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Short Communication

Prevalence of white spot virus and monodon baculovirus in shrimp culture systems of West Bengal, India

Thangapalam Jawahar Abraham • Anjan Mondal • Avijit Patra • Harresh Adikesavalu

Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, India

Correspondence
Thangapalam Jawahar Abraham; Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, India

abrahamtj1@gmail.com

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Abstract

The global shrimp aquaculture is impacted by episodes of viral diseases resulting in huge income losses. This communication presents the results of the polymerase chain reaction (PCR) based surveillance of white spot virus (WSV) and monodon baculovirus (MBV) in shrimp culture systems of West Bengal, India. The WSV was detected in 14.87% of the total samples (*N* = 121) by first PCR and 16.53% of samples by nested PCR. The WSV infection was noticed in 12 of 65 *Penaeus monodon*, 6 of 39 *Litopenaeus vannamei* and 2 of 11 *Macrobrachium rosenbergii* samples. The MBV was detected in 8 of 65 *P. monodon* samples by non-nested PCR and all were also positive for WSV, thus indicating concurrent infection of shrimp. The results emphasized the need to observe strict quarantine measures during the seed selection to prevent the introduction of viral pathogens in grow-out systems.

Keywords: *Penaeus monodon*; *Litopenaeus vannamei*; *Macrobrachium rosenbergii*; white spot virus; monodon baculovirus; polymerase chain reaction

Development of coastal aquaculture in West Bengal is centred on shrimp farming. Commercial culture of shrimp started in West Bengal during the mid-1980s and by 2010 more than 54,000 ha area has been brought under culture through traditional, improved traditional, extensive, improved extensive, semi-intensive and intensive methods in South 24 Parganas, North 24 Parganas and East Midnapore districts (Abraham and Sasmal 2008; Ananda-Raja *et al.* 2012a, 2012b; Anon 2019). This State contributes nearly 25% of farmed shrimp in the country and is the 2nd largest producer of cultured shrimp after Andhra Pradesh (Anon 2019). The rapid growth of the shrimp farming industry halted suddenly in 1996–97, attributed mainly to the environmental and health problems resulting in the outbreak of viral diseases (Abraham and Sasmal 2008). Viral pathogens are responsible for huge economic loss in the shrimp aquaculture industry (Shinn *et al.* 2018; Rahman *et al.* 2020). Several viral diseases have been reported in Asia and widespread mortalities have been reported mainly due to white spot virus (WSV) and monodon baculovirus (MBV) (OIE 2003; Mishra *et al.* 2005; Ananda-Raja *et al.* 2012a, 2012b; Kalaimani *et al.* 2013; Shinn *et al.* 2018). In Asia alone, the impact of WSV of shrimp has been estimated at $US 4–6 billion during the

10 years after its emergence in 1992. In India, the gross economic losses due to shrimp diseases were estimated at Rs. 10,221 million (USD 135 million) in 2006–08 (Kalaimani *et al.* 2013) and loss continue even now. The incidences of shrimp viral diseases in Indian shrimp culture system (Karunasagar *et al.* 1997; Sahul-Hameed *et al.* 2005; Jose *et al.* 2010; Kalaimani *et al.* 2013) including sites adjacent to the Sunderban ecosystem in West Bengal (Mishra *et al.* 2005; Abraham and Sasmal 2008; Ananda-Raja *et al.* 2012a, 2012b; Dutta *et al.* 2015) have been well documented. The present study presents the results on the surveillance of WSV and MBV infection in West Bengal shrimp culture systems.

Specimens of cultured and wild crustaceans were collected from nineteen different sampling areas comprising 121 samples from normal (*n* = 84) and disease-affected grow-out ponds (*n* = 20) and one *Macrobrachium rosenbergii* hatchery in South 24 Parganas, North 24 Parganas and East Midnapore districts of West Bengal between 2012 and 2018. A few farms in South 24 Parganas district were close to the Indian Sunderban ecosystem. A total of 121 pooled samples comprising *Penaeus monodon* (*n* = 65), *Litopenaeus vannamei* (*n* = 39), *M. rosenbergii* (*n* = 11), *Metapenaeus monocerus* (*n* = 1), *Fenneropenaeus penicillatus* (*n* = 2), *F. indicus* (*n* = 1), red crab *Ocypoda* spp. (*n* = 1) and paddler crab *Varuna* spp. (*n* = 1) were collected. From each pond/hatchery, at least 10 specimens for each species, except the wild crustaceans, were collected, pooled together and preserved in absolute alcohol. Nested PCR assay was followed for the screening of WSV by WSV detection kit (Bangalore Genei). Non-nested PCR assay was carried out for the detection of MBV by MBV detection kit (Bangalore Genei). Conventional PCR assay was also followed using primers pairs - Forward F1 (5′-GACAGAGATATGCACGCCAA-3′) and Reverse R1 (5′-ACCAGTGTTTCGTCATGGAG-3′) for the detection of WSV (Mishra *et al.* 2005). The reactions condition were 50 – 100 ng of DNA, 12.5 µL of 2x PCR master mix (Thermo Fisher Scientific, USA), 1 µL of each primer at a final concentration of 10 pmol, and nuclease-free water to a final volume of 25 µL. The PCR cyclic conditions consisted of an initial denaturation at 95°C for 5 min followed by 30 cycles of 95°C for 30 sec, 52°C for 1 min, 72°C for 1 min and a final extension at 72°C for 5 min.

Several diagnostic tests are available for the detection and screening of WSV and MBV (OIE 2003). Among them, the PCR technique has been found to be a more specific and sensitive method. The affected shrimp, *P. monodon* and *L. vannamei* of the present study were of uneven size, anorectic, exhibited inadequate escape response. The gut was empty and the contents pale. The muscle, gills and hepatopancreas were discoloured. All the infected *P. monodon* from Lahiripur and Kashiabad had white spots on the interior carapace and reddish discolouration of the body and appendages, which experienced 100% mortality. The WSV infection was noticed in 12 out of 65 *P. monodon* (18.46%) and 6 out of 39 *L. vannamei* (15.38%) samples. Further, two out of 11 samples of *M. rosenbergii* from extensive culture system, which stocked wild seeds, were positive for WSV. They were apparently healthy and did not show any clinical signs of WSV infection, but carriers of WSV. *Penaeus monodon* of these ponds were, however, negative for WSV. Out of 121 pooled samples, 18 samples (14.87%) from infected ponds were positive for WSV by first PCR and 20 samples (16.53%) by nested PCR, as well as by conventional PCR. The positive samples yielded a specific amplicon product of 650 bp and 300 bp by first PCR and nested PCR, respectively by commercial kit and 643 bp by conventional PCR. The results indicated that the conventional PCR could also detect WSV in lightly infected shrimp. According to Lo *et al.* (1996) techniques such as nested PCR allow gradation of viral infection, with highly infected shrimps being positive in first PCR, but lightly infected ones positive in nested PCR. Further, 8 samples of the grow-out *P. monodon* from Lahiripur and Kashiabad of Indian Sunderban were positive for MBV, which yielded a specific product of 361 bp. All these MBV positive *P. monodon* samples were also positive for WSV, indicating concurrent infection of shrimp. Other samples such as *F. penicillatus*, *F. indicus* and *M. monocerus*, and wild stocks of red crab *Ocypoda* spp. and paddler crab *Varuna* spp. caught in the grow-out ponds of Indian Sunderban were, however, negative for WSV (Table 1).

In East Midnapur district, none of the *P. monodon* specimens screened (*n* = 33) during the early period of the surveillance work was positive for WSV. On the other hand, 4 out of 22 samples of *L. vannamei* (18.18%) were WSV positive. These results together with the earlier observations (Mishra *et al.* 2005; Abraham and Sasmal 2008; Ananda-Raja *et al.* 2012a, 2012b; Dutta *et al.* 2015) indicated the fact that the WSV is still a major problem in different shrimp culture systems of West Bengal, possibly because of the use of poor quality, non-certified hatchery-raised shrimp seeds, use of wild seeds of uneven size and over-dependence of consultants for seeds and aqua drugs and lack of shrimp seed quality testing facilities. Besides these, environmental factors such as temperature fluctuations, intensive culture practices and inappropriate farm management would further favour the transmission of viral pathogens. The PCR testing of shrimp seeds together with the adoption of better management practices (BMP) would help prevent the viral outbreak and the spread of the viral diseases in West Bengal shrimp culture systems. The results of the present study emphasized the need to observe strict quarantine measures during shrimp culture especially during seed selection to prevent the introduction and spread of viral pathogens into the fragile Sunderban ecosystem.

TABLE 1 Prevalence of white spot virus (WSV) and monodon baculovirus (MBV) in cultured shrimp and wild crustaceans of West Bengal.

Sampling area	Species	*N*	Positive (No.)	
			WSV	MBV
South 24 Parganas District (29 ponds)				
Lahiripur	*Penaeus monodon*	7	7	7
Kashiabad	*P. monodon*	3	2	1
Kakdwip	*P. monodon*	3	0	0
Kakdwip	*Fenneropenaeus indicus*	1	0	-
Kakdwip	*Metapenaeus monoceros*	1	0	-
Kakdwip	*F. penicillatus*	2	0	-
Kakdwip	*Ocypoda* spp.[a]	1	0	-
Kakdwip	*Varuna* spp.[a]	1	0	-
Kakdwip	*Litopenaeus vannamei*	2	0	-
Jharkhali	*P. monodon*	6	0	0
Jharkhali	*Macrobrachium rosenbergii*	2	2	-
Amratala	*L. vannamei*	1	0	
Gangadharpur	*L. vannamei*	5	0	
Krishnanagar	*L. vannamei*	2	2	0
North 24 Parganas District (20 ponds)				
Basirhat	*P. monodon*	5	0	0
Hasnabad	*L. vannamei*	6	0	-
Paschim Goberia	*L. vannamei*	1	0	-
Hasnabad	*P. monodon*	8	3	0
East Midnapur District (55 ponds)				
Digha[b]	*M. rosenbergii*	9	0	-
Heria	*P. monodon*	17	0	0
Raghunathpur	*P. monodon*	16	0	0
Norghat	*L. vannamei*	2	2	-
Ramnagar	*L. vannamei*	12	0	-
Rasulpur	*L. vannamei*	8	2	-
Total		121	20	8

[a], *wild stocks caught in grow-out ponds; –, not done;* [b], *Macrobrachium rosenbergii hatchery*

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CONFLICT OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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CONTRIBUTION OF THE AUTHORS

TJA research design, data analysis and manuscript preparation; **AM, AP & HA** sample collection, laboratory analysis, data generation and interpretation

TJ Abraham https://orcid.org/0000-0003-0581-1307
A Mondal https://orcid.org/0000-0001-6285-427X
A Patra https://orcid.org/0000-0003-2034-9552
H Adikesavalu https://orcid.org/0000-0002-2258-1470