WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 17, 745-762. Research Article ISSN 2277– 7105

AQUACULTURE OF SHRIMP: WITH REFERENCE TO PCR DIAGNOSIS OF WSSV IN THE EGGS OF *PENAEUS INDICUS*

P. Karnan[1]* and Dr. R. Usha[2]

[1]Ph.D Research Scholar, Presidency College.

[2]Associate Professor and Head, Presidency College.

Article Received on
06 August 2018,

Revised on 27 August 2018,
Accepted on 17 Sept. 2018

DOI: 10.20959/wjpr201817-12628

***Corresponding Author**
P. Karnan
Ph.D Research Scholar,
Presidency College.

ABSTRACT

Aquaculture, an important technique practiced in India and all over the world is the cultivation of not only fishes but also other valuable aquatic organisms such as prawns, algae, crabs, lobsters, shell fishes and pearls. The infection of WSSV in the eggs of P.indicus is diagnosed by a biotechnological tool PCR. The tests conducted from November to January show less than 40% of infection with 2 positive results of 5 tests undertaken. From March, the percentage of WSSV infection is slowly increased (March 50%, April 67%) and reaches the peak of more than 80% in May and June. This percentage is then decreased in July 67% August and September 50% and October shows less than 40% of infection (33%). This Variation in percentage of WSSV infection is mainly depended the temperature. Fluctuation due to high temperature May and June shows high percentage of infection, July to November show average value of WSSV infection and December and January show very low percentage of infection due to lesser temperature than other months. Hence it is clear that the infection of WSSV from the broadstock to larval stages is through the genome of the eggs in *P.indicus*. It is believed that the transmission of WSSV from the brooders to the larvae may be by the vertical transmission through the genome of the eggs.

KEYWORDS: WSSV, PCR, R-PCR, DNTP, Bp, cDNA, P.indicus, Transmission, Life Cycle.

INTRODUCTION

Shrimp culture refers to the cultivation of marine prawns in controlled conditions. *Penaeus indicus* is one among about 28 species of the genes *Penaeus* Fabr (Hall, 1962). This is one of the major commercial species of the world. It is the most important shrimp caught off the

East African coast and is probably the most important Indian commercial species, especially for the inshore fisheries and for the rice field culture in Kerala. (Fig.1). *P. indicus* is an important species in Iran, Bangladesh, Malaysia, Thailand, Indonesia and the Philippines. Due to its good quality, good flavour this shrimp is popular with importers in Japan, Western Europe and U.S.A. Unfortunately recent heavy fishing and diseases outbreak in India and Western Indo Pacific areas have declined production of this shrimp, (Khalsan, 1998).

P. indicus is also known as Indian white shrimp, captured and cultured from most of Asian countries and all over the world. Currently India, Iran, Saudi Arabia have taken more interest for cultivating this prawn successfully. The shrimp farming sector suffered a serious setback during 1995-96. This is due to some diseases caused by pathogens. Two dozen diseases of shrimp occur sporadically in shrime farms, but none of these have caused serious harm to the industry. However the recent disease caused by a viral pathogen (WSSV) 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 (Sangamaheswaran and Jayaseelan, 2001). 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 (Bonami & Lightner, 1991). 13 Viral diseases of cultured penaeid shrimp are now recognised (Lightner, 1993, 1999). Among them white spot syndrome viral disease and associated mortalities are emerging as the major threat to penaeid shrimp culture (Sindermann, 1990). Recently disease outbreaks have caused mass mortalities among cultured penaeid shrimps worldwide, especially in Asian countries (Kim, et.al, 1998). WSSV caused high mortalities and severe damage to the shrimp culture industry in China (Huang, et.al., 1994), Thailand (Wong teerasupaya et.al., 1995) Japan (Takahashi, et.al., 1994), Taiwan (Wang et.al., 1995) and Indonesia and India (Anon, 1994). White spot viral disease caused severe mortalities of cultured shrimp *P.monodon* and *P.indicus* along the east coast of India (Anon, 1994). Karunasagar, et. al., (1997) reported the WSSV disease oubreak on the west coast of India (Sangamaheswaran and Jayaseelan, 2001). White spot viral disease infects not only penaeid prawns but also other crustaceans like fresh water prawn. *Macrobrachium rosenbergii* (Anitha. 2006), fresh water crab Scylla serreta (Hameed, et.al. 2003, 2005) and lobsters (Peng, et.al., 1998). All the life stages of *P. indicus* have been infected by this viral pathogen. The infected shows some symptoms like refused feed, pushed at pond margins, exhibited broken antennae and had damaged appendages. The most conspicous feature of the

syndrome is large to small white spots on the inner side of the carapace especially in the cephalothoracic region. Shrimp mortality starts gradually, but within 5 to 7 days from the appearance of the first gross signs mass mortality results (Mohan, et.al., 1998). White spot syndrome virus was detected in post larvae, juveniles, sub-adults and broodstock of *P. indicus* obtained from shrimp hatcheries and farms located near Chennai, Tuticorin (TN), Gundur, Nellore and Kota (AP). The presence of WSSV is confirmed by PCR, bioassay experiments, transmission electron microscopy insitu hybridization and Elisa. Many of the brooders did not exhibit any external symptoms of WSSV infection. PCR amplified products are seen in WSSV infected muscle tissues, hepatopancreas, heart, gills, hindgut and in eyestalk of P. *indicus* (Mohan, et. al., 1998 ; Ramasamy, 2006).

MATERIALS AND METHODS

Sample Collection

Eggs of *Penaeus indicus* used in this study were collected from the hatcheries around Chennai area. Eggs were gently collected from the spawner reared in the spawning tank. The fertilized eggs were separated by microscopicall examination. The eggs were always kept immersed in sea water to avoid mechanical damage. The samples were transported in polythene bags to the laboratories for further analysis. A monthly sample of 500 eggs was randomly selected to use from October 2006 to September 2007.

EQUIPMENTS AND MATERIALS USED

Agarose gel

Buffer solution

Centrifuge

Chloroform

Ethanol

Electrophoresis equipment and reagents

Pipettes

PCR Kit

Thermal cycler

UV Transilluminator

Kit Components

(a) Reagents for DNA extraction are stored in room temperature.

(i) DNA extraction buffer 75 Ml tube.

(b) Reagents for PCR reaction stored at 20^0C

(i) Ready to use master mix in PCR, 1 tube 7.5 ml / tube.

(ii) Primer solution 1 tube 500 ml / tube

(iii) Market 1 tube 20 ml / tube

(iv) Negative control 1 tube 20 ml / tube

(v) Positive control template 1 tube 20 ml / tube

DNA extraction From Samples

Samples of 500 eggs were used each time to WSSV analysis by PCR as described by Hossain, et. al. (2001), Otta, et. al. (2003) and Hameed, et. al. (2005). The egg sample were transferred to a UV sterilized plastic sachet and crushed well. To this 1 ml of guanidine hydrochloride buffer (10 mM Tris – HCL. pH 8.0, 0.1 M EDTA, pH 8.0, 6M guanidine hydrochloride and 0.1 M sodium acetate) was added and allowed to react for 30 minutes.

The sample was transferred to a microphage tube and centrifuged at 500 rpm for 5 minutes. The supernatant was transferred to fresh microphage tube and 500 ml Ethanol was added to this, mixed well and again centrifuged at 14000 rpm for 20 minutes. The pellet obtained was washed with 95% ethanol followed by 70% ethanol. The DNA pellet was dried in a vacuum drier and dissolved in 100µl sterile distilled water (otta, et. al., 2003).

The infection of WSSV was confirmed by the PCR technique using four pairs of WSSV primers as described by Hossain, et. al.(2001). The reaction mixture consisting of 75µl DNA buffer, 10 P.mol of each primer, 200 µM of dNTPS, 2.25 units of Tag DNA polymerase and 3 µl of DNA extraction.

Denaturation of target DNA was done first at 94^0C for 2 minutes, annealing of primers at 550C for 1 minute, elongation of primers at 72^0C for 1 minute ending with an additional elongation steps to minutes at 72^0C. The programme included in initial delay of 5 minutes at 94^0C and final delay of minutes at 72^0 before and after 30 cycles respectively.

The PCR products were than analysed by electrophoresis on a 0.8% agarose gel stained with ethidium bromide and visualized by ultraviolet transillumination. Visualization of viral DNA band classified the status of viral attack (Santiago, et. al., 2000). PCR analysis of the DNA extracts of egg sample a distinct band of amplified DNA of 310bp was observed in positive

control and suspected sample. No band was observed in negative control sample. The value of this analysis is shown in the result.

RESULT`

Distribution of *Penaeus indicus*

The Indian White prawn *Penaeus indicus* inhabits the coasts of East Africa, South Africa, India and Srilanka including Madagascar and the Redesa. It is also distributed in Bangladesh, Thailand, Malaysia, Philippines, Saudi Arabia and northern coast of Austrilia (Holthius and Rosa, 1965; Pannikkar and Ayyar 1937; FAO, 2007). In India, *P.indicus* distributed widely in all the coastal states. This prawn is put in aquaculture practices in India and all the world fishering producing countries. Currently, *P.indicus* is mainly cultured in Saudi Arabia, Vietnam, Iran and India (Fig. 2). Though in India, Ceylon, Malaya, Singapore and much of east Africa this species exists as a commercial fishery, it is reported to have scattered its distribution in Australia also.

Biology of *Penaeus indicus*

P.indicus is non-burrowing, active at both day and night, and prefers a sandy mud bottom. Adults are normally found at depths less than 30m but have been caught in even 90m. The shrimp mature and breed mostly in marine habitats and spend the juvenile and sub adults stages of 30 to 120 mm total length in coastal estuaries, backwaters or lagoons. (Menon, 1955; Menon and Raman, 1961).

Almost all the Penaeid prawns having some typical life cycles (Silas, 1985). All the Penaeid prawns are bisexual, normally females are larger than the males. Maximum lengths of females are 23 cm and males are 18.4 cm, usually less than 17 cm (FAO. Org., 2008). *P.indicus* are highly fecund laying 68000 to 254200 eggs in females of 140-200 mm total length. *P.indicus* belongs to the closed thelycum group and mating takes place immediately after the females moult. Mating occurs always at night, the sperm packs are deposited by the hard shelled male into the thelycum of newly moulted, soft shelled female. Pannikar and Menon (1956) has indicated the existence of two breeding periods namely October to November and May to June in this species. George (1961) has recorded the spawning season of this shrimp from October to May with two peak of spawning periods in November to December and February to April in India. In Singapore the spawning season of the species is record in February to April (Hall, 1962).

Subramaniyam (1963) has studied the gonad index of the species from Madras (Chennai) and observed that the breeding activity appeared in the months of May, July, August and September and that the lesser breeding activity in March. George, et.al. (1968) states that this species breeds throughout the fishing season with two peaks as observed earlier. Rao (1968) has observed that *P.indicus* has prolonged breeding period extending from October to April in Cochin waters. This species also has an extended spawning period in Madagascar waters, with a peak in March and April, the months in which the highest water temperatures are recorded (Crosnier, 1965). Subramanyam (1965) has collected freshly spawned eggs and nauplii from the Chennai coast in May to August. Fertilization is external and the ripe ova released by the female become fertilized by the sperm extruding simultaneously from the stored spermatheca in the thelycum. Depending upon the temperature, hatching takes place within 8-12 hours after hatching.

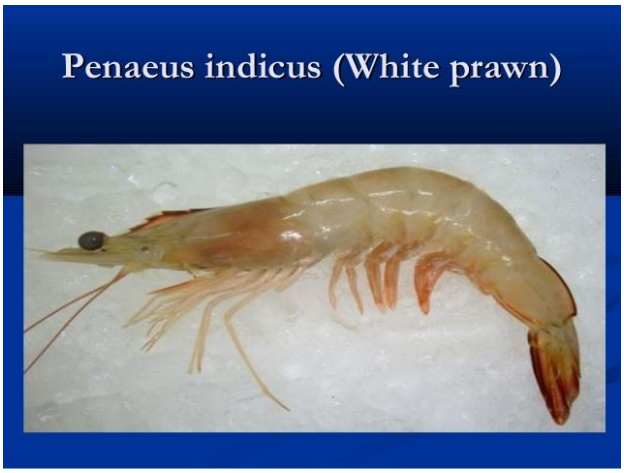

Figure – 1.

Life cycle of *Penaeus indicus*

There are 7 distinct life stages during the reproduction of *P.indicus* such as eggs, nuplius, protozoea post larva, mysis, juvenile or sub-adults and adult. The nauplii are free swimming and non-feeding and pass through six moults. The larvae further pass through protozoea (3 stages), mysis (3 stages) and they metamorphosis into postlarvae which resemble almost the adult. The postlarvae migrate into the estuaries, settle and feed on benthic detritus, polucheate worms and small crustaceans and remain there until they attain 110-120 mm in TL. The sub adults then return to the sea and life cycle continued. (Fig.2).

Eggs

The eggs of *P.indicus* are perfectly spherical with considerable pervitelline space and measure 0.45-0.47 mm in diameter. In the blastula stage the embryonic mass encircled by a thin membrane measures 0.23 mm in diameter. The embryonic development is rapid, the nauplius larva hatches out 10-14 hrs after spawning depending on the temperature of the sea water (Silas, 1985).

Nauplius larvae

The nauplius is pear shaped with 3 pairs of appendages. The nauplius moults every 3-5 hours and passes through 6 sub stages. The total length of nauplius is from 0.33 mm to 0.48 mm. It swims actively towards a weak source of light. It does not take any external feed and it subsits on the yolk material still present inside the body. After about 2 days the nauplius metamorphosis into the protozoa. (Silas, 1985).

Protozea

The protozoa has 3 sub stages. It has a broad head and a narrow tail with a forked end. Protozea has a alimentary canal, mouth and feeding appendages and starts feeding on the unicellular algae present in sea water. It swims actively in the water and strongly attracted towards light. This stage lasts for 3-4 days and is succeeded by the mysis larvae stage (Menon, 1957; Silas, 1985; Anonymous, 1976).

Mysis

The mysis stage also has 3 sub stages which appear very much alike the adults and the 3 sub stages last for 3 days. In this stage pleopod buds are developed. It also retain the filtering mechanism for feeding on algal cells. Total length of mysis is about 1.8 to 4.4 mm in diameter. After third substage, mysis metamorphosis into post larvae (Mohemed, 1983; Silas, 1985).

Post larvae

Total length of PL is 3.2 to 5.33 mm. This is characterised by the shrimp like body shape. Swimming state, chela and first walking legs are developed. This stage loss the filter feeding habit and become capable of catching prey with the help of chelate legs. The post larval stage graudually transforms into juvenile phase undergoing 20-30 successive moults, depending on the availability of food.

Juvenile

The intermediate phase between Pl and adults is juvenile. Juveniles are almost changed in all body characters and developed body appendages. The juveniles (sub-adults) migrate to sea for further development maturation and breeding. The total length is about 30-120 mm (Menon, 1955; Silas, 1965; Hall, 1962).

Adults

Penaeid prawns are the biosexual and the females are larger than the males. They are easily distinguished by the external modifications in breeding seasons. Maximum body length of female is 23 cm and male is 18.4 cm, usually less than 17 cm. The species attains sexual maturity at about 130 mm, when about one year old (George, et. Al., 1968). George and Mohamed (1966) have observed that the prawn fishery of Kanyakumari district is exclusively supported by large-sized mature *P.indicus.*

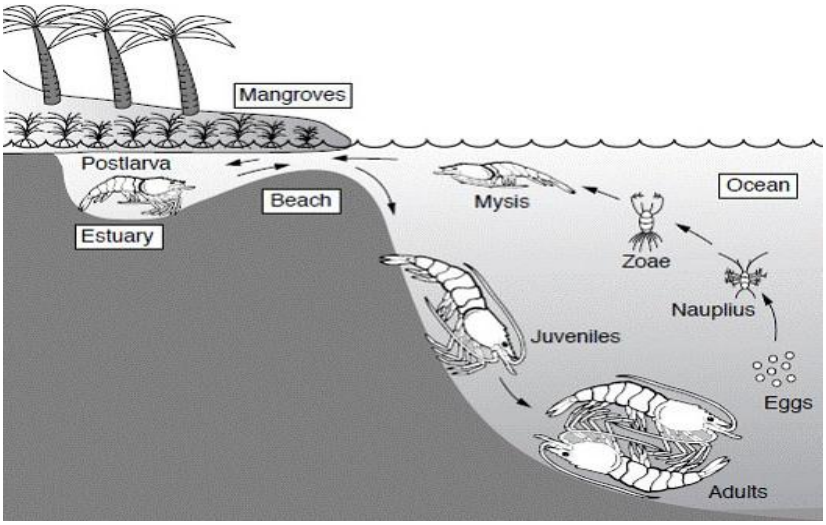

Figure: 2.

VIRAL DISEASES IN SHRIMP SPECIES

Disease may be defined as the state of illness of the body, during that time the normal function of the physiological condition is disturbed. Diseases may be caused by various reasons like heredity, infection through causing organisms such as bacteria, algae, fungi and virus. Among them viral pathogens are prone to infect the shrimp species and cause mass mortality within the short period after infection.

The major viral diseases are Baculovirus Penaeie (BP), Taura Syndrome Virus (TSV) Yellow Head Virus (YHV), Infectious Hypodermal Hemopoietin Necrosis Virus (IHHNV) Monodon

Baculo Virus (MBV) Mouriliyan Virus (MOV), Systemic Ectodermal Monodon Baculo Virus (SEMBV), and White Spot Syndrome Virus (WSSV). These important viral diseases are caused severe loss in the shrimp production. Among them white spot syndrome virus is one of the most devastating virus and causes morality in most of the penaeid prawns.

BP virus is confirmed by Overstreet, et. al., (1998) in adults of *P.vannamei* by PCR and also detected in PLs and larvae by PCR in india (Ramasamy, et. Al., 1995) and Costa Rica (Lighter, 1985). The juveniles of P.japonicus shows positive results in Japan by using PCR (Sano, et. Al., 1982). TSV is diagnosed in *P.vannamei* and adult *P.japonicus* using histopathology (Johnson, et.al.,1992). PLs of *P.japonicus* and *P.stylisostris* brood stocks have shown positive infection detected by gene probes and bioassay respectively (Sano and Fakuda, 1997; Lightener, 1997).

Yellow Head Virus (YHV) is one of the most important virus causes severe loss in production of all penaeid shrimp. YHV detected in adults of *P.indicus* using PCR by Karunasagar (1996) from the south east coast of india. YHV infects most of the life stages of *P.monodon* and diagnosed juveniles and sub adults of *P.monodon* using PCR in India (Flegel, 2002) and USA (Lightner, 1985).

IHHNV is diagnosed by using Various techniques like histopathology give positive results in juvenailes of *P.stylirostris* (Lightner, 1985). The brood stocks of *P.vannamei* and *P.monodon* show positive infection to IHHNV in bio-assay technique (Bell, et.al., 1990; Lightner, 1983).

Monodon Baculo Virus (MBV) is one of the major viral diseases causing high mortality in all penaeid shrimps especially in *P.monodon.* It is also diagnosed in and around Chennai using different techniques like PCR by Manohar (1996) and Manivannan, et. al. (2002), histopathology by Maria Charles (1997) and Karlo Dante, et. al. (2003) detected MBV in post larvae of *P.monodon* using duplex PCR technique in Philippines.

MBV is also detected in sub adults and mysis of *P.monodon* using PCR by Lightener, et. al. (1983) and Chen, et. al. (1992). In *P.indicus* histopathology test by Vijayan (1995) gives positive results to MBV. Hepatopancreatic Parvo Virus(HPV) is detected in adult. *P.monodon* and *P.indicus* from Chennai area by different aquaculturist using different techniques. It is diagnosed in PLs of *P.monodon* using histopathology by Manivannan. et,al.(2002) and adults by Manju Naik, et.al.(2005).

White Spot Syndrome Virus is one of the most devastating viral pathogen caused mass mortalities among the cultured penaeid shrimps worldwide especially in Asian countries (Kim, et.al.1998; Sangamaheswaran and Jeyaseelan, 2001). Various techniques are used to confirm this virus in India and all over the world. Preliminary test such as bio-assay, electronmicroscopy and histopathology give positive results to WSSV infection. WSSV infects most of the important cultivable penaeid shrimps and cause loss of production in 1990s. In *P.monodon* WSSV diagnosed using PCR in West coast of India give positive results (Hossain, et. al., 2001; otta et.al., 2003). In south east coast of India it is detected using PCR by Hammeed, et.al. (2005), Uma et. al. (2005) Rajendran et. al. (1999) and Rajendran (2007). In Iran PCR Technique give positive results to WSSV detection (Manson Sayari, 2005; Afsharnasab, 2007) Karlo Dante et. al., (2003) has detected WSSV in *P.monodon* of post larvae using duplex PCR technique (Table 1).

Different Viral Diseases in *P.indicus*

Among the above listed viral diseases some of them are prone to infect all the life stages of *P.indicus*. MBV detected in adults of *P.indicus* in India using TEM by Santiago, et. al. (1996) and histopathology by Vijayan et.al. (1995). Mourilian Virus is detected in adults using PCR by Karunasagar (1996). Yellow head virus is detected in adults by histology (Mohan, et.al.1998) and PCR (Karunasagar,1996). Hepatopancreatic parvovirus detected in adults and PLs using histology and HPV gene probe by Chang and Loh (1984) and Lightner and Bell (1997) respectively (Table 2).

Table 1: Viral diseases detected in Penaeid prawns.

S.No.	Viral Disease	Shrimp	Life Stages	Test Used	Place	Reference
1	BP	*P.monodon*	P.L.	PCR	India	Ramasamy et.al,.1995.
		P.monodon	Larval Stage	PCR	Coastia Rica	Lightner, 1985.
		P.vannamei	Adult	PCR	Australia	Overstreet, et.al.,1988.
2.	TSV	*P.vannamei*	Adult	Histopath -ology	Tucson (USA)	Johnson et.al.,1992
		P.japonicus	Post-Larvae	Geneprobes	Japan	Sano and fukuda 1997.
3.	YHV	*P.japonicus*	Juvenile	PCR	Australia	Spann and Lester 1997.
		P.vannamei	Adult	PCR	USA	Tang & Lightner 2000
		P.stylirostris	Adult	PCR	Thailand	Bediar et.al.,200.
		P.indicus	Adult	PCR	India	Karuna sagar 1996.
		P.monodon	Sub-Adult	PCR	USA	Lightner, 1996.
		P.monodon	Juvenile	PCR	India	Flegel, 2002.
4.	IHHNV	*P.stylirostris*	Juvenile	Histopath -ology	South East Asia	Lightner et.al.,1985
		P.vannamei	Brood Stock	Biopsy	Mexico	Bell.et. al., 1990
		P.monodon	Brood Stock	Biopsy	Mexico	Lightner et.al.,1983

White Spot Syndrome Virus in Penaeid Shrimps

WSSV infects most of the cultivate penaeid prawns. In *P.Monodon* histopathology and PCR give positive results to WSSV (Maria Charles and Shyamala, 1999; Hossain, et.al., 2001; otta, et, al., 2003; Uma, et.al., 2005; Hammeed, et.al., 2005; Rajendran, 2007) WSSV in *P.indicus* give positive results by PCR in India by Maria Charles and Ephrem (2006) and Brightsingh, et.al., (2005), in Thailand by Alabi, et.al., (1999). Other panaeid prawns also infected by WSSV is confirmed by different diagnostic techniques. In *P.japonicus* (Itami, et.al., 1998), *P.semisulcatus* (Rajendran et. al., (1999), *P.stylirestis* (Nunan,, et. al., 1998) and *P.duorarun* (Lightner and Redman, 1999) (Table 3).

Table 2: Different tests used to diagnose viral diseases in *P.indicus.*

S. No	Viral Disease	Life stage of *P.indicus*	Technique used	Place	Reference
1.	**MBV**	Adult	Histopathology	South east coast of India	Vijayan 1995.
		Adult	TEM	South east coast of India	Santiago et.al., 1996.
2.	**Mourilian Virus**	Adult	PCR	India	Karunasagar, 1996.
3.	**YHV**	Adult	Histopathology	West coast of India	Mohan et. al., 1998
		Adult	PCR	India	Karunasagar 1996.
4.	**HPV**	Post Larvae	HPV Geneprobe	Tucson USA	Lightner and Bell, 1997.
		Adult	Histopathology	Taiwan	Chang & Loh, 1984.
5.	**WSSV**	Post Larvae	PCR	India	Marioacharles and Ephrem, 2006.
		Adult, PL Juvenile	PCR	Kerala, India	Bright singh et.al., 2005.
		Juvenile	PCR	Thailand	Albi et.al., 2005.
		Post Larvae	PCR	Bushehr, Iran	Afsharnasab 2007.
		Adult	PCR	South east coast of India	Hameed et.al., 2002.
		Juvenile	PCR	Iran	Mansousayari 2005.
		Juvenile	PCR	East coast of India	Vaseeharan et.al., 2003.

Table 3: WSSV diagnosed in penaeid prawns using different tests.

S. No	Penaeid Prawns	WSSV Diagnosis		Place	Reference
		Test used	Result		
1.	*P.monodon*	Histopathology	+	South east coast of India	Maria and Charles and Shymala.,1999
		PCR	+	West coast of India	Hossain, et.al., 2001.
		PCR	+	West coast of India	Otta, et. al., 2003.
		PCR	+	South east coast of India	Uma, et. al., 2005.
		PCR	+	South east coast of India	Hammed, et.al., 2005.
		PCR	+	South east coast of India	Rajendran 2007.
2.	P.indicus	PCR	+	India	Bright Singh, et.al., 2005.
		PCR	+	Thailand	Alaibi et.al., 1999.
		PCR	+	South east coast of India	Maria Charles and Ephrem, 2006.
3.	P.japonicus	Histopathology	+	Japan	Mamoyama et.al., 1993.
		PCR	+	Bangkok	Itami, T.et.al.,1998.
4.	P.vannamei	Situ hybridisation	+	Thailand	Wongteera supaya. 1996.
5.	P.semi-sulcates	Histopathology and Bio assy	+	South east coast of India	Rajendran, et.al., 1999.
6.	P.Stylirestis	PCR	+	Tucson, USA	L.M.Nunan, et.al., 1999
7.	P.duorarum	Histology	+	USA	Lightener and Redman 1999.

Detection of white spot syndrome virus in *P.indicus*

Most of the life stages of *P.indicus* are detected with the prsence of WSSV using different diagnostic techniques. The infection of WSSV is confirmed in adults of *P.indicus* using PCR by Hameed et.al. (2002), Alabi at.al. (1999) and Bright Singh, et.al. (2005). In juveniles WSSV is confirmed by PCR in India (Bright Singh, et. al. 2005), Iran (Mansou Sayari, 2005). The post larval stage show high mortality due to the infection of WSSV. Infection of WSSV in PLs is detected using PCR by Maria Charles and Ephrem (2006). Bright Singh, et. al. (2005), Afsharnasab, (2007) and Vasaaharan, et.al.,(2003) have detected WSSV in juvenile of *P.indicus* using PCR technique. However, there is no reported infomation about the infection of WSSV in the eggs and early larval stages of *P.indicus* (Table 4).

Table 4: WSSV Detected in *Penaeus indicus* using different diagnostic techniques.

S. No	Life stage	Test used	Result	Place	Reference
1.	**PL**	PCR	+	India	Maria Charles and Ephrem, 2006.
2.	**PL**	PCR	+	Kerala, India	Bright Singh, et.al., 2005
3.	**Adult**	PCR	+	Thailand	Alabi et.al., 1999
4.	**Adult**	PCR	+	Kerala, India	Bright Singh, et.al., 2005
5.	**PL**	PCR	+	Bushehr Iran	Afsharhasab, 2007.
6.	**Juvenile**	PCR	+	Iran	Mansou sayari, 2005
7.	**Juvenile**	PCR	+	Kerala, India	Bright Singh, et.al., 2005
8.	**Adult**	PCR	+	South east coast of India	Hammed et.al., 2002.

Detection of WSSV in eggs of P.indicus

The infection of WSSV in the eggs of P.indicus is diagnosed by a biotechnological tool PCR. The egg samples are collected from the hatcheries near Chennai area for a period of 12 months from November 2006 to October 2007. Breeding and spawning of P.indicus is noticed high from the month of March to August. The tests conducted from November to January show less than 40% of infection with 2 positive results of 5 tests undertaken. From March, the percentage of WSSV infection is slowly increased (March 50%, April 67%) and reaches the peak of more than 80% in May and June. This percentage is then decreased in July 67% August and September 50% and October shows less than 40% of infection (33%). This Variation in percentage of WSSV infection is mainly depended the temperature. flurtuation due to high temperature May and June shows high percentage of infection, July to November show average value of WSSV infection and December and January show very low percentage of infection due to lesser temperature than other months. (Table 5).

Table 5: WSSV infection in eggs of *P.indicus* of the present study.

S. No	Period of Test	No.of.eggs used	No. of test undertaken	Positive results	Percentage infection
1.	November 2006	500	6	2	33%
2.	December 2006	500	6	1	17%
3.	January 2007	500	6	1	17%
4.	February 2007	500	5	2	40%
5.	March 2007	500	6	3	50%
6.	April 2007	500	6	4	67%
7.	May 2007	500	6	5	83%
8.	June 2007	500	6	5	83%
9.	July 2007	500	6	4	67%
10.	August 2007	500	6	4	50%
11.	September 2007	500	6	3	50%
12.	October 2007	500	6	2	33%

Hence it is clear that the infection of WSSV from the broadstock to larval stages is through the genome of the eggs in *P.indicus*. It is suggested that this infection may not be caused by horizontal transmission through the infected live feed and improper pond construction etc. It is believed that the transmission of WSSV from the brooders to the larvae may be by the vertical transmission through the genome of the eggs.

DISCUSSION

Aquaculture is one of the fastest growing industries to produce valuable aquatic organisms in large scale all over the world (Yap, 2000; FAO, 1996; Subortinghe, et.al., 1998). According

to FAO statistic 80% of world's fishery production comes from Asia valuing at 38.855 billion (FAO, 1996). Thailand leads first among the Asian countries with high production (Yap, 2000). Among all the aquatic organisms, shrimps leads first with high production due to its high nutritive and export value. Most of the penaeus species have been used in shrimp culture practices. Among them *P.indicus* stands next to *P.mondon*, cultivated in India and all over the world mainly from the western Asian countries. various culture techniques like intensive, extensive, polyculture and monoo sex culture are adopted successfully. In Kerala, *P.indicus* is cultivated in rice fields also. In Asia during the last one and a half decades 1984 to 1997, 63 countries are listed to produce shrimp. In total the major regions of the world southeast Asia is still the leading shrimp producing country with 506,035 mt or 53% of the total productivity. Asia is recorded to contribute 737,380 mt of 78% of worlds shrimp farming. America contributes only 198,925 mt or 21% of the total world productivity.

In Asia, Thailand leads first among the other shrimo producing coubtries. Next to Thailand, China and Indonesia stand the second and thrid position respectively (Yap,2000). Iran, Saudi Arabia and Vietnam reporting the production of *P.indicus* with 6700 tonnes in 1990. This just below 2000 tonnes in 2000 to make it the largest producer in 2005 amounting nearly 11300 tones. The production value has many up and downs during the last decade. The declined production is due to severe viral diseases. The shrimp farming industry is suffered a serious setback between 1993-1997. This is mainly due to some diseases caused by viral pathogens. 13 viral diseases are identified to infect the shrimp industry. Among them white spot syndrome virus is one of the most important to cause severe loss in the shrimp production not only in Asia but also all over the world (FAO, 2000; Yap,2000).

Monodon Baculo Virus (MBV), Taura Syndrome Virus (TSV), Baculovirus penaie (BP), Hapatopancreatic Parvo Virus (HPV), Yellow Head Virus (YHV), Mourilian Virus (MV), Infectious Hypodermal Heamopoietic Necrosis Virus (IHHNV) and White Spot Syndrome Virus (WSSV) are some important viruses, cause heavy loss to the shrimp industry. Among them WSSV is one of the important virus to decline the production of shrimp from Asia and all over the world in 1990's. WSSV is highly pathogenic not only to shrimp but also in freshwater crabs (Hameed, et. al., 2005) freshwater prawns (Anitha, 2006) and lobsters too (Rajendran, 2002). white spot syndrome virus is one of the most devastating virus in the world and affect most of the commercially cultured shrimp species (Inoye, et. al., 1994; Span

and lester, 1997) WSSV is first diagnosed in Japan in 1993 (Rajendran, 2007) and spread all over the world. In India it is first identified in 1994 (Karunasagar, 1995).

Various diagnostic techniques are used to confirm the presence of WSSV. The technique include histopathology, immunological assay, gene probe, insitu hybridization, polymerase chain reaction (Takahashi et. al., 1994; Wang, et. al., 1997; Nadala, et. al., 1997; Hameed, et al., 1998; Yoganathan et. al., 2005; Nunan et.al., 1998; Hossain, et. al., 2001; Otta, et. al., 2003; Maria Charles, 2006, Anitha, 2006; Rajendran, 2007). Among these technique PCR is considered as the most sensitive and important technique to detect the WSSV in the ganome level (Lightner and Red man, 1998; Niraj Kumar, et.al., 2006). *P.indicus* is highly infected by WSSV. The earlier studies reveal that the species have specific resistance to WSSV. However, now a days application of advanced techniques have confirmed that the P.indicus and its life stages are easily infected by WSSV. The Infection of WSSV in Adults is confirmed using PCR by Hammed, et. al., (2002), Alabi, et,al. (1999) and) Bright Singh, et.al., (2005) and in juveniles using PCR by Bright Singh, et.al., (2005) and Mansau Sayari (2005). Thepost larvae of *P.indicus* also show positive results to PCR tests Maria Charles and Ephrem, 2006; Bright Singh, et.al., (2005) and Afsharnasab, 2007. There is no reported information in India and other Asian shrimp farming countries that the infection if WSSV in early larval stages and eggs of *P.indicus.*

Although the previous informations reveal that the infection of WSSV adults and brooders through the infected live feed and improper maintenance of culture ponds etc., the transmission of WSSV is from the vrood stock to the early larval stages through the vertical transmission via genome of eggs of *P.indicus*. The infected broodstock have the virus in their germinal cells and occytes. Meanwhile the eggs having the WSSV in their genome hatch out successfully and the mortality begins from the larval stages. as it is identified in most of the larval stages of *P.indicus*. Therefore in the present study, to diagnose WSSV in eggs of *P.indicus* tests have been undertaken using advanced biotechnological tool of PCR. The samples for this investigations are collected from Chennai area. The end products of PCR are analysed in agarose gel electrophoresis and bands were classified at 210 bp.

From the investigation period of November 2006 to October 2007, May and June months shows more than 80% of WSSV infection due to high temperature. December and January months shows less than 40% of infection, and decrease (less than 80%) of WSSV infection. It is clear that the infection of WSSV in eggs of *P.indicus* is mainly depend upon the

temperature (or) temperature acts as an important role in the infection of WSSV. The results of the present study shows that the eggs from the WSSV infected brooders without any clinical signs are having WSSV in their genome. It is highly suspected that they might be the carrier of WSSV to the larval stages. Therefore the present study exposes or suggest that the brooders must be screened to WSSV by PCR and pathogen are very important before stocking the brooders.

CONCLUSION

Aquaculture is the cultivation of valuble aquatic organisms not only fishes but also prawns, lobsters, crabs, pearls etc., under controlled condition. Shrimp culture is one of important branch of aquaculture for cultivate the prawn for its nutritive value. Shrimp culture is undertaken in all over the world. Asia leads always first in the production of aquatic organisms. *Penaeus indicus* is one of most important penaeid prawns cultivated in India and worldwide. West Asian countries like Iran and Saudi Arabia are now take more interest in the culture of *P.indicus.* Te aquaculture production was declined due to disease outbreaks mainly by viruses. White Spot Syndrome virus is one of most important viral diseases caused heavy loss in shrimo production in 1990's. The first outbreak of WSSV is reported from Japan in *Penaeus indicus i*n 1994 and has subsequently all over the world. WSSV is detected in India first in 1994. Most of the infected penaeid prawns show mortality within 5-7 of infection. Various diagnostic techniques are used to confirm the presence of WSSV. Polymerase chain reaction (PCR) is the modern technique used worldwide to confirm the presence of WSSV. The egg samples used in this investigation raised from the hatcheries present in and around Chennai area are tested by PCR as described by Hossain, et. al. (2001).

The data shown are the results of the monthly test for a period 12 months from November 2006 to October 2007. May and June 2006 shows high percentage of WSSV infection. April and July 2007 shows moderate percentage of infection. The rest of the months show less percentage of infection. The results clearly states that the infection of WSSV from the brooders to the larvae through the genome of eggs of *Penaeus indicus.*

REFERENCES

1. Adams J.R. Introduction and classification of viruses of invertebrates, 1-8, In J.R. Adams and J.R. Bonami (eds.) Atlas of invertebrate viruses. CRC Press, Boca Raton, FL. P, 1991; 1-8.
2. Anitha, L., Aquaculture of prawn larvae: with reference to PCR diagnosis of WSSV in *Macrobrachium resenbergii*, M.Phil dissertation, University of Madras, Chennai, 2006; 76.
3. Bonami, J.R. and D.V. Lightner, Unclassified viruses of crustacean, J.R. Adams and J.R. Bonami (eds.) In: Atlas of invertebrate viruses, CRC Press, Boca Raton, 1991; 597-622.
4. C.V. Mohan, K.M. Shankar, S. Kulkarni, P.M. Sudhga, Histopathalogy of cultured shrimp showing gross sings of yellow head syndrome and white spot syndrome during 1994 in Indian Epizootics. Dis. Aqua, Organ, 1998; 34: 9-12.
5. Flegel, T.W., Detection of major penaeid shrimp viruses in Asia. A historical prospective with emphasis on Thailand. Aquaculture, 2006; 258: 1-33.
6. George, M.J., Studies on the prawn fishery of Cochin and Alleppay Coast. Indian J. Fisheries, 1961; 8(1): 75-95.
7. Hall, D.N.F., Observations on the taxonomy and biology of some Indo-West pacific penaeid (crustacean, Decapoda). Fish Publs Colon. off., 1962; 17: 229.
8. Hossain, M. S., Chakraborty, A., Joseph, B., Otta, S.K., Karunasagar, I. and Karunasagar, I., Detection of new hosts for white spot syndrome virus of shrimp using nested polymerase chain reaction. Aquaculture, 2001; 198: 1-11.
9. Itami, T., Asano, M., Tokushige, K., Kubono, Nakagawa, A., Takeno, N., Nishimura, H., Maeda, M., Kondo, M., Y. Takahashi. Enhancement of disease resistance of Kuruma sshrimp, *Penaecus japonicus* after oral administration of peptidoglycan derived from Bifidobacterium thermophilum. Aquaculture, 1998; 164: 277-288.
10. Kim, C.K., P.K. Kim, S.G. Sohn, D.S. Sim, M.A. Park, M.S. Heo, T.H. Lee. J.D. Lee, H.K.Jun and K.L. Jung., Development of polymerase Chain Reaction (PCR) procedure for the detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimp, J.Fish Dis., 1998; 21: 11-17.
11. Lightner, D.V., Redman, R.M., Williams, R.R., Mohney, L.L., J.P.M. Clerx, T.A. Bell and J.A.Br., Recent advances in penaeid virus disease investigations. J.World Mariculture Soceity, 1985; 16: 267-274.
12. Menon, M.K., on the paddy field prawn fishery of Travanlore – Cochin and an experiment in prawn culture. Proc. Indo – Pafic. Fish. coun., 1955; 5(2): 131-5.

13. Mohan, C.V. and K.M. Shankar, Recent findings on white spot syndrome (WSS) of cultured shrimp: Management implications. Fishing chimes, September, 1998; 35-37.
14. Mansour sayari., White spot disease in Iran. Disease Information, 2005; 18(31).
15. Mohan C.V, Shankar K.M. Kulkarni.S, Sudha P.M., Histopathology of cultured shrimp showing gross sings of Yellow head syndrome and WSS during 1999, Indian Epizootics, Dis.Aqu.org, 1998; 34(1): 9-12.
16. Mohamed, K.H., Synopsis of biological data on the Indian white prawn *Penaeus indicus* H.Milne Edward 1837. FAO Fisheries synopsis, 1970; 94: 20 PP.
17. Maria Charles, P., Report on SEMBV infection *in Penaeus monodon* of Indian forms. In: Proceedings of second Asia – Pacific marine Biotechnology Conference, Thailand, 1997.
18. Mohan, C.V, Integrated risk reduction strategies for management of white spot syndrome in shrimp farming some thoughts, MPEDA Newsletter, March, 2000; 2000; 22-24.
19. Misra, S.S., Shekhar, M.S. and Azad, I.S., 2005. Concurrent infection with WSSV and WBV in Tiger prawn Penaeus monodon in West Bengal and their detection using PCR and DNA dot – blot hybridization techniques. Indian J.Biotechnology, 4: 506-515.
20. Nunan, L.M., Poulos, B.F.,Lightner, D.V., The detection of white spot syndrome virus (WSSV) and yellow head virus (YHV) in imported commodity shrimp. Aquaculture, 1998; 160: 19-30.
21. Vijayan, K.K. Rajendran K.V. and Santiago T.C.,– Rapid molecular diagnosis of Indian WSSV using PCR. Fishing chimes, 2000; 20(3): 45-46.