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Histopathological and bacteriological studies of monodon slow growth syndrome (MSGS) affected shrimps

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ABSTRACT

Shrimps affected by monodon slow growth syndrome (MSGS) were sampled from culture ponds in Amalapuram and Bhimavaram areas of Andhra Pradesh during 2005-2010 and subjected to bacteriological as well as histopathological investigations. Three species of *Vibrios* were identified in the bacterial isolates from haemolymph *viz., V. alginolyticus*, *V. fluvialis* and *V. harveyi.* Histopathological studies revealed major changes in the hepatopancreas as well as gill tissue and the presence of monodon baculovirus (MBV), heptopancreatic parvo virus (HPV) and Infectious hypodermal and hepatopancreatic necrosis virus (IHHNV). Fifty percent of the MSGS affected shrimps showed single infections with MBV, 20% with HPV and 30% had dual infections of HPV and MBV.

Keywords: Bacteriology, Histopathology, Monodon slow growth syndrome (MSGS), *Penaeus monodon*, Shrimp viruses, *Vibrio* spp.

Introduction

Occurrence of diseases is one of the major threats to sustainable shrimp farming. Infectious deseases caused by bacteria, viruses, fungi, protozoans and disease conditions of non-infectious etiology such as nutritional and environmental factors, occur in shrimp culture systems. Further, certain new and emerging diseases are also appearing. Monodon slow growth syndrome (MSGS) is one such disease condition affecting the tiger shrimp *Penaeus monodon* in culture ponds in many areas. The disease is characterised by brittle antennae, bamboo segments and retarded growth in the affected shrimps. Slow growth of farmed *P. monodon* was first detected in Thailand in the year 2002 and was subsequently reported throughout the shrimp growing areas of Thailand and figures indicated that annual production volume was down by approximately 36% (Flegel, 2008). Chayaburakul *et al.* (2004) recorded HPV as a contributing factor of MSGS but not as the overriding factor responsible for MSGS. Prevalence of HPV and combined HPV/MBV infections in the affected shrimps were significantly higher as compared to normal shrimps (Chayaburakul *et al*., 2004).

Sritunyalucksana *et al.* (2006) identified Laem-Singh virus (LSNV) in MSGS affected *P. monodon* collected from ponds in Thailand. LSNV has also been detected in healthy shrimps and there was apparently no correlation between LSNV infection and slow growth (Chayaburakul *et al.*, 2004; Anantasomboon *et al*., 2006; Sritunyalucksana *et al.*, 2006). Sittidilokratna *et al.* (2009) made a survey on the prevalence of LSNV infection in *P. monodon* in Andhra Pradesh, India, and observed 56.8% of shrimps were infected with LSNV. Pratoomthai *et al.* (2008) suggested that retinopathy associated with LSNV may be linked to stunting of *P. monodon* in MSGS ponds.

Rai *et al.* (2009) recorded IHHNV, MBV, HPV and WSSV in shrimp infected with MSGS and also stated that IHHNV could be one of the causes of slow growth in cultured *P. monodon*. Panphut *et al.* (2011) reported a novel RNA, integrase-containing element (ICE) in giant tiger shrimp from MSGS ponds along with Laem-Singh virus (LSNV) and he also stated that they may act together as component causes of MSGS. Although many viral pathogens have been detected from MSGS affected shrimp, so far the exact aetiological agent has not been identified. In the present study, an attempt was made to identify the bacterial and viral pathogens associated with MSGS affected shrimps and to investigate their prevalence and pathogenicity.

Materials and methods

A survey on the prevalence of MSGS in shrimps in modified extensive and semi-intensive culture ponds in East and West Godavari districts of Andhra Pradesh was

undertaken during 2005-2010. Ten culture ponds were selected randomly for collection of samples. Ten MSGS affected shrimps from each pond (one hundred in total) were sampled and transported to the laboratory in live condition for microbiological and histopathological analyses. Haemolymph was drawn from the infected shrimp and pour plated using tryptone soya agar (TSA) and thiosulphate citrate bile sucrose agar (TCBS) to estimate total bacterial and total *Vibrio* counts. Pure isolates were subjected to taxonomical tests as given by Lightner (1996). Virulence studies were carried out by injecting the bacterial isolates (3 X10 ^{6} CFU g^{-1}) in healthy juvenile shrimps at different concentrations following Liu *et al*. (1996). For histopathological studies, the tissues were fixed in Davidson's fixative, processed, wax embedded and sectioned (5 µ thickness) and stained with Haematoxylin and Eosin (Lightner, 1996).

Results

Symptoms of MSGS

MSGS is characterised by brittle antennae, bamboo segments and retarded growth in affected shrimps. MSGS affected shrimp record a very slow growth rate of less than 0.1g per day after attaining weight of 10-15 g (Fig. 1)

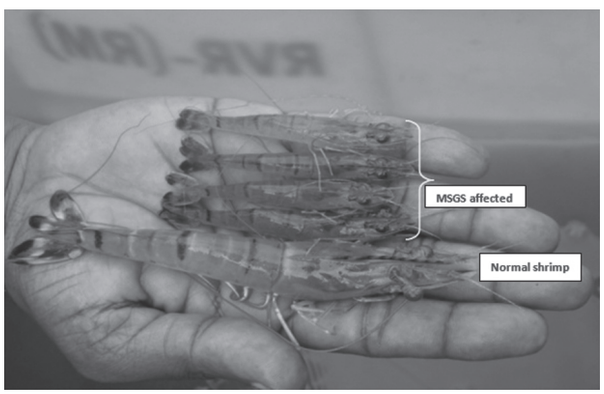

Fig. 1. *Penaeus monodon* affected by monodon slow growth syndrome (MSGS)

Prevalence of infection

The results of the survey undertaken on prevalence of MSGS in culture ponds of East and West Godavari districts revealed that the disease first appeared in the year 2005 affecting 5% of the stock. Over the years there is a gradual increase in the incidence, presently reaching 12% (Fig. 2). The prevalence of MSGS was very low in West Godavari District, where the salinity was lower in culture farms (below 15 ppt). The prevalence of infection varied between 12-28% in West Godavari District and 34-52% in East Godavari district. There was no incidence of MSGS in tiger shrimp grown in freshwater culture ponds of West Godavari District of Andhra Pradesh.

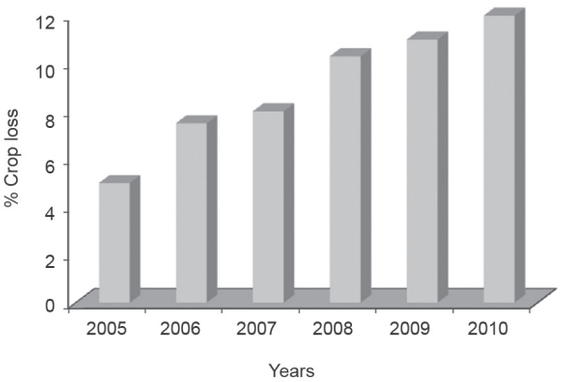

Fig. 2. Crop loss due to MSGS in shrimp culture ponds in Andhra Pradesh

Microbiological analysis

Th total bacterial count of MSGS affected shrimps from East Godavari District ranged from 0.7 x 10^{4} to 1.3 x 10^{6} cfu ml^{-1} and between 1.6 x 10^{3} to 2.4 x 10^{4} cfu ml^{-1} in West Godavari District. Average total *Vibrio* counts of MSGS affected shrimp from East Godavari District ranged between 0.8 x 10^{3} to 1.3 x 10^{4} cfu ml^{-1} and those from West Godavari District varied between 4.2 x 10^{2} to 3.3 x 10^{3} cfu ml^{-1}. Based on various morphological and biochemical characterisation tests, isolated bacteria were identified as *Vibrio harveyi, Vibrio alginolyticus* and *Vibrio fluvialis* (Table 1).

Table 1. Characterisation tests performed on bacterial isolates from monodon slow growth syndrome affected shrimp

Name of the test	Isolate 1	Isolate 2	Isolate 3
Gram's staining	-	-	-
Shape	Rod	Rod	Rod
Motility	+	+	+
Oxidase	+	+	+
Catalase	+	+	+
O/F Test	F	F	F
Acid production from glucose	+	+	+
NaCl tolerance test			
2%	+	+	+
4%	+	+	+
6%	+	+	+
8%	+	+	+
10%	+	+	+
Temperature tolerance test			
4 ^{0}C	-	-	-
20 ^{0}C	+	+	+
30 ^{0}C	+	+	+
40 ^{0}C	+	+	+
Decarboxylation of amino acids			
Arginine	-	-	+
Ornithine	+	+	-
Lysine	+	+	-

MR test	+	+	+
VP test	-	+	-
Indole test	+	+	-
Starch hydrolysis	+	+	-
Urea hydrolysis	+	+	+
Esculin hydrolysis	-	-	+
Gelatin liquefaction	+	+	+
Utilisation of carbohydrates			
L-Arabinose	+	-	+
Dextrose	+	+	+
Fructose	+	+	+
Lactose	-	-	-
Mannose	+	-	+
Galactose	+	+	+
Sucrose	+	+	+
Trehalose	+	+	+
Cellobiose	+	-	+
Melibiose	-	+	-
Salicin	+	-	+
Xylose	-	-	-
Citrate utilisation	+	+	+
Nitrate reduction	+	+	+
ONPG hydrolysis	+	-	+
Growth on TCBS	Y	Y	Y
Inhibition by 0/129 phosphate			
10 µg	R	R	R
150 µg	S	S	S
Luminescence	-/+	-	-
	V. h	*V. alg.*	*V. fluv.*

V. h = Vibrio harveyi, V. alg. = V. alginolyticus, V. fluv.= V. fluvialis

Among the *Vibrio* spp. isolated from MSGS affected shrimps collected from West Godavari District, *V. alginolyticus* was more prevalent (70%) followed by *V. harveyi (*25%), *V. fluvialis* (3%) and other vibrios (2%) . In East Godavari District, *V. harveyi* was more prevalent with 52% infection followed by *V. alginolyticus* (38%), *V. fluvialis* (6%) and other vibrios (4%) (Fig. 3).

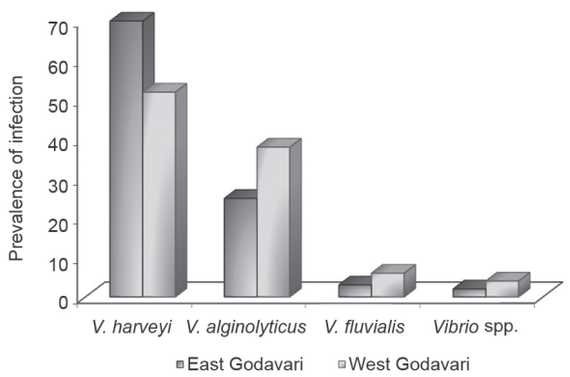

Fig. 3. Prevalence of *Vibrio* infections in MSGS affected shrimps in East and West Godavari Districts of Andhra Pradesh

Histopathological studies

Histopathological investigations of the hepatopancreatic tissues from the affected shrimps revealed the presence of basophilic intranuclear inclusion bodies with crescent shaped nuclei pushed to one side of the nucleolus characteristic of HPV infection (Fig. 4) and the presence of polyhedral eosinophilic occlusion bodies characteristic of MBV infection (Fig. 5) in the hepatopancreatic cells. Prevalence of WSSV, HPV and MBV infections varied between the samples collected from the two districts. Fifty percent of MSGS affected shrimps collected from West Godavari District and 30% of those from East Godavari District were found to be infected with MBV. HPV infection was more prevalent (40%) in East Godavari District. Dual infections with MBV, HPV and IHHNV (Fig. 6 and 7) were observed in both East and West Godavari districts, whereas multiple infections were recorded only from East Godavari District and the prevalence was very low (Fig. 8).

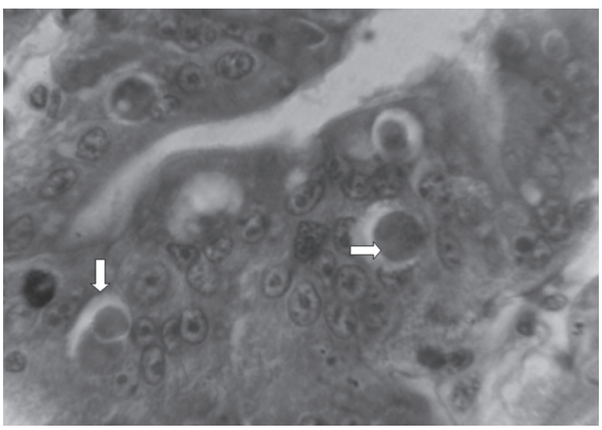

Fig. 4. Section of hepatopancreas showing HPV infection (H & E; X 400)

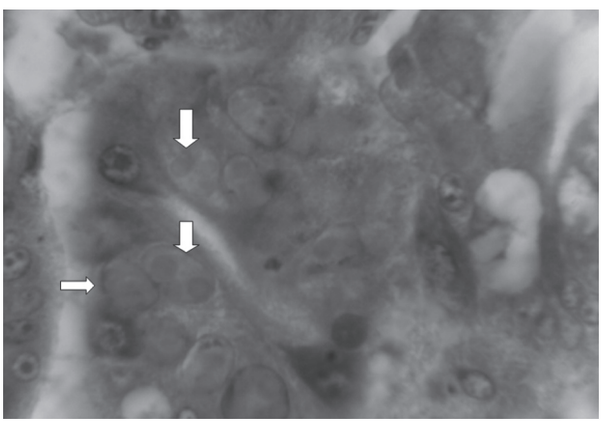

Fig. 5. Section of hepatopancreas showing MBV infection (H & E; X 400)

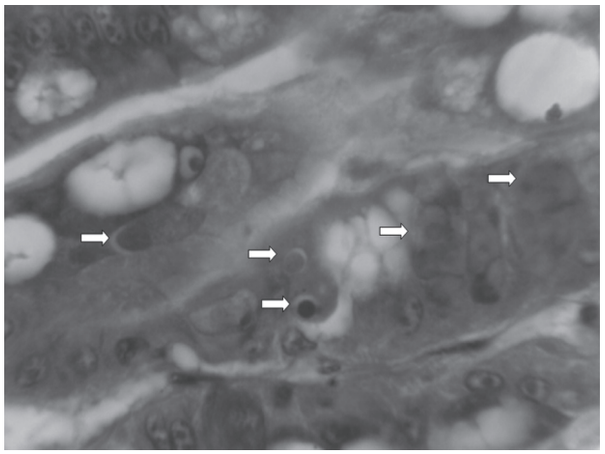

Fig. 6. Section of hepatopancreas showing MBV as well as HPV infections (H & E; X 400)

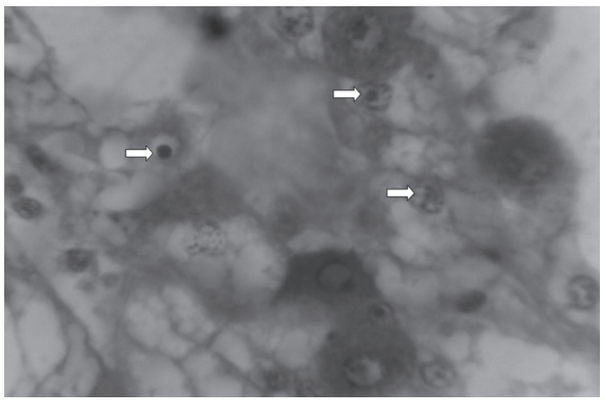

Fig. 7. Section of gill showing IHHNV infection (H & E; X 400)

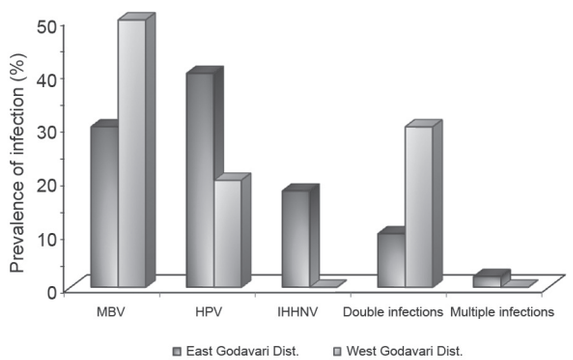

Fig. 8. Prevalence of viral infections in MSGS affected shrimps in East and West Godavari districts of Andhra Pradesh

Discussion

Monodon slow-growth syndrome (MSGS) is characterised by abnormally slow growth and coefficients of size variation was greater than 35% (Sritunyalucksana *et al*., 2006). In Thailand, the average growth rate in MSGS ponds is approximately half that normally expected by shrimp farmers (Pratoomthai *et al.*, 2008). Chayaburakul *et al*. (2004) made investigations on the occurrence of MSGS in relation to the viral pathogens *viz.*, MBV, HPV and IHHNV and found that the disease was not correlated with these viruses. Studies by Sritunyalucksana *et al*. (2006) on MSGS in *P. monodon* revealed the possibility that LSNV could act in concert with other agents or under certain environmental conditions to cause MSGS. Findings of Pratoomthai *et al*. (2008) suggested that LSNV might be causally associated with MSGS in a complex manner.

In India, MSGS was encountered during 2005 and investigations on the viral pathogens involved in MSGS revealed the presence of IHHNV, MBV, HPV and LSNV (Rai *et al.,* 2009). Their study revealed the presence of IHHNV in India and indicated that this virus could be one of the causes of slow growth in cultured *P. monodon*. Madhavi *et al.* (2002) recorded multiple viral infections in shrimp with stunted growth. The present investigation on MSGS in shrimp culture ponds of East and West Godavari districts also revealed occurrence of IHHNV, HPV, MBV as single, dual as well as multiple infections. IHHNV and HPV were more prevalent in East Godavari District and MBV was dominant in West Godavari District. Occurrence of multiple infections with all the three viruses was more predominant in East Godavari District and dual infections were more prevalent in West Godavari District. Rai *et al*. (2009) observed the presence of IHHNV, MBV and HPV along both coasts of India but LSNV was recorded only in the east coast. They also observed that, prevalence of IHHNV was highest (25%) followed by HPV (6.9%), LSNV (4.2%) and MBV (4.2%), and also observed that the occurrence of dual infections was very low. Prevalence of infection with all the three viruses was very low (2%) and was recorded only in East Godavari District. Among bacterial infections, *V. alginolyticus* was the most dominant species among the vibrios identified from West Godavari District, whereas in East Godavari District, *V. harveyi* was most dominant. Variations in the prevalence of infections between the two districts could be attributed to the differences in the environmental conditions of the two areas. The present study suggests that MSGS in India may be caused by multiple infections with viral and bacterial pathogens. Further studies are needed to investigate the involvement of unknown pathogens.

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