Prevalence of white spot syndrome virus and monodon baculovirus in *Penaeus monodon* broodstock and postlarvae from hatcheries in southeast coast of India

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During the period March 2000 to May 2002 broodstock and postlarvae (PL) collected randomly during different months from the shrimp hatcheries in the southeast coast of India were screened for white spot syndrome virus (WSSV) and monodon baculovirus (MBV) by polymerase chain reaction (PCR). Nested PCR was performed for detection of WSSV, whereas wet-mount method and one-step PCR were carried out for MBV. Prevalence of WSSV ranged from 25 to 50% in the broodstock and 10–13% in PL by nested PCR. MBV prevalence ranged from 25 to 60% in the broodstock and 34 to 39% in the PL by non-nested PCR. A follow-up study showed that successful culture was obtained in 87% of the shrimp farms stocked with the PL that were negative for WSSV and MBV by PCR.

Keywords: Broodstock, hatcheries, monodon baculovirus, postlarvae, *Penaeus monodon*, white spot syndrome virus.

VIRAL diseases are a challenging problem for the global shrimp culture industry. Disease caused by white spot syndrome virus (WSSV) has resulted in heavy mortalities and consequent production losses to the shrimp culture industry in many countries in Asia and Latin America[1–5]. WSSV is extremely virulent with a wide host range and targets various tissues of ectodermal and mesodermal origin[6–8]. All age groups and sizes of shrimp are affected by WSSV in all kinds of aquaculture systems (extensive, semi-intensive and intensive.[4]) Monodon baculovirus (MBV) causes an epizootic disease in larval and adult shrimp[9–11]. This virus has been reported to be widely present in shrimp broodstock and larvae in several parts of Asia[12,13], but there are few reports from India[11,14–16]. Presence of WSSV in broodstock has been reported from different countries[17–19].

Transmission of WSSV occurs vertically from infected broodstock to larvae or horizontally through water or infected animals. In the case of MBV, transmission occurs horizontally through fecal oral route. Prevalence of MBV in *Penaeus monodon* broodstock has been reported from several countries in Asia, but there is little information from India. Data on prevalence of viruses in broodstock would be important to develop strategies for health management. In the case of WSSV, virus positive broodstock may yield either virus negative or virus positive larvae depending on degree of infection[20,21]. Techniques such as nested PCR allow gradation of viral infection, with highly infected animals being positive in non-nested PCR and lightly infected ones being positive only in nested PCR[8].

There are about 72 *P. monodon* hatcheries located along the southeast coast of India, with an annual production capacity of 2933 million postlarvae (PL) accounting for 27% of the total national PL production (Fishing Chimes 2001). This study was conducted with an aim to know the prevalence of WSSV and MBV in the broodstock and PL from these commercial hatcheries.

P. monodon broodstock were randomly collected from either commercial hatcheries or distributors involved in the collection and distribution of broodstock to the hatcheries. Broodstock that were not used for spawning before were selected for the study. *P. monodon* PL samples were randomly collected from different commercial hatcheries in Chennai, southeast coast of India, during March 2000 to May 2002. Each sample consisted of a minimum of 1000 live PL (PL12-20). The total length (from the rostral tip to the telson) of the PL varied from 10 to 18 mm. About 500 PL were randomly selected from each sample and fixed in 95% ethyl alcohol for detection of WSSV and MBV by PCR. About 100 PL were randomly selected from each sample and kept in live condition for diagnosis of MBV by wet mount method. After excising a portion of hepatopancreas from live broodstock for the preparation of wet mount squash, broodstock were fixed individually in 95% ethyl alcohol for diagnosis of WSSV and MBV by PCR.

The hepatopancreas from the PL/broodstock was dissected and placed on clean glass slides. The tissues were stained with 0.05% aqueous malachite green and squashed gently with a cover slip. The slides were observed under the microscope.

Diagnostic PCR was carried out for WSSV and MBV in PL and broodstock. A commercial PCR diagnostic kit (Ampli-WSSV and Ampli-MBV kit, Mangalore Biotech Laboratory, Mangalore) was used. About 20–30 whole shrimp PL or 150 mg of gills/pleopods and hepatopancreas from the broodstock were used for extracting DNA as recommended by the manufacturer of the kits. Next, 2 µl of template DNA was taken for detection of WSSV or MBV by PCR. Nested PCR amplification was carried out for WSSV following a temperature profile, with an initial denaturation for 5 min at 94°C followed by 30 cycles at 94°C for 30 s; 55°C for 30 s; 72°C for 30 s and a final extension at 72°C for 5 min. Non-nested PCR amplification was carried out for MBV detection. A temperature profile with an initial denaturation for 5 min at 94°C followed by

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30 cycles at 94°C for 30 s; 60°C for 30 s; 72°C for 30 s and a final extension at 72°C for 5 min was followed. In PCR amplification, WSSV-positive samples yielded a fragment of either 486 bp in the first step or 310 bp in the nested step. MBV positive samples yielded a 361 bp fragment by non-nested PCR. PCR products were separated by agarose gel (1.2%) containing 0.5 μg ml^{-1} ethidium bromide and observed in a DNA transilluminator.

Among the 94 broodstock samples examined, 37 (39.4%) were positive for WSSV either in the first step PCR or nested PCR (Table 1). Presence of WSSV in broodstock has been reported in many countries[17–19]. The 39.4% prevalence of WSSV recorded in broodstock suggests that WSSV is highly prevalent in the wild shrimp population in India, as reported by Hossain *et al.*[19]. The prevalence of WSSV in broodstock noted in this study is lower compared to 75% prevalence reported by Otta *et al.*[18] from the west coast of India. Observations in this study show that 13% of the brooders in the southeast coast had fairly high level of infection with WSSV, being positive by non-nested PCR. Since WSSV can infect oocytes and follicle cells in the connective tissue in the ovary, it has been suggested that the virus can be transmitted vertically[20]. It has been further noted that larvae obtained from broodstock that are WSSV-positive by PCR, could be positive in non-nested reaction or PCR-negative[20,21]. It would be important to know the level of infection in the broodstock, so that the risk of producing infected larvae could be assessed. Table 1 shows that the prevalence of WSSV during the three years ranged from 25 to 50%.

Among the 1451 PL samples examined, 180 were positive for WSSV either in the first-step PCR or in the nested PCR, giving an overall prevalence of 12.4% (Table 2). A total of 56 samples (3.8%) were positive for WSSV in the one-step PCR, indicating high degree of infection and 124 samples (8.5%) were positive for WSSV by nested PCR (Table 2). Yearly prevalence of WSSV PL varied from 10 to 13% during the period March 2000 to May 2002 (Table 1). It is interesting to note that the prevalence of WSSV in PL was much lower compared to the prevalence in the broodstock. This could be due to production of WSSV-negative larvae from broodstock that was mildly infected (positive only in nested PCR). This has implications for hatchery operations. In case broodstock are positive by WSSV only by nested PCR, it could be recommended that they be used for spawning and nauplii could be tested again. If found negative, the hatchery could continue rearing the larvae.

Results in Table 1 suggest that during the three-year study, the percentage of broodstock positive for WSSV (nested and non-nested) ranged from 25 to 50. Though the prevalence of WSSV as indicated by nested PCR increased during 2001 and 2002, the corresponding increase in animals positive to non-nested PCR was marginal. This might explain the similar prevalence of WSSV observed during the three years by PCR.

Among the broodstock samples tested, 27 (28.7%) were positive for MBV by wet mount method and 39 (41.5%) were positive for MBV by non-nested PCR. Out of 1451 PL samples examined for MBV, 378 (26%) samples were MBV-positive by wet mount method. Microscopic observation of wet mount squashes prepared from the hepatopancreas had characteristic single/multiple intranuclear MBV occlusion bodies. When all the samples, including those examined by wet mount method were subjected to non-nested PCR, 544 (37.5%) were positive for MBV.

Table 1. Prevalence of WSSV and MBV in *Penaeus monodon* brooder samples collected during March 2000 to May 2002

Period	Total brooders diagnosed (no.)	WSSV positive (no.)		Prevalence of WSSV (%)	MBV positive by PCR (no.)	Prevalence of MBV (%)
		One step PCR	Nested PCR			
March to December 2000	28	3	4	25.0	12	42.8
January to December 2001	30	4	8	40.0	18	60.0
January to May 2002	36	6	12	50.0	9	25.0
Total	94	13	24	39.4	39	41.5

Table 2. Prevalence of WSSV in *P. monodon* postlarvae collected from commercial hatcheries during March 2000 to May 2002

Period	Total samples diagnosed (no.)	WSSV-positive (no.)		Prevalence of WSSV (%)
		One step PCR	Nested PCR	
March to December 2000	306	12	19	10.1
January to December 2001	560	23	49	12.9
January to May 2002	585	21	56	13.1
Total	1451	56	124	12.4

Table 3. Prevalence of MBV in *P. monodon* postlarvae collected from commercial hatcheries during March 2000 to May 2002

Period	Total samples diagnosed (no.)	MBV-positive by PCR (no.)	Prevalence of MBV (%)
March to December 2000	306	105	34.3
January to December 2001	560	211	37.7
January to December 2002	585	228	39.0
Total	1451	544	37.5

The yearly prevalence of MBV (arrived based on the PCR results) was observed to show an increasing trend and ranged from 34 to 39% in PL samples (Table 3).

This study further shows that MBV is highly prevalent in both broodstock and larvae along the southeast coast of India. Many researchers in India have reported higher prevalence of MBV compared to WSSV. Ramasamy *et al.*[11] have reported MBV prevalence of 68–92% in PL from the southeast coast of India by wet mount squash method. Otta *et al.*[16] have reported MBV in 54% PL samples in the west coast by PCR. MBV infection in broodstock has been reported by a number of workers[21–24]. Ramasamy *et al.*[24] have reported prevalence of up to 20% in the wild caught broodstock in the southeast coast of India. Karunasagar *et al.*[15] reported a prevalence of 20% in the broodstock collected from the west coast. In this study, prevalence of MBV recorded in broodstock samples ranged from 25 to 60% during the period March 2000 to May 2002.

This study showed prevalence of MBV (34–39%) to be higher than WSSV (10–13%) in PL, whereas there was no wide variation in the prevalence ranges of MBV (25–60%) and WSSV (25–50%) in broodstock. Most of the shrimp hatcheries along the southeast coast follow PCR to screen for WSSV in broodstock and in various larval stages of *P. monodon* to know the WSSV-free status of the PL. However, much attention is not being paid by the hatcheries in screening the broodstock for MBV due to its lesser impact on culture and production. Higher prevalence of MBV in the PL could be attributed to the lack of adopting effective screening and prevention strategies. A lower prevalence of WSSV in PL (12.4%) compared to the broodstock (39.4%) could be due to avoidance of WSSV-infected brooders by the hatcheries, as many hatcheries have set up PCR facilities for screening brooders and larvae for viruses with the financial support from the government.

A follow-up study was conducted in shrimp farms stocked with PL that were negative for WSSV and MBV by PCR. A total of 83 shrimp farms were randomly selected for the study. About 73 (87%) shrimp farms had a successful harvest and 12 farms failed in different days of culture due to disease outbreaks that could be attributed to contamination from the creek and neighbouring farms. The successful crops in shrimp farms stocked with PCR tested virus-free PL show that PCR is a good diagnostic tool that could be employed for screening of PL and broodstock, as suggested by Peng *et al.*[25].

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ACKNOWLEDGEMENTS. Services of the Bioinformatics Centre, Department of Biotechnology, College of Fisheries, Mangalore are acknowledged.

Received 14 March 2005; revised accepted 11 July 2005