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White spot syndrome virus infection: Threat to crustacean biodiversity in Vembanad Lake, India

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ABSTRACT

The Vembanad Lake located on the south-west coast of India, an ecological hotspot is the nursing ground of many economically important crustaceans. The prevalence of white spot syndrome virus (WSSV) among crustaceans from farmed, estuarine and marine environments surrounding the Vembanad Lake, India was detected using PCR. A total of 308 samples from aquaculture ponds consisting of six species of crustaceans collected from five different farms were tested for the presence of WSSV. Of these, 67% were found to carry the virus. A total of 258 samples of crustaceans from the Cochin backwater system that forms a part of the Vembanad lake viz., *Metapenaeus dobsoni, Metapenaeus monoceros, Penaeus monodon and Penaeus indicus* were found to contain WSSV in 62% of the samples. Fifteen species of crustaceans caught from the seas off Cochin were also screened for the presence of WSSV. Out of these, twelve species had WSSV incidence levels ranging from 6–23%. WSSV was not detected from three species of deep sea crustaceans tested. The black tiger shrimp, *Penaeus monodon* had the highest incidence of WSSV among the species screened in farmed, estuarine and marine environments.

1. Introduction

Vembanad Lake is the largest estuarine system located on the south-west coast of India. The Lake regularly supports 20,000 residential as well as migratory waterbirds, prawns and fishes. Many crustaceans spent part of their lifecycle in the estuarine system before migrating to the sea. The borders of the Vembanad Lake support extensive as well as semi-intensive aquaculture.

White spot syndrome virus (WSSV) is the causative agent of a disease that causes high mortality in crustaceans. WSSV is highly virulent and targets various tissues and organs that originate from the ectoderm or mesoderm [1]. The virus can infect almost all commercially important species of penaeid shrimp [5] and has been isolated from a wide range of crustaceans [13,19,11,17]. The virus can induce 100% mortality in infected shrimp within 3–5 days [10]. WSSV is a member of the genus *Whispovirus* within a new virus family called Nimaviridae [16]. It is an enveloped, rod shaped virus containing double stranded DNA [18]. The simplest method to detect WSSV infection in shrimp is to observe for local lesions and white spots on the carapace [2]. Sometimes a pink to reddish-brown coloration is seen on the shrimp due to the expansion of sub-cuticular chromatophores [5]. Since the shrimps die after the appearance of symptoms, this method cannot be used for diagnosis. Histological lesions include distinct hypertrophied nuclei containing marginated chromatin and amphophilic central inclusions in the cuticular epithelial cells, connective tissue cells and hemocytes [5]. Molecular methods like polymerase chain reaction [4,14,15] are sensitive for detection of WSSV infection even in carrier animals that do not show any gross symptoms of the disease. WSSV infection of penaeid shrimp has a negative influence on the shrimp aquaculture in the Vembanad estuarine system. Interventions in the form of reclamation and discharge of pollutants to the Vembanad Lake also have an adverse impact on the potential of the aquatic ecosystem that used to support high levels of bioproductivity and biodiversity [9]. This study was undertaken to determine the prevalence of WSSV among the crustaceans in the Vembanad estuary, the shrimp aquaculture farms surrounding the estuary, and the sea off Cochin coast, India.

2. Materials and methods

2.1. Study area

The site chosen for the study was Vembanad Lake, the largest estuarine system located on the south west coast of India and the

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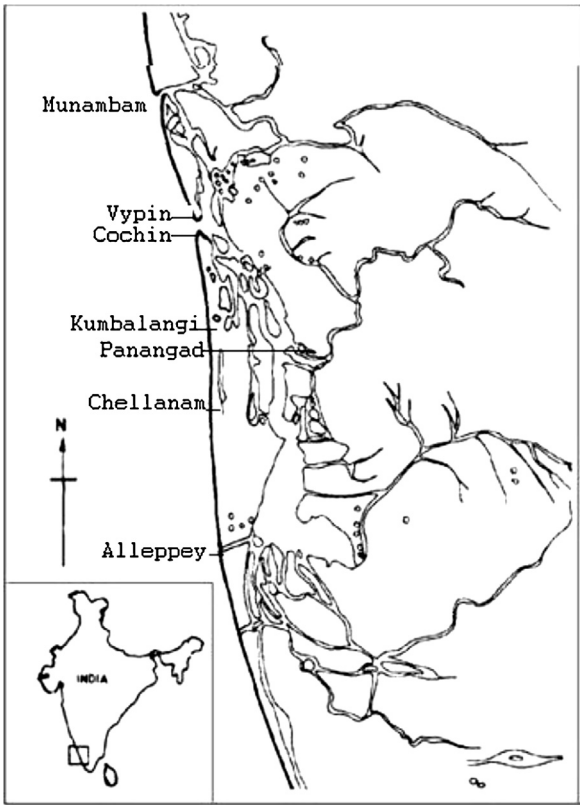

Fig. 1. Vembanad estuary along the south west coast of India, showing the location of sampling sites.

aquaculture farms adjoining the Vembanad Lake (Fig. 1). Vembanad Lake extends between 9°00′ and 10°40′N and 76°00′ and 77°30′E. The area includes low lying swamps and tidal creeks with mangroves which support larvae and juveniles of many crustacean species due to their nutrient rich environment. Cultured crustaceans collected from the aquaculture farms of Vypin, Panangad, Kumbalangi, Chellanam and Allepey and wild crustaceans of estuary and from the seas off Cochin, Kerala state, India were used in this study.

The specimens were checked for the presence of symptoms of WSSV before storage. One peapod from the each specimen was excised and stored at −70 °C until use.

2.2. Extraction of viral DNA

The viral DNA was extracted from the tissue of the crustaceans as described by Otta et al [12]. Total DNA extracted from infected *Penaeus monodon* was used as positive control.

Table 1
Primers used for the detection of WSSV.

Name of primer	Sequence	Size (bp)	Reference
IK1	5′TGG CAT GAC AAC GGC AGG AG 3′	486	[15]
IK2	5′GGC TTC TGA GAT GAG GAC GG3′		
IK3	5′TGT CAT CGC CAG CAC GTG TGC3′	310	
IK4	5′AGA GGT CGT CAG AGC CTA GTC3′		
WSSV1 out	5′ATC ATG GCT GCT TCA CAG AC 3′	982	[4]
WSSV2 out	5′GGC TGG AGA GGA CAAGACAT3′		
WSSV1 in	5′TCT TCA TCA GAT GCT ACT GC3′	570	
WSSV2 in	5′TAA GGC TAT CCA GTA TCA CG3′		

Table 2
Prevalence of WSSV in farmed shrimp and other crustaceans from aquaculture ponds by nested PCR.

Common name	Scientific name	One step PCR	Two step PCR
Tiger prawn	*Penaeus monodon*	54/112(48)	88/112(79)
White prawn	*P. indicus*	29/84(35)	46/84(55)
Brown shrimp	*Metapenaeus dobsoni*	8/22(36)	15/22(68)
Speckled shrimp	*M. monoceros*	13/31(42)	22/31(71)
Mud crab	*Scylla serrata*	2/15(13)	5/15(33)
Mud crab	*S. tranquebarica*	3/18(17)	7/18(39)
Total		118/308(38)	207/308(67)

Figures indicate the number of animals positive for WSSV to number of animals tested.
Figures in parenthesis indicate the percentage of animals tested positive for the virus.

2.3. Detection of WSSV by PCR

For detection of WSSV, a nested PCR reaction was done with primer pairs (IK1 and IK2, IK3 and IK4) as described by Umesha et al [15]. A second set of primers was also used for the detection of WSSV using primers and PCR conditions as described by Kimura et al. [4] (Table 1). To eliminate the incidence of false reactions, a template free reagent control, a known negative control and a known positive control were run in all the reactions. The PCR amplified products were analysed in 1.5% agarose gels containing ethidium bromide at a concentration of 0.5 μg/mL and visualized under UV transilluminator.

3. Results

3.1. Detection of WSSV in crustaceans from aquaculture ponds

Out of 308 samples collected from cultured ponds, 118 (38%) were positive by first step PCR while 207 (67%) were positive by nested PCR (Table 2). The lowest incidence of WSSV was found in mud crab (*Scylla serrata*) were the infection was present in only 33% of the samples. Two *Metapenaeus* spp., (*Metapenaeus dobsoni* and *Metapenaeus monoceros*) that are not usually cultured but might have entered the aquaculture ponds during water exchange are found to carry WSSV in 15/28 (54%) and 22/32 (71%) of samples, respectively.

The farm wise prevalence of WSSV is given in Table 3. The maximum percentage of infected individuals was detected in Farm No. 5 by both first step PCR and nested PCR. In Farm No. 5, 44/73 (60%) of the samples were positive by first step PCR and 61/73 (84%) samples were positive by nested PCR. After 10 days of collection of samples, Farm No. 5 had an outbreak of WSSV infection and an emergency harvest was done. The lowest incidence of WSSV was found in Farm No. 1 with only 49% of the samples infected by nested PCR.

Table 3
Farm wise prevalence of WSSV among crustaceans.

Location	One step PCR	Nested PCR
Farm No. 1	25/75(33%)	37/75(49%)
Farm No. 2	10/44(23%)	26/44(59%)
Farm No. 3	18/56(32%)	39/56(70%)
Farm No. 4	21/62(34%)	44/62(71%)
Farm No. 5	44/73(60%)	61/73(84%)

Figures indicate the number of animals positive for WSSV to number of animals tested.
Figures in parenthesis indicate the percentage of animals tested positive for the virus.

Table 4
Prevalence of WSSV in shrimps from Cochin backwater system by nested PCR.

Common name	Scientific name	One step PCR	Two step PCR
Tiger prawn	(*Penaeus monodon*)	36/82(44)	59/82(72)
White prawn	(*Penaeus indicus*)	11/43(26)	19/43(44)
Brown shrimp	(*Metapenaeus dobsoni*)	25/79(32)	46/79(58)
Speckled shrimp	(*M. monoceros*)	21/54(39)	36/54(67)
Total		93/258(36%)	160/258(62%)

Figures indicate the number of animals positive for WSSV to number of animals tested.
Figures in parenthesis indicate the percentage of animals tested positive for the virus.

3.2. Detection of WSSV in shrimps from Cochin backwaters of the Vembanad estuary

The major species of shrimps found in the estuary viz., *P. monodon, Penaeus indicus*, *M. dobsoni and M. monoceros* collected from different locations of the Vembanad estuary showed the presence of WSSV by PCR (Table 4). Out of the 258 shrimp samples tested, 93 (36%) were positive for the presence of WSSV infection by first step PCR while 160 (62%) were positive by nested PCR. Samples from the backwaters had WSSV prevalence rates ranging from 44% to 72%. The lowest incidence of WSSV infection was found in *P. indicus* 19 (18%) of the 43 samples and highest in *P. monodon* 59 (72%) of the 82 samples.

3.3. Detection of WSSV in wild captured decapods

WSSV was prevalent in crustaceans collected from seas off Cochin. Fifteen species of crustaceans were analysed for the presence of WSSV. Out of them, twelve species had WSSV incidence levels ranging from 6 to 23%. WSSV was not detected from any of the three species of deep sea crustaceans tested viz., *Heterocarpus gibbosus, Plesionika spinipes and Puuerulus* spp. Of the 504 crustacean samples tested, WSSV was present in 21 (4%) of the samples by first step PCR and 65 (13%) by nested PCR (Table 5). The aquaculturally important *P. monodon* had the highest incidence of WSSV among the species from wild tested with 12(23%) samples infected out of 52 samples tested. WSSV infection levels were found to be lowest in specimens from the wild compared to the estuarine and aquaculture environments.

Table 5
Prevalence of WSSV in shrimps and other decapods from sea landings off Cochin by nested PCR.

Common name	Scientific name	One step PCR	Two step PCR
Marine Shrimp	*Parapenaeopsis stylifera*	2/78(3)	7/78(9)
Brown shrimp	*Metapenaeus dobsoni*	4/54(7)	12/54(22)
Tiger prawn	*Penaeus monodon*	5/52(10)	12/52(23)
White prawn	*Penaeus indicus*	3/38(7)	7/38(18)
King prawn	*Metapenaeus affinis*	2/45(4)	5/45(11)
Indian nylon shrimp	*Heterocarpus woodmasoni*	1/23(4)	3/23(13)
	H. gibbosus	0/34(0)	0/34(0)
	Plesionika spinipes	0/21(0)	0/21(0)
Mud crab	*Scylla serrata*	1/14(7)	3/14(21)
Mud crab	*Scylla tranquebarica*	0/16(0)	2/16(13)
Blood spotted crab	*Portunus sanguinolentus*	0/43(0)	4/43(9)
Blue swimming crab	*Portunus pelagicus*	2/33(6)	6/33(18)
Sea crab	*Charybdis cruciata*	1/18(6)	3/18(17)
Deep sea lobster	*Puuerulus* spp	0/17(0)	0/17(0)
Scalloped spiny lobster	*Panulirus homarus*	0/18(0)	1/18(6)
Total		21/504 (4%)	65/504 (13%)

4. Discussion

Among the infectious diseases affecting crustaceans, WSSV is one of the most challenging problems responsible for huge production losses to shrimp culture worldwide. PCR has become the method of choice for the detection of WSSV because it exceeds the sensitivity limits of other DNA based methods like dot blot or southern blot hybridization. Detection of WSSV by nested PCR method was found to be 10^3–10^4 fold more sensitive than the one step PCR method [6,7]. In this paper we report the occurrence of WSSV in crustaceans in the aquaculture farms and natural waters off Cochin.

A nationwide screening in Philippines indicated widespread occurrence of WSSV in 79% of juveniles in grows out ponds [8]. Vaseeharan et al. [17] reported WSSV in 62.5% of the juveniles of *P. monodon* in cultured ponds in various locations in South India. In this study, WSSV was found to be present in 67% of crustaceans in aquaculture farms adjoining the Cochin backwaters.

The Cochin backwater which forms part of the Vembanad Lake is an ecological hot spot for the breeding of many shrimp species. The adult gravid female shrimps lay the eggs in the estuary and the young ones spend part of their life cycle in the estuary before migrating to the sea. Factors that directly or indirectly affect this process cause deterioration of shrimp stock. This is all the more relevant to aquaculture industry in India since the production of postlarvae for aquaculture purpose is solely dependant on the wild brood stock. This study revealed the presence of WSSV in 160/258 (62%) samples collected from Cochin backwaters including *P. monodon, P. indicus, M. dobsoni and M. monoceros*.

White spot syndrome virus has been isolated from a wide range of wild crustaceans living in marine and fresh water such as crabs, lobsters, shrimps, fresh water prawn and cray fish [13,19,11,3]. The incidence of WSSV was 23% in wild caught crustaceans from south-west and south-east coast of India that include *Scylla serrata*, *Squilla mantis*, *P. indicus* juveniles and *Metapenaeus* spp. [17]. This study indicates the presence of WSSV in 65/504 (13%) of the wild caught crustaceans off Cochin coast. WSSV was present in 12 species of crustaceans out of the 15 species screened.

The presence of WSSV in wild population of crustaceans in Cochin backwaters and sea is a matter of concern as they may act as carriers of the infection. Aquaculture farms in and around Cochin are situated adjacent to the backwaters. The effluents from infected farms are usually released directly into the estuary without proper treatment. The effluent water carries along with it the dead and diseased shrimp into the natural environment thereby threatening the natural crustacean population. The backwaters are the sites of breeding of many economically important shrimp species. Juveniles of these species migrate to seas for maturation and could carry the infection to the sea. Infection may not occur in the sea because of the absence of stress factors that induces multiplication of the virus in host. It is a common strategy by governmental agencies to ranch hatchery reared postlarvae into the Vembanad estuary for replenishment of *P. monodon* stock. This practice may be carried out with utmost caution since hatchery reared postlarvae can be carriers of WSSV. The proportion of WSSV infected individuals among cultivated species of shrimp in estuary and sea viz. *P. monodon and P. indicus* is high compared to the non-cultivated species. This result indicates that infected shrimps from the aquaculture farms had found their way into the natural waters. Farmers should take care not to release farm reared shrimps during water exchange or during harvesting as it may have a negative impact on the natural population of shrimps. The presence of WSSV in wild crustaceans pose a potential threat to the very survival of their population as well as it affects the shrimp aquaculture. Efforts should be urgently initiated to control the spread of WSSV and there by protect the

extremely fragile ecosystem of the Vembanad Lake. This will go along way in saving many species of crustaceans from extinction and maintaining the biodiversity of the Vembanad Lake.

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References

[1] P.S. Chang, C.F. Lo, Y.C. Wang, G.H. Kou, Identification of white spot syndrome associated baculovirus (WSBV) target organs in the shrimp *Penaeus monodon* by *in situ* hybridization, Dis. Aquat. Org. 27 (1996) 131–139.
[2] H.Y. Chou, C.Y. Huang, C.H. Wang, H.C. Chiang, C.F. Lo, Pathogenecity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan, Dis. Aquat. Org. 23 (1995) 163–173.
[3] S. Hossain Md, A. Chakraborty, B. Joseph, S.K. Otta, I. Karunasagar, I. Karunasagar, Detection of new hosts for white spot syndrome virus of shrimp using nested polymerase chain raction, Aquaculture 198 (2001) 1–11.
[4] T. Kimura, K. Yamano, H. Nakano, K. Momoyama, M. Hiraoka, K. Inouye, Detection of penaeid rod-shaped DNA virus (PRDV) by PCR, Fish Pathol. 31 (1996) 93–98.
[5] D.V. Lightner, A Handbook of Shrimp Pathology and Diagnostic Procedures for Disease of Penaeid Shrimp, World aquaculture society, Baton Rouge, LA, 1996.
[6] C.F. Lo, J.H. Leu, C.H. Ho, C.H. Chen, S.E. Peng, Y.T. Chen, C.M. Chou, P.Y. Yeh, C.J. Huang, H.Y. Chou, C.H. Wang, G.H. Kou, Detection of baculovirus associated with white spot syndrome in penaeid shrimps using polymerase chain reaction, Dis. Aquat. Org. 25 (1996) 133–141.
[7] C.F. Lo, C.H. Ho, S.E. Peng, C.H. Chen, H.C. Hsu, Y.L. Chiu, C.F. Chang, K.F. Liu, M.S. Su, C.H. Wang, G.H. Kou, White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods, Dis. Aquat. Org. 27 (1996) 215–225.
[8] F.O. Magabanua, K.T. Natividad, V.P. Migo, C.G. Alfafara, F.O. de la Pena, R.O. Mirinda, J.D. Albaladejo, E.C. Nadala Jr., P.C. Loh, L.M. Tapay, White spot syndrome virus (WSSV) in cultured *Penaeus monodon* in the Philippines, Dis. Aquat. Org. 10 (2000) 77–82.
[9] N.N. Menon, A.N. Balchand, N.R. Menon, Hydrobiology of the Cochin backwater system – a review, Hydrobiologia 430 (2004) 149–183.
[10] H. Nakano, H. Koube, S. Umezaea, K. Momoyama, M. Hiraoka, K. Inouye, N. Oseko, Mass mortalities of cultured kuruma shrimp, *Penaeus japonicus* in Japan in 1993: epizootiological survey and infection trials, Fish Pathol. 29 (1994) 135–139.
[11] S.K. Otta, G. Shubha, B. Joseph, A. Chakraborty, I. Karunasagar, I. Karunasagar, Polymerase chain detection (PCR) of white spot syndrome virus (WSSV) in cultured and wild crustaceans in India, Dis. Aquat. Org. 38 (1999) 67–70.
[12] S.K. Otta, I. Karunasagar, I. Karunasagar, Detection of monodon baculovirus and white spot syndrome virus in apparently healthy *Penaeus monodon* postlarvae from India by polymerase chain reaction, Aquaculture 220 (2003) 59–67.
[13] S.E. Peng, C.F. Lo, C.H. Ho, C.F. Chang, G.H. Kou, Detection of white spot baculovirus in giant freshwater prawn, *Macrobrachium rosenbergii*, using polymerase chain reaction, Aquaculture 164 (1998) 253–262.
[14] Y. Takahashi, T. Itami, M. Maeda, N. Suzuki, J. Kasornchandra, K. Supamattaya, R. Khongpradit, S. Boonyaratpalin, M. Kondo, K. Kawai, R. Kusuda, I. Hirono, T. Aoki, Polymersae chain reaction (PCR) amplification of bacilliform virus (RVPJ) DNA in *Penaeus japonicus* Bate and systemic ectodermal and mesodermal baculovirus (SEMBV) DNA in *Penaeus monodon* Fabricus, J. Fish Dis. 19 (1996) 399–403.
[15] K.R. Umesha, B.K.M. Dass, B.M. Naik, M.N. Venugopal, I. Karunasagar, I. Karunasagar, High prevalence of dual and triple viral infections in black tiger shrimp ponds in India, Aquaculture 258 (2006) 91–96.
[16] M.C. Van Hulten, J. Witteveldt, S. Peters, N. Kloosterboer, R. Tarchini, M. Fiers, H. Sandbrink, R.K. Lankhorst, J.M. Vlak, The white spot syndrome virus DNA genome sequence, Virology 286 (2001) 7–22.
[17] B. Vaseeharan, R. Jayakumar, P. Ramasamy, PCR-based detection of white spot syndrome virus in cultured and captured crustaceans in India, Lett. Appl. Microbiol. 37 (2003) 443–447.
[18] C.H. Wang, C.F. Lo, J.H. Leu, C.M. Chou, P.Y. Yeh, H.Y. Chou, M.C. Tung, C.F. Chang, M.S. Su, G.H. Kou, Purification and genome analysis of baculovirus associated with white spot syndrome (WSBV) of *Penaeus monodon*, Dis. Aquat. Org. 23 (1995) 239–242.
[19] Y.C. Wang, C.F. Lo, P.S. Chang, G.H. Kou, Experimental infection of white spot baculo virus in some cultured and wild decapods in Taiwan, Aquaculture 164 (1998) 221–231.