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Prevalence of shrimp viral pathogen (WSSV) in marine ecosystem

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Abstract. Wild Crustacean samples were collected for a period of 8 months from two stations, Station 1 was the Vellar estuary situated at Parangipettai, and Station 2 the Uppanar estuary situated at Cuddalore, Tamilnadu, India. The DNA of the larvae were isolated and tested for the presences of White Spot Syndrome Virus by Polymerize Chain Reaction technique. The above study revealed that the extent of contamination caused by virus is governed mainly by the presence of shrimp farms. The study also indicates that the dispersal of WSSV into the wild environment is maximum during April, May and minimum during the months of January, February, July and August. The high viral load could be due to the draining of the untreated infected effluents from the shrimp farms directly into the water bodies. The minimal viral load may be due to the dilution brought about by the incoming fresh water during the rainy season and reduced discharge of shrimp farm effluents.
Key Words: shrimp, WSSV, PCR, viral load, marine ecosystem.

Introduction. WSSV outbreaks have caused mass mortalities among cultured penaeid shrimps worldwide, especially in Asian countries (Kim et al 1998). White spot viral disease has caused high mortalities and severe damage to the shrimp culture industry in China (Huang et al 1994), Thailand (Wongteerasupaya et al 1995), Japan (Takahashi et al 1994), Taiwan (Wang et al 1995), Indonesia and India.

WSSV is a highly contagious viral disease of penaeid prawns (Penaeidae), characterized by the rapid onset of high levels of mortality in farmed prawn populations. Although WSSV infection occurs in a wide variety of both wild and farmed crustaceans, this is essentially a disease of farmed penaeid prawns. Prawns can acquire white spot infection by either vertical or horizontal routes of transmission. Horizontal transmission through water and feeding of infected shrimps has been suggested by Chou et al (1996) and Mohan et al (1997) as the probable route for the spread of white spot disease virus. Lo et al (1997) and Mohan et al (1997) proved that vertical transmission of this viral agent is possible from brooders to off springs.

Materials and Methods

Site selection. The selection of sites for the present study was done in accordance with the location and the density of ponds surrounding the site. The crustacean larval samples were collected from two different stations. Station 1 is a high density area encompassing approximately 500 ponds. It is in Parangipettai and is familiar by the name Vellar estuary. Station 2 is a low density area encompassing approximately 50 ponds. It located in Cuddalore and is known as Uppanar estuary.

Collection of sample. The surface water plankton from a selected area was filtered by using plankton net (0.35m mouth diameter) that is made up of bolting silk (no.10 mesh size 158µm). During the study period, samples were collected at fortnight intervals. The collected samples were brought immediately to the laboratory. Crustacean larvae samples were isolated by screening through mesh. Isolated samples were washed

repeatedly to reduce contamination. After rinsing, the Crustacean larvae were sorted out for PCR detection.

Detection of viral DNA. DNA from infected animals were isolated by method of Nelson & Fangman (1979) and Saline citrate solution method.

Polymerase chain reaction. Polymerase chain reaction is an invitro method for specific DNA amplification. The purpose of PCR is to make huge number of copies of the desired gene. Certain readymade WSSV detection PCR kits are available in market. The isolated DNA sample was brought to ENBIOSIS PCR Lab present in Marakannam. And the IQ 2000 readymade kit was used for the detection of WSSV from sample.

Result

January & February. Two samples were selected from each station and none of them showed the presence of virus, indicating the absence of infected crustacean larvae in the environment during these months. According to Flegel et al (1997), Fegan & Clifford (2001) the viral expression in the latently infected populations often follow deterioration in the pond environment. Triggers for the expression of clinical disease may include rapid changes in variables such as water temperature and dissolved oxygen concentration, hardness or salinity.

The stocking of seeds is done during these 2 months. The culture is not usually exposed to stress conditions in this initial period. It does not require any kind of water exchange from the sources and there is no release of waters from ponds into the environment. This explains the reason for obtaining negative result during the first 2 months of study.

March. During this month, one of the samples collected from Vellar estuary showed positive result whereas both samples from Uppanar estuary continued to show negative result. The positive results are due to the activation of latently infected population in to infectious forms. In this stage approximately 30% of the ponds had been infected with the WSSV virus. The release of effluents from such infected ponds may causes contamination to the environment. As the uppanar area has a few ponds and conversion of latent forms to patent forms is minimal, the samples taken from this area did not show any viral contamination (Tables 1-2).

April & May. The samples collected from Vellar estuary were positive for virus in two consecutive months. The samples collected from Uppanar estuary showed different results in two months. The sample showed the absence of virus in the month of April but one of the samples taken in May was positive for virus.

The results obtained in Vellar estuary is due to the fact that, as the days of culture reaches approximately 100 days, there is a rapid multiplication of virus and mortality rate of the organism increases drastically. The number of ponds infected with the virus also shows an ascending pattern.

The sample taken from Station II showed negative results in the month of April due to low pond density in that area. But there is a significant variation in the result obtained during the month of May due to the transfer of virus to other ponds. The increased rate of viral multiplication may be due to the various stress factors triggered in the ponds. The draining of waters from such infected ponds resulted in the contamination of the wild environment.

June to August. The change in season from summer to rainy had a significant impact on the results obtained. The samples showed the presence of virus initially but later showed only negative results. This may be explained as during the rainy season, the incoming fresh water carried the source water containing crustacean larvae in to the sea resulting in the dilution of virus in the environment.

Contrarily, in Station II a positive result is seen in July as backwaters have minimal chances of being carried in to the seas. Hence a positive result is seen during this season. However, in August the samples were negative for the virus (Tables 1-2).

Table 1

Vellar estuary (Station I)

S.No	*Months*	*Noumber of samples tested*	*PCR detection*	
			WSSV (+ve)	WSSV (-ve)
1	January 2007	1		✓
		1		✓
2	February 2007	1		✓
		1		✓
3	March 2007	1		✓
		1	✓	
4	April 2007	1	✓	
		1	✓	
5	May 2007	1	✓	
		1	✓	
6	June 2007	1	✓	
		1		✓
7	July 2007	1		✓
		1		✓
8	August 2007	1		✓
		1		✓

Discussion. Various investigations on white spot diseases revealed that the similar trends of carrier diseases were noticed. The most surprising feature of this virus is its wide range of potential hosts (Flegel 1997). It infects not only several species of penaeid shrimp including those cultivated in the Western Hemisphere (Lo et al 1997) but apparently also a wide range of other decapods, including crabs and other related crustaceans (Cai et al 1995; Hameed et al 1998).

In Taiwan, Peng et al (1998), Chang et al (1998), and Wang et al (1998) carried out polymerase chain reaction (PCR) analysis to confirm that many of the suspected carriers are indeed infected. Some carriers have been shown to transmit the virus to *Penaues monodon*. These carriers include penaeid shrimps, other shrimps, crabs, lobsters, copepods and insect larvae. Similar studies in Thailand have confirmed that local crabs can be carriers.

Table 2

Uppanar estuary (Station II)

S.No	*Months*	*Number of samples tested*	*PCR detection*	
			WSSV (+ve)	WSSV (-ve)
1	January 2007	1		✓
		1		✓
2	February 2007	1		✓
		1		✓
3	March 2007	1		✓
		1		✓
4	April 2007	1		✓
		1		✓
5	May 2007	1		✓
		1	✓	
6	June 2007	1	✓	
		1		✓
7	July 2007	1		✓
		1		✓
8	August 2007	1		✓
		1		✓

Various wild decapod crustaceans, such as prawns (*Metapenaeus* spp), grass shrimp (*Acetes* spp), mud crabs (*Scylla serrata*) and blue swimmer crabs (*Portunus pelagicus*), can carry WSSV infection into prawn ponds when they enter via intake water or, in the case of some crab species, by migrating overland. Evidence from tank studies shows that crustacean carriers may infect prawns via water or after death when prawns ingest infected tissue (Supamattaya et al 1998; Kanchanaphum et al 1998; Fegan & Clifford 2001). While the actual risk of transmission of infection from non-prawn crustaceans to prawns in commercial ponds remains unclear, it probably depends in part on the prevalence of infection and virus load in these carriers.

All decapods crustaceans, including prawns, lobsters and crabs from marine, brackish water or freshwater environments are considered susceptible to infection, but WSSV has mainly been a problem in farmed penaeid prawns. Although WSSV infection is present in wild prawn populations in countries where WSSV is endemic on farms, there is

no evidence that the virus causes significant mortalities in these populations. Factors which contribute to the absence of an observable impact include lower stress levels in the wild, lower levels of infection (Lo et al 1997) and lower host densities (Lotz & Soto 2002).

Contrarily, a general hypothesis on the infectivity of WSSV is common and increasing in prevalence in wild prawns in countries where farms are affected by WSSV. Some studies have also found an association between season and prevalence of WSSV infection in wild prawn populations (Lo et al 1997).

Rajendran et al (1999) conducted experimental studies on the southeast coast of India by injecting or feeding white spot disease virus obtained from infected *P. monodon* to five species of shrimp (*P. monodon, P. indicus, P. semisulcatus, Metapenaeus monoceros* and *M. dobsonii*), two species of freshwater prawns (*Macrobrachium rosenbergii* and *M. idella*), four species of crab (*Scylla serrata, S. tranquebarica, Metapograpsus* sp. and *Sesarma* sp.) and three species of lobster (*Panulirus homarus, P. ornatus* and *P. polyphagus*). All species examined were susceptible to the virus.

All three species of lobster survived the infection for 70 days without clinical symptoms. However, bioassay and histology using healthy *P. monodon* revealed that crabs, prawns and lobsters may act as asymptomatic carriers/reservoir hosts of white spot disease virus.

The infection may be transmitted from brooders to offspring (Lo et al 1997 and Mohan et al 1997). The vertical transmission can be controlled by screening the fry before stocking. The poor maintenance of the pond leads to rapid multiplication of the virus and hence the percentage of infected shrimps.

Final Remarks. Crustacean samples were collected monthly for a period of 8 months ie. January 07 to August, 07. The DNA of the larvae were isolated and tested for the presences of WSSV by PCR technique at Enbiosis PCR lab at Marakkanam. This was repeated for all the samples collected during the period. This study revealed that the extent of contamination caused by virus is governed mainly by the presence of shrimp farms. The study also indicates that the dispersal of WSSV into the wild environment is maximum during April & May and minimum during the months of July & August. The high viral load could be due to the draining of the untreated infected effluents from the plants directly in to the water bodies. The minimal viral load may be due to the dilution brought about by the incoming fresh water during the rainy season.

Environmental pollution by WSSV is dangerous as this virus has many crustaceans in the wild population as its carriers. These asymptomatic carriers further spread the disease to the larvae and farm prawns by vertical transmission, affecting the entire aquaculture industry. Hence an efficient treatment plan is very much essential to save the wild environment from this kind of biotic pollution.

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