Sasmal, 2008). The present communication reports the bacteriological, histopathological and scanning electron microscopic results of these bacterial diseases which affected cultured *P. monodon.*

A severe epizootic of bacterial diseases was recorded between 45 and 70 days of stocking in a number of

commercial penaeid shrimp farms rearing *P. monodon* in Contai, Soula, Balisai and Digha regions of East Midnapur District, West Bengal, India in 2004. In severely affected farms cumulative mortality rates approached 60-90% within 70 days of stocking. Moribund shrimps, displaying clinical signs of disease, were taken from several farms, wrapped individually in U-V sterilised polythene bags, placed in icebox and brought to the laboratory. Few moribund shrimps were also brought to the laboratory in live condition in oxygen filled polythene bags or fixed in Davidson's fixative. The fixed shrimps were processed and sectioned as as per Bell and Lightner (1988) for histopathological studies. The sections were examined and photomicrographs were taken using a trinocular research microscope (Olympus, Model BX51, Japan).

For scanning electron microscopy (SEM), both normal and moribund shrimps were collected and fixed in glutaraldehyde. In brief, pleopods, gills and exoskeleton of both moribund and normal shrimps were cut in to small pieces (1 mm) and washed in phosphate buffered saline (PBS). The cut pieces were fixed in 2.5% glutaraldehyde solution for 30-60 min (100 ml PBS + 2.5 ml glutaraldehyde, Merck, India),washed again in PBS and kept in 2.5 % glutaraldehyde solution for 12-16 h. Samples were then washed again in PBS and dehydrated in graded alcohol series (70%, 90%, 100% ethanol) and finally in iso-amyl acetate for 30-90 min depending on the tissue characteristics. The soft tissues and gills were dried in critical point dryer (CPD) and the hard tissues, like shell and pleopods, were air-dried. After drying, the samples were separately placed on metal stab for gold coating in a gold sputter coater. The gold-coated samples were viewed in a scanning electron microscope and photomicrographs were taken.

The bacterial flora associated with hepatopancreas, hemolymph, gill, intestine and exoskeleton of diseased shrimps were isolated by streak plating on tryptic soy agar supplemented with 1% sodium chloride (TSAS), thiosulfate citrate bile salt sucrose agar (TCBS) and seawater complex agar (SWC). Enumeration of total plate counts (TPC) on TSAS, presumptive vibrio counts (PVC) on TCBS and luminous bacterial counts (LBC) on SWC from the hepatopancreas of moribund shrimps was done by spread plating as described in Gomez-Gil *et al*. (1998) and Lavilla-Pitogo *et al*. (1998). Randomly picked bacterial isolates from hepatopancreas, hemolymph, gills, intestine and exoskeleton of diseased shrimps on the basis of dominance, were identified by performing a series of biochemical reactions as described in MacFaddin (1980). Taxonomic keys proposed by Alsina and Blanch (1994) and Lechevallier *et al*. (1980) were followed for identification of vibrios and non-vibrios, respectively.

The gross signs displayed by the multiple disease affected shrimps from penaeid farms of West Bengal during the present study include red discoloration, parasitic infestation, shell disease, vibriosis, hepatopancreatitis and several other pathophysiological abnormalities.

Histopathological examination of shrimp muscle showed bacterial infection resulting in edema, haemocytic infiltration (Fig. 1), degeneration of cells and necrosis (Fig. 2). Severe necrosis in hepatopancreas was noticed in

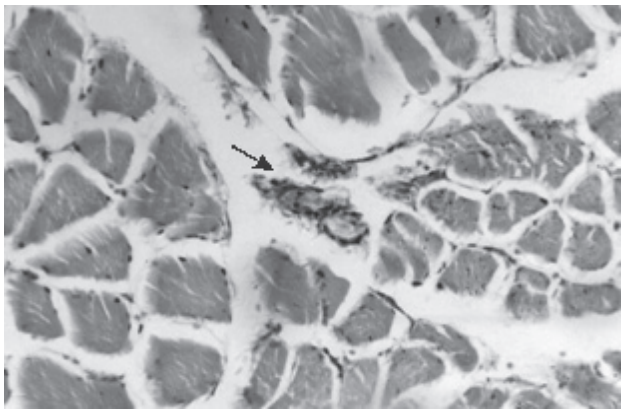

Fig. 1. Histological section of shrimp muscle tissue showing bacterial infection with edema and infiltration of haemocytes (H&E; X 100)

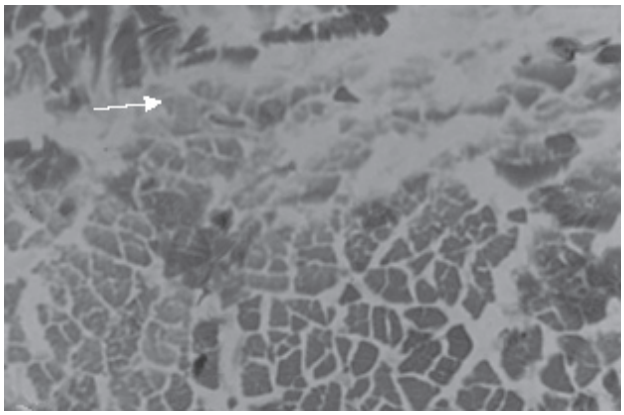

Fig. 2. Photomicrograph of shrimp muscle tissue section showing degeneration of muscle and necrosis due to bacterial infection (H&E; X 100)

the diseased shrimps (Fig. 3), with hepatopancreatic epithelial cell damage and accumulation sloughed cells in the lumen (Fig. 4). The hepatopancreas contains a variety of carotenoid pigments (mainly beta-carotene) and damaged hepatopancreas loses its ability to retain the stored beta-carotene as well as other carotenoids which are released in to the hemolymph, and leads to reddening of the entire body of the shrimp, a characteristic feature of red disease.

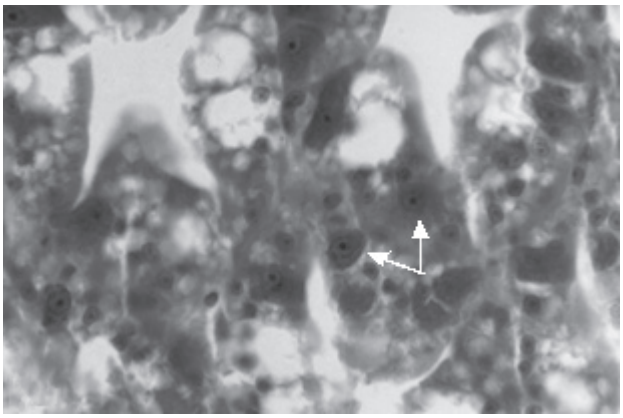

Fig. 3. Hepatopancreatic pathology in infected shrimp (H&E; X 400)

Hstopathological examination revealed extensive hepatopancreatic lesions, characterised by inflammatory sinuses with bacterial plaques and cell debris (Fig. 5), thickening of basal laminae of invaded tubules and separation of cell lining from the basal lamina (Fig. 6). Development of characteristic black or brown colouration observed in the affected parts of the exoskeleton was observed to be due to bacterial infection, as evident from the scanning electron micrographs (Fig. 7 a- d), which in turn induced formation of melanin and discolouration of exoskeleton

During the present study, *Vibrio* spp., *Aeromonas* spp. and *Pseudomonas* spp. were isolated from the hepatopancreas, hemolymph, intestine, gills and eroded portion of the exoskeleton. Luminous *V. harveyi* was the dominant bacterial flora in the affected organs, followed by non-luminous *V. harveyi, V. parahaemolyticus, V. alginolyticus,* other *Vibrio* spp., *Aeromonas* spp. and *Pseudomonas* spp. (Table 1). The hepatopancreas (HP) of diseased shrimp with mixed infection recorded total heterotrophic counts (THC), presumptive vibrio counts (PVC) and luminous bacterial counts (LBC) ranging from

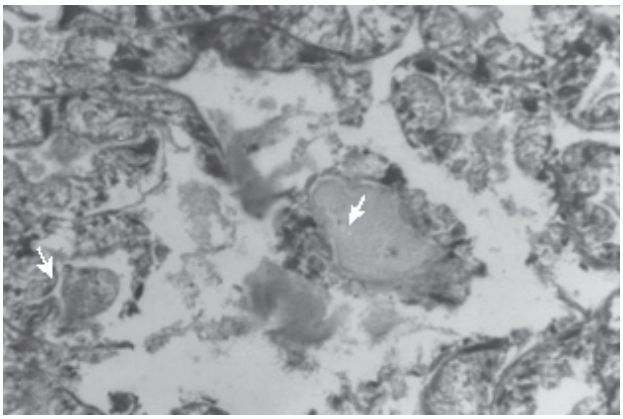

Fig. 5. Inflammatory sinuses with bacterial plaques, cell debris and necrosis in the hepatopancreas of infected shrimp (H&E; X 200)

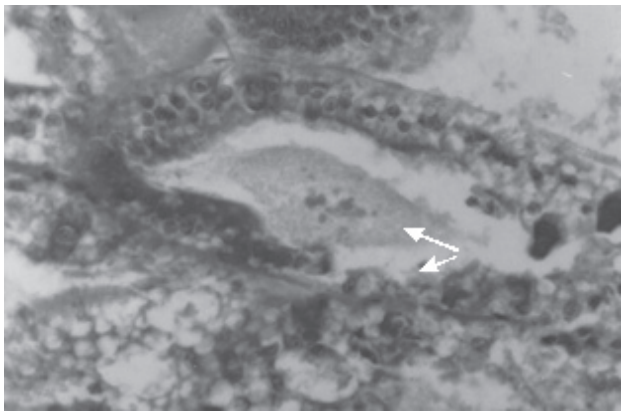

Fig. 4. Hepatopancreatic tissue with bacterial infection showing damage in the tubular epithelial layer and accumulation of sloughed cells in the lumen (H&E; x 400)

2.45×10^4 to 3.10×10^6 g HP $^{-1}$, 1.70×10^4 to 2.05×10^6 g HP $^{-1}$, 7.00×10^2 to 1.85×10^6 g HP $^{-1}$, respectively. *Vibrio* spp. were the single dominant population (65-92%). The results corroborate the findings of Lavilla-Pitogo *et al*. (1998). Chen *et al.* (1992), in their studies on two cases of epizootics with high mortalities in Taiwanese penaeid farms recorded high proportions of *Vibrio* spp. (73.4 - 84.6%) and majority of the isolates were of *V. damsela* and *V. harveyi*. The findings of Gomez-Gil *et al*. (1998) suggested that the presence of bacteria in the hepatopancreas is not necessarily indicative of disease and diagnosticians should expect to find a wide range of *Vibrio* isolates in the hepatopancreas of healthy *P. vannamei*.

From the observations on high cumulative mortalities (90%) and extensive damages in hepatopancreas and other organs caused by the mixed bacterial infections, it is concluded that the bacterial flora associated with the infection are highly virulent and capable of causing extensive damage to the hepatopancreas, a vital organ of shrimp, leading to high levels of mortality.

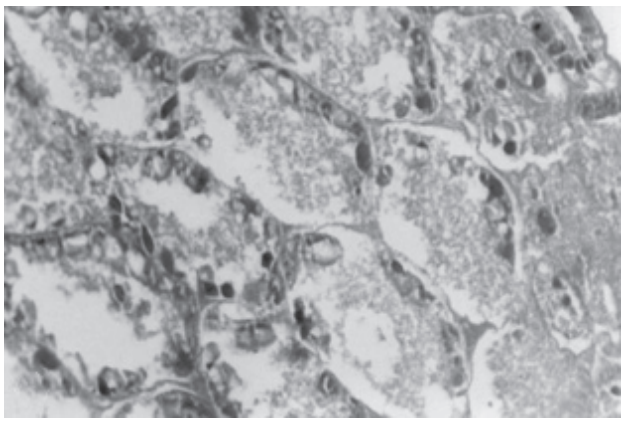

Fig. 6. Degeneration and massive necrosis in hepatopancreas of infected shrimp (H&E; X 200)

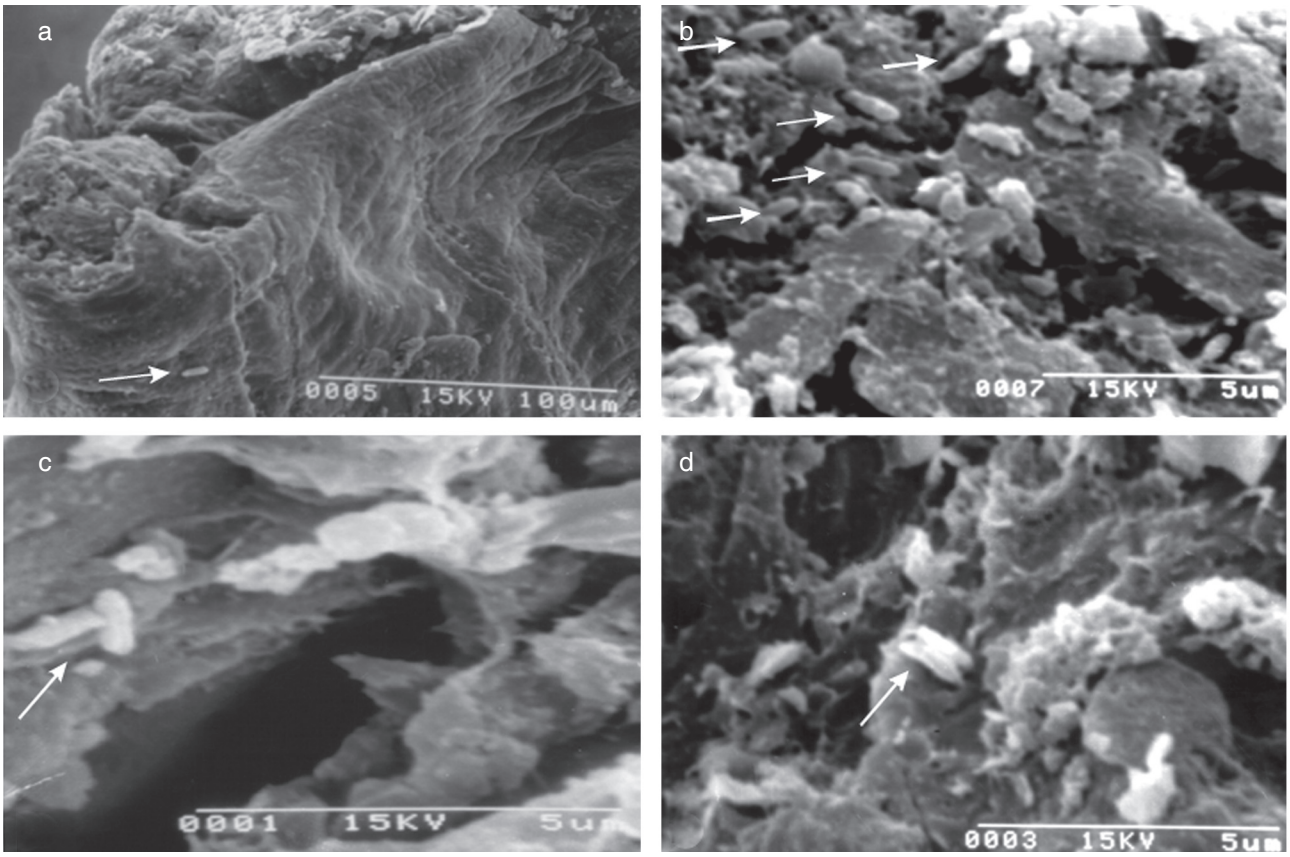

Fig. 7 a-d. Scanning electron micrographs of shell disease affected shrimp showing bacterial attachment on the granular core of damaged epithelium

Table 1. Bacterial flora associated with different organs of diseased *Penaeus monodon*

Species of bacteria	Hepatopancreas	Hemolymph	Gills	Intestine	Exoskeleton
Aeromonas spp.	-	+	+	+	+
Pseudomonas spp.	-	-	+	+	+
Vibrio alginolyticus	+	+	+	-	-
V. cholerae	-	-	-	+	-
V. fluvialis	+	-	+	+	+
V. furnissii	-	-	+	+	+
V. gazogenes	-	-	+	-	-
V. harveyi (luminous)	+	+	+	+	-
V. harveyi	+	+	+	+	+
V. parahaemolyticus	+	+	+	+	+
V. metschnikovii	-	+	-	+	-
V. splendidus I	-	-	+	+	-
V. pelagius II	-	-	+	-	-
Unidentified *Vibrio* spp.	+	-	+	+	+
Unidentified Gram negative group	-	-	+	+	+

- (Absent), + (Present)

Similar observations on the signs and symptoms of shrimps have been reported earlier from India (Abraham and Manley, 1995; Abraham and Sasmal, 2008; Karunasagar *et al.* (1997, 2007). Karunasagar *et al.* (1997) reported role of bacteria in the aggravation of pathological conditions in white spot syndrome virus (WSSV) affected shrimp.

It has been speculated that the typical 'red disease' syndrome of shrimps may be due to the effects of aflatoxins and microbial toxins present in the rancid or spoiled feeds or in the detritus of organically rich ponds (Lightner, 1993) or due to Gram positive and Gram negative bacteria (Limsuwan, 1993; Alapide-Tendencia and Dureza, 1997). These findings are in conformity with earlier reports on the hepatopancreas of diseased shrimp (Chen *et al*., 1992; Lightner and Redman, 1994; Loy *et al.,* 1996).

Shell disease can be characterised by single or multiple eroded areas in general on body cuticle with brownish to black colouration (Lightner, 1993). In the present study, such brown to black coloured multiple melanised and eroded areas were found on the dorsal surface of the exoskeleton. A number of factors have been suggested for the manifestation of shell disease, which includes involvement of various bacterial species that produce extracellular lipase, protease and chitinase enzymes (Lightner, 1993; Abraham and Manley, 1995). Chen *et al.* (1992) opined the route of invasion might be the stomach to the primary duct and the secondary duct, extending up to hepatopancreatic lumen. According to them, in naturally diseased *P. monodon*, these pathogenic bacteria invaded the hepatopancreatic tubules and caused extensive lesions, in association with or with out other pathogens, such as viruses and parasites.

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