

# White Spot Viral Disease in Penaeid Shrimp – A Review

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## Abstract

The white spot viral disease in penaeid shrimp affects the development of the global shrimp industry. This paper reviews the viruses that cause the disease, the transmission of the virus, diagnosis and preventive measures.

## Introduction

The global aquaculture of penaeid shrimp has grown rapidly during the past two decades. In 1998, world shrimp farmers produced an estimated 737 200 t of whole shrimp (this includes 530 200 t from the eastern hemisphere and 207 000t from the western hemisphere), a record 12% increase from the 660 200 t produced in 1997 (Rosenberry 1998, 1999). The shrimp farming sector suffered a serious setback during 1995-96. Lack of technically qualified manpower, improper site selection, defective farm design, rapid intensification, overcrowding of farms in restricted locations and disproportionate development of the industry relative to supply of quality farm inputs paved the way for poor environmental conditions in ponds. The ultimate effect of the above-mentioned problems was serious disease outbreaks. Two dozen diseases of shrimp occur sporadically in shrimp farms, but none of these have caused serious harm to the industry. However, the recent disease caused by a viral pathogen (white spot viral disease) during 1993–1994 delivered a lethal blow to the global shrimp farming industry, which could not

recover immediately. In other words, this specific viral pathogen still plays a key role in biotically controlling the development of the global shrimp farming industry.

Brackishwater shrimp farming is carried out in culture systems with millions of microorganisms per ml of water/gram of soil. These microorganisms include beneficial, benign, opportunistic pathogenic and primarily virulent and pathogenic forms. Several microbial investigations have been carried out in shrimp farms over the past 20 years by many scientists from various countries in diverse fields. The available literature pertaining to shrimp viral diseases, with special reference to white spot viral disease, is reviewed here.

## Shrimp Viruses

According to Adams (1991) more than 1100 viruses of invertebrates have been reported. More than 30 viral diseases are now known to occur in crustaceans, including penaeids. Major groups of viruses reported in Crustacea include the Reoviridae, Picornaviridae, Parvoviridae, Togaviridae, Baculoviridae, Paramyxoviridae, Rhabdoviridae and Iridoviridae (Bonami and

Lightner 1991). Viral diseases and associated mortalities are emerging as the major threat to penaeid shrimp culture (Sindermann 1990). Though just a few nanometers in diameter and when laid side by side, 310 million of them stretch a little less than half an inch, they can virtually wipe out the farm raised shrimp crop and cause anxiety to shrimp aquaculturists about the future (Otta et al. 1998).

According to Lightner (1993, 1996) at least 13 viral diseases of cultured penaeid shrimp are now recognised. They include the following:

1. *Penaeus monodon* type baculovirus (MBV) (Lightner and Redman 1981), which includes Plebejus baculovirus (PBV) (Lester et al. 1987)
2. Baculovirus penaei (BP) (Couch 1974)
3. Baculoviral midgut gland necrosis virus (BMNV) (Sano et al. 1981)
4. Type C baculovirus of *P. monodon* (TCBV) (Brock and Lightner 1990)
5. Haemocyte-infecting baculovirus of *P. monodon* and *P. esculentus* (HB)
6. Infectious hypodermal and haematopoietic necrosis virus

- (IHHNV) (Bonami et al. 1990)
- 7. Hepatopancreatic parvo-like virus (HPV) (Lightner and Redman 1985)
- 8. Lymphoidal parvo-like virus (LOPV) (Owens et al. 1991)
- 9. Lymphoid organ vacuolization virus (LOW) (Bonami et al. 1992)
- 10. Reo virus: Reo – 3 (Bonami et al. 1992)
- 11. Reo virus: Reo – 4 (Tsing and Bonami 1987)
- 12. Yellow head virus (YHV) (Boonyaratpalin et al. 1993) and
- 13. White spot disease virus or Systemic Ectodermal and Mesodermal Baculovirus (Wongteerasupaya et al. 1995).

Each virus type is almost certainly comprised of a multitude of individual strains, some of which are highly pathogenic to some penaeids, while being of little importance to others.

### White Spot Viral Disease

Recently, disease 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 (Anon 1994). The white spot disease virus is believed to have been transmitted through seed brought to India clandestinely from Southeast Asian countries, where the virus has been amplified before (Shankar and Mohan 1998). During 1994-95, white spot viral disease caused severe mortality of cultured shrimp *P. monodon* and *P. indicus* along the

east coast of India (Anon 1994). Karunasagar et al.(1997) reported the white spot viral disease outbreak on the west coast of India.

Acutely affected shrimps are reported to show a rapid reduction in food consumption, become lethargic and have a loose cuticle with white spots (hence, the name “White spot” disease) of 0.5 to 2.0 mm in diameter, which are more apparent on the inside surface of the carapace. The white spots represent abnormal deposits of calcium salts by the cuticular epidermis. Populations of shrimp showing these signs display high mortality rates with cumulative mortalities reaching 100% within 3 to 10 days of the onset of clinical signs (Momoyama et al. 1994; Takahashi et al. 1994; Anon 1994; Anon 1995; Chou et al. 1995; Wang et al. 1995; Lightner 1996).

White spot disease viruses are cylindrical to elliptical or obovate and measure 121±9 nm in width at the widest point and 276±26 nm in length (Wongteerasupaya et al. 1995). Wang et al. (1995) described the genome of the white spot disease virus as a double-stranded DNA molecule longer than 150 kbp.

Based on the morphological characteristics and genomic structures of the virus, the authors classified the virus into the genus belonging to non-occluded baculovirus (NOB) of the sub-family Nudibaculovirinae of the Baculoviridae.

Lightner (1996) compiled the information pertaining to white spot causing viruses from different geographic regions along with the reported mean virion size and mean nucleocapsid size of these viral strains (Table 1).

The particulars provided in Table 1 help explain the confusion prevailing in the global scientific arena regarding the white spot viral disease. In addition to this, various names used by different scientists from different geographical regions to identify the white spot viral disease are summed up and presented in Table 2.

White spot viral disease in penaeid shrimp is characterized histopathologically by widespread and severe nuclear hypertrophy, chromatin margination and eosinophilic (in the early stages they are Cowdry A-like) to large basophilic intranuclear inclusions

**Table 1. Viruses that cause white spot disease with mean virion size and mean nucleocapsid size of the viral strains.**

Name of virus	Mean virion size	Mean nucleocapsid size
1. HHHNV (Hypodermal and haematopoietic necrosis baculovirus)	120 x 360 nm	Not reported
2. RV-P # 1 (Rod shaped nuclear virus of <i>P. japonicus</i> )	Not reported	84 x 226 nm
3. RV-PJ #2 (Rod shaped nuclear virus of <i>P. japonicus</i> )	83 x 275 nm	54 x 216 nm
4. SEMBV (Systemic ectodermal and mesodermal baculovirus)	121 x 276 nm	86 x 201 nm
5. WSBV (White spot baculovirus)	70-150 x 250-380 nm	58-67 x 330-350 nm

(routine haematoxylin and eosin (HE) staining) with variable multifocal necrosis in most tissues of ectodermal and mesodermal origin. These tissues include the gills, haemocytes and haematopoietic tissue, lymphoid organ, connective tissues, subcuticular epidermis, stomach, foregut and hindgut epithelium, heart, striated muscle, midgut and ovary walls, antennal gland and the nervous tissues (Anon. 1994; Wongteerasupaya et al. 1995)

A survey on the incidence of white spot viral disease in shrimps in Tamil Nadu, India was carried out by Ruby et al. (1999). The authors observed histopathological lesions in various ectodermal and mesodermal organs such as exoskeleton, heart, gonads, gut epithelium, lymphoid organ, antennal gland and eyestalk. It is of

interest to note that the authors also reported lesions from the hepatopancreas of infected shrimp.

The virulence of different geographic isolates of white spot disease virus (shrimps obtained from China, India, Thailand and Texas and Carolina of the USA and crayfish from the USA) was compared by Wang et al. (1999) using *Litopenaeus vannamei* postlarvae and *Farfantepenaeus duorarum* juveniles. The Texas isolate caused mortalities more rapidly than did the other viral isolates, the isolate from crayfish had the lowest virulence. The authors reported slight differences in virulence among geographic isolates of white spot disease virus tested. According to Wang et al. (1999), susceptibility could vary with species and life stages of the host.

## White Spot Virus – Nature of Infection

Wang et al. (1998a) classified the white spot viral infection into two types. Type I is an acute infection that causes high mortality within two weeks in species such as *P. monodon*, *P. indicus* and *P. penicillatus*, as described by Chou et al. (1995) and Nakano et al. (1994). Type II is latent and the individuals harbouring this specific virus remain alive as in the case of *Macrobrachium* sp., wild crabs and wild lobsters (and also do not exhibit any disease symptoms) as described by Peng et al. (1998).

Sudha et al. (1998) and Mohan and Shankar (1998) classified white spot viral disease outbreaks in penaeid shrimps into three types (Type I, II and III) based on the clinical manifestation. In a type I outbreak (acute or subacute) the tissue level severity of infection was moderate to high, significant mortalities occurred within 7-10 days and the affected shrimp had prominent white spots on the carapace as the principal clinical sign. In a type II outbreak (parachute), the affected shrimp displayed massive reddening, the tissue level severity of infection was very high and mass mortalities occurred within 2-3 days. A type III outbreak (chronic) has a low tissue level severity of infection, white spots and reddening were absent, and the mortalities of shrimp were spread over a duration of 15-28 days.

## Carriers of White Spot Disease Virus

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

**Table 2. Names used for denoting white spot causing viral disease in penaeid shrimp.**

SI.No.	Disease Name	Reference
1.	White spot viral disease (WSVD)	Wang et al. (1997)
2.	White spot syndrome (WSBV)	Kim et al. (1996) Lo et al. (1996)
3.	White spot syndrome associated baculovirus (WSBV)	Chang et al. (1996) Chou et al. (1998)
4.	White spot baculovirus disease	Durand et al. (1996, 1997) Mohan et al. (1997) Peng et al. (1998) Wang et al. (1998a)
5.	White spot syndrome (WSSV)	Nunan et al. (1998) Supamattaya et al. (1998)
6.	White spot syndrome (WSS)	Chou et al. (1995) Karunasagar et al. (1997) Sudha et al. (1998) Wang et al. (1999)
7.	White spot viral infection	Wongteerasupaya et al. (1996) Flegel et al. (1997)
8.	White spot disease	Shankar and Mohan (1998) Wang et al. (1998b) Ruby et al. (1999)
9.	White patch disease	Chanratchakool et al. (1995)
10.	Systemic ectodermal and mesodermal baculoviral (SEMBV) disease	Anon. (1994) Wongteerasupaya et al. (1995) Sahul Hameed et al. (1998)
11.	Hypodermal and haematopoietic necrosis baculovirus (HHMBV)	Cai et al. (1995)
12.	White spot virus (WSV)	Tapay et al. (1999)
13.	White spot syndrome virus (WSSV)	Rajendran et al. (1999)

Hemisphere (Lu et al. 1997) but apparently also a wide range of other decapods, including crabs and other related crustaceans. In Taiwan, Peng et al. (1998), Chang et al. (1998a) and Wang et al. (1998a) carried out polymerase chain reaction (PCR) analysis with detailed histology, including transmission electron microscopy and *in situ* hybridization, to confirm that many of the suspected carriers are indeed infected. Some carriers have been shown to transmit the virus to *P. 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. One of these studies by Supamattaya et al. (1998) showed that the swimming crab *Portunus pelagicus* and the mud crab, *Scylla serrata* could be infected with white spot disease virus by injection or feeding. Moreover, these crabs subsequently showed typical white spot viral disease histopathology by light and electron microscopy. Lesions were positive by *in situ* hybridization with a DNA probe specific for white spot disease virus (Wongteerasupaya et al. 1996).

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 (*S. 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. Experimental infections in the shrimp had the same clinical symptoms and

histopathological characteristics as in naturally infected *P. monodon*. A cumulative mortality of 100% was observed within 5-7 days in shrimp injected with white spot disease virus and 7-9 days in shrimp fed with infected tissue. Two species of mud crab (*S. serrata* and *S. tranquebarica*) survived the infection for 30 days without any clinical symptoms. 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. This is the first report with evidence of the carrier/reservoir capacity of these hosts through histological and bioassay evidence.

### Transmission of White Spot Disease Virus

Horizontal transmission through water and feeding of infected shrimps has been suggested by Chou et al. (1995) 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 offspring.

### Diagnosis

DNA hybridization probes for the white spot disease virus have been developed by several laboratories (Chang et al. 1996; Durand et al. 1996; Wongteerasupaya et al. 1996). The primers for detection of this virus by PCR technology have also been developed (Lo et al. 1996). Several methods are available for the detection of the white spot disease virus, including PCR (Kim et al. 1998; Nunan et al. 1998; Peng et al.

1998), *in situ* hybridization (Chang et al. 1996; Durand et al. 1996; Wongteerasupaya et al. 1996; Chang et al. 1998a), Dot blot hybridization (Wongteerasupaya et al. 1996; Sahul Hameed et al. 1998) and ELISA (Sahul Hameed et al. 1998).

Tapay et al. (1999) developed primers for PCR based on the sequence of a cloned fragment of the white spot disease virus genome and used the primers to detect white spot disease virus from both experimentally and naturally infected shrimp. They developed one-step and two-step PCR protocols with the sensitivities of 10-100 pg and 100 femtograms respectively. The authors recommended the two-step PCR protocol as a very sensitive and specific alternative protocol to Western blot assay for the detection of white spot disease virus.

### Virucidal Activity

Virucidal effects of ultraviolet (UV) irradiation, heat, pH, ozone, salinity and some chemical disinfectants (sodium hypochlorite, povidone iodine and benzalkonium chloride) on white spot disease virus were investigated by Chang et al. (1998b), by infectivity assay using juvenile *P. monodon*.

## Conclusion

For sustainable shrimp farming, the industry should aim at undertaking ecologically sound management practices. Intensification of aquaculture and disproportionate growth of the industry relative to infrastructure development could create both environmental and social problems. A good source of uncontaminated water, sufficient aeration and better management practices can prevent the outbreak of diseases to a large extent. "Prevention is better than

cure” is definitely an appropriate maxim for shrimp farming. The immune system of shrimp is of great research interest and the use of different immunostimulants has proved effective in improving the immune system of shrimp and thereby reducing mortality. However, such practices may not help in heavily contaminated areas due to various reasons. It is to be expected that in the long run, immunostimulants will help sustain shrimp aquaculture.

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