Indian Journal of Marine Sciences
Vol. 40(3), June 2011, pp. 403-406

Detection of white spot syndrome virus (wssv) in brood stock of tiger shrimp, *penaeus monodon* and other crustaceans of Andaman waters

S.N. Sethi, V. Mahendran, K. Nivas[1], P. Krishnan, S. DamRoy, Nagesh Ram & Shalini Sethi

Central Agricultural Research Institute, Post Box No. 101, Port Blair-744101, A&N Islands,
[1] Department of Biotechnology, Marudupandiyar College, Thanjavur-613403, Tamil Nadu
[Email: sethisatyanarayana@yahoo.co.in]

Received 25 May 2009; revised 13 July 2010

Survey had been convened off South & North Andaman Seas to find out the presence of WSSV. In the present investigation the WSSV was detected in wild caught *P. monodon* brood stocks. This virus were also detected in other crustaceans such as Mud crab, *Scylla serrata*, and Banana shrimp, *Fenneropenaeus merguiensis* collected from South and North Andaman during the year 2006 to 2007. While the prevalence of WSSV was 26.38% in *P.monodon* brood stocks, it was 31.66% for other crustaceans.

[**Keywords**: White spot syndrome virus, *Penaeus monodon*. Brood stock, Andaman.]

Introduction

Aquaculture is the world's fastest growing industry. Disease outbreaks have caused serious economic losses to this industry in many South East Asian countries. According to a recent World Bank report, global losses as a result of disease are around US $ 3000 million[1] . The disease caused by White Spot Syndrome Virus (WSSV) is characterized by the presence of white spots in the exoskeleton of infected shrimp and losses have been estimated to be several million dollars in different parts of India[2].

The virus belongs to a unique genus Whispovirus and now commonly known as white spot syndrome virus (WSSV). It is a double stranded DNA virus. Affected shrimp show rapid reduction in food consumption, become lethargic with reddening and loose cuticle. Characteristic white spot of 0.5 to 2 mm diameter at inner side of carapace is common in moribund and dead shrimp. Mortality rates reach up to 100% in 3-10 days. The virus infects all sizes and species of Penaeid shrimp in all types of culture systems[3] . Since 1993, white spot disease (WSD/ WSSV) has been causing significant production losses in cultured shrimp all over South East and South Asia. WSSV is extremely virulent with a wide host range and targets various tissues of ectodermal and mesodermal origin. This virus has been reported to be widely present in shrimp brood stock and larvae in several parts of Asia[4,5] but there are few reports from India[6,7,8]. Presence of WSSV in brood stock has been reported from different countries[9]. Transmission of WSSV occurs vertically from infected brood stock to larvae or horizontally through water or infected animals. In the case of WSSV, virus positive brood stock may yield either virus negative or virus positive larvae depending on degree of infection Lo *et al.* [10] . 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. Since brood stocks collection to larval production still depends on wild catch, it is necessary to estimate the degree of infection.

Materials and Methods

Tiger Shrimps, *Penaeus monodon* brood stock and other crustaceans were collected from different landing centers and hatcheries of Andaman. Totally 103 samples were collected consists of Tiger Shrimp, *P. monodon* , Mud crab, *Scylla serrata* and Banana Shrimp, *Fenneropenaeus merguiensis*. Pleopod muscle of all the shrimps and leg meat of mud crab were collected and kept in absolute ethanol during transportation. Specimens were stored –80^{o}C until required. Diagnostic PCR was carried out. A commercial PCR diagnostic kit (WSSV detection kit, Genei, Bangalore and CIBA, Chennai) was used. To extract DNA 20-30 mg of pleopod muscle or leg meat of crabs was ground with plastic pestle in PCR tubes containing 1 ml of extraction buffer. Samples were

incubated at 95°C for 10 minutes and centrifuged at 10,000 rpm for 10 minutes. About 50-100 µl of supernatant containing sample DNA collected in a 0.5 ml vial for detection of WSSV. The amplification was carried out in 25 µl reaction volume in 0.2 ml PCR tubes. For the 1st Step amplification, 23 µl of first PCR premix (contain $MgCl_2$, dNTPs, and WSSV specific and external primers) and 1 µl of *Taq* DNA polymerase were added to each tubes followed by 1 µl of template DNA. Positive and Negative controls were included in the reaction. The PCR cycle consisted of an initial denaturation at 95°C for 3 minutes, followed by 27 cycles of 95°C for 30 seconds, 58°C for 30 seconds and last cycle of 72°C for 5 minutes. The reactions were stored –20°C for nested PCR. Nested PCR was carried out in 0.2 ml of PCR tubes containing 23 µl of nested PCR premix (containing PCR reaction buffer, $MgCl_2$, dNTPs, and WSSV specific internal primers) 1 µl of *Taq* DNA polymerase, and 1 µl of first step PCR. The reaction was amplified in the thermocycler ((DNA Engine (BIO-RAD): 1,200 × 1,600 pixels, file size: 614 KB, MIME type: image/jpeg) with the similar cycle conditions as in the 1st Step PCR reaction. The amplified DNA (12 µl) was mixed with 6X gel loading dye (2 µl) and run on 2% agarose gel using 1X TAE running buffer. The ethidium bromide (10 mg/ml) stained gel was visualized under UV transilluminator.

Results

Among the 255 samples analysed, 35 brood stocks and 16 other crustaceans were positive for WSSV either in the first-step PCR or in the nested PCR, giving an overall prevalence of 17% (Table 1).

The Tiger Shrimp, *Penaeus monodon* was negative in first step PCR but positive in second step with infection rate of 55%. Banana shrimp, *Fenneropenaeus merguiensis* showed 18% prevalence, where as Mud crabs *Scylla serrata* showed 8% positive in. The average length and weight of Tiger Shrimp of Betapur and Chouldari were 19.625 ± 2.69 mm, 60.17± 24.96 gm and 18.70 ± 2.12 mm, 56.30 ± 18.07 gm respectively. During 2006, two brood stock shrimp collected from the wild clinically showed white spot on their carapace and throughout the body whereas three brooders showed same clinical sign during 2007 (Fig. 1) Tiger Shrimp, *Penaeus monodon* of Betapur, North Andaman shows four White spot syndrome virus specific bands in nested PCR reaction (Fig. 2).

Table 1—Detection of WSSV in brood stock of Tiger Shrimp and its susceptible carrier using PCR technique

Sl. No	Species	Total no. of Sample tested		Positive for WSSV		Total % Prevalence of WSSV	Clinical Signs of WSSV
		2006	2007	2006	2007		
1.	*Penaeus monodon*	50	85	13	22	25	Five brooders of Tiger shrimp, showed white spot on the carapace and other parts of the body.
2.	*F. merguensis*	30	30	6	5	18	
3.	*Scylla serrata*	30	30	3	2	8	
	Total	110	145	24	33	17	

Fig. 1—WSSV infected brood stocks of Tiger Shrimp, (*Penaeus monodon*) of Andaman waters

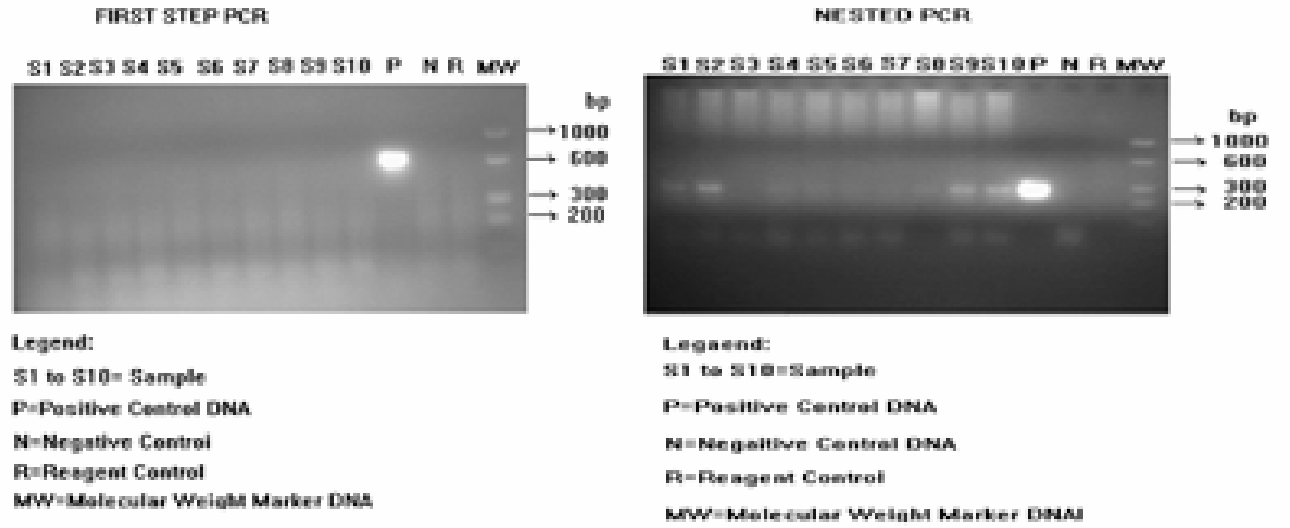

Fig. 2—Tiger Shrimp, *Penaeus monodon* shows four White Spot Syndrome Virus (WSSV) specific bands in nested PCR reaction. (Betapur, North Andaman).

Discussion

Various diagnostics methods have been developed to monitor and control WSSV. PCR techniques particularly nested PCR have been found to be most sensitive among the diagnostic method. The level of infection has been considered to be important for the management of WSSV in culture system and in this context; the PCR techniques are very useful to screen the level of WSSV infection. Magbanua *et al*. (2000)[11] reported that the transmission of virus was WSSV-positive spawners to their offspring. Screening and selecting WSSV-negative brooders markedly reduces the chances of a subsequent outbreak of WSSV[9,10,11] . A recent study conducted by Hossain *et al*. (2001)[12] revealed that an infection of WSSV observed in India was 55% and actual incident could be even higher than that WSSV prevalence was also reported to the quite high in wild decapods populations[11] . WSSV can be vertically transmitted from WSSV positive shrimps to their offspring[13,11].

Conclusion

The present study found that the WSSV was present in crustaceans (*P. monodon* and Carriers: Mud crab, *Scylla serrata*, Banana shrimp, *Fenneropenaeus merguiensis*) collected from South and North Andaman. A preliminary survey carried out by Central Institute of Brackish water Aquaculture, CIBA, Chennai during 2004, the prevalence of WSSV in brood stock of Tiger Shrimp, *P.monodon* was 25% where as other crustaceans (*F. merguensis* and *Portunus pelagicus*) showed low prevalence of WSSV 13% was found in Andaman water. Findings of present study are in support of the study carried out by CIBA, Chennai during 2004.

Acknowledgement

Authors express their deep gratitude to Director, CARI, Port Blair for his valuable guidance and help for carrying out the above research work.

References

1 Lundin CG (1996). Global attempts to address shrimp disease. Marine/Environmental Paper No. 4 Land, Water and Natural Habitats Division, Environment Department, The World Bank, p 45

2 Anonymous. Report of marine products export development agency. The Hindu, June 4, (1996) pp 17.

3 Karunasagar, I., Otta, S. K. and Karunasagar, I. Monodon baculovirus (MBV) and bacterial septicemia associated with mass mortality of cultivated shrimp (*Penaeus monodon*) from east coast of India. *Indian J. Virol*., 14 (1998) 27-30.

4 Baticados, M. C. L., Pitago, C. R. L., Paner, M. G., Pena, L. D. and Tendencia, E. An Occurrence and pathology of *Penaeus monodon* baculovirus infection in hatcheries and ponds in the Philippines. *Isr. J. Aquacult.*, 43 (1991) 35-41.

5 Ramasamy, P., Brennan, G. P. and Jayakumar, R., A record and prevalence of monodon baculovirus (MBV) from post larval *Penaeus monodon* in Madras, India. *Aquaculture*, 130 (1995), 129-135.

6 Manivannan, S., Otta, S. K., Karunasagar, I. and Karunasagar, I. Multiple viral infections in *Penaeus monodon* shrimp post larvae in an Indian hatchery. *Dis. Aquat. Org*., 14 (2000) 48, 233-236.

7 Otta, S. K., Shuba, G., Joseph, B., Chakraborthy, A., Karunasagar, I. and Karunasagar, I. Polymerase chain

reaction (PCR) detection of white spot syndrome virus (WSSV) in cultured and wild crustaceans in India. *Dis. Aquat. Org.*, 38 (1999) 67-70.

8 Tsai, M. F. *et al.* Long-term presence of white spot syndrome virus (WSSV) in a cultivated shrimp population without disease outbreaks. *Dis. Aquat. Org.*, 38 (1999) 107-114.

9 Itami, T. *et al.* Possible prevention of white spot syndrome in kuruma shrimp (*Penaeus japonicus*) in Japan. In *Advances in Shrimp Biotechnology* (ed. Flegel, T. W.), National Center for Genetic Engineering and Biotechnology, Bangkok, (1998) pp. 291-295.

10 OLo C.F., Ho C.H., Peng S.E., Chen C.H., Hsu H.C., Chiu Y.L., Chang C.F., Liu K.F., Su M.S., Wang C.H & Kou G.H. White spot syndrome baculovirus (SWSDBV) detected in cultured and captured shrimps, Crabs and other arthropods. *Dis.Aquat. org.,* 27 (1996) 215-225.

11 Magbanua F.O., Natividad K.T., Migo V.P., Alfafara C.G., de la Pena F.O., Miranda R.O., Albaladejo J.D., Nadala E.C.Jr, Loh P.C. & Mahilum-Hossain, S., Chakraborty, A., Joseph, B., Otta, S.K., Karunasagar, I., Karunasagar, I., (2001). Detection of new hosts for white spot syndrome virus of shrimp using nested polymerase chain reaction. Aquaculture 198, 1-11.

12 Lo C.F. Chen C.H., Liu K.F., Chiu Y.L., Yeh P.Y., Peng S.E., Hsu H.E., Lo.H.C., Chang C.F., Wang C.H & Kou G.H. Detection and tissue tropism of white spot syndrome (WSBV) in captured brooders of *Penaeus monodon* with a special emphasis on reproductive organs. *Dis. Aquat. org.,* 30 (1997) 53-72.