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Prevalence of Diseases caused by White Spot Virus and *Enterocytozoon Hepatopenaei* in *Penaeus vannamei* Shrimp Farms in Nagapattinam District, Tamil Nadu, India

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A B S T R A C T

Disease is one of the major factors affecting the development and sustainability of aquaculture. White spot disease caused by white spot syndrome virus (WSSV) results in severe production and economic losses to the shrimp farming industry worldwide. Recently, Hepatopancreatic microsporidiosis caused by *Enterocytozoon hepatopenaei* (EHP) a microsporidian parasite has been reported to cause severe growth retardation and losses in commercial *Penaeus vannamei* farming. In the present study, we report the prevalence of white spot syndrome virus and hepatopancreatic microsporidiosis in the *P. vannamei* shrimp farms in Nagapattinam district, Tamil Nadu, India. This study was undertaken in a total number of 57 selected *P. vannamei* farms in Nagapattinam district, Tamil Nadu during the period from October 2016 to September 2017. *P. vannamei* samples collected at fortnight intervals were screened for WSSV and EHP by PCR. The results showed that the prevalence of diseases caused by WSSV and EHP were 49.12% and 66.66% respectively. This report alerts the farmers for the adoption of better management practices to be followed so as to prevent these diseases and improve the production and sustainability in *P. vannamei* farming.

Introduction

Total shrimp production in the world is dominated by *P. vannamei* and *P. monodon* which contributes around 80% of total shrimp production (FAO, 2009). In the worldwide the dominance of *P. vannamei* over *P. monodon* is due to its availability of SPF, SPR brood stock, fast growth rate and low protein requirement. The global production of crustaceans was about 4128 million tonnes in quantity. China remains to be the top producer of crustaceans globally with 32.5% of production and India stands at sixth place with around 7.0% of production (FAO statistics, 2016). In the shrimp culture system, most of the disease incidence has been attributed to viral pathogens (Kiran and Salim, 2012). But in recent years, there has been incidence of new parasitic pathogens

that has caused severe economic losses to the farmers. Such emerging diseases include WSSV and hepatopancreatic microsporidiosis (HPM), and so on. Since July 1994, the Indian shrimp industry has been under the clutch of disease mainly WSSV and it has washed out most of the farms in India. White spot disease (WSD) was first reported in 1992 in cultured kuruma shrimp (*P. japonicus)* in the Fujian province of china and in nearby Taiwan (Zhan *et al.,* 1998 and Jiang 2001) and later it had spread to most shrimp farming countries throughout south and south-east Asia. In India, WSSV was first reported in 1994 in black tiger shrimp (*P. monodon*) from Visakhapatnam of Andhra Pradesh to sirkali of Tamil Nadu. It cause disease and mortality reaching upto 100% within 2-10 days after the onset of symptoms (Lightner 1996 and Xu *et al.,* 2006).WSSV has emerged as a major threat to the commercial penaeid shrimp farming globally as it has caused mortalities and consequent serious damage to the shrimp culture industry since, 1992 (Inouye *et al.,* 1994, Chou *et al.,* 1995, Wongteerasupaya *et al.,* 1995, Lo *et al.,* 1996, and Karunasagar *et al.,* 1997). WSSV infection results in a rapid onset of the disease and high mortality of upto 100% within 3-10 days in *P. monodon* (Cai *et al.,* 1995) and *P. vannamei*. The major targets of for WSSV infection are tissues of ectodermal and mesodermal origin such as the gills, lymphoid organ, cuticle epithelium, nervous tissue and muscle (Chang *et al.,* 1996). *Enterocytozoon hepatopenaei* (EHP) is a microsporidian parasite that was first characterized and named from the giant or black tiger shrimp *P. monodon* from Thailand in 2009 (Tourtip *et al.,* 2009). It was discovered in slow growing shrimp but was not statistically associated with slow growth at that time. EHP is confined to the shrimp hepatopancreas (HP) and morphologically resembles an unnamed microsporidian, previously reported in the HP of *P. japonica* from Australia in 2001. Together, these studies suggest that EHP is not an exotic pathogen but that it is endemic to Australia. Later, it was found that EHP could also infect exotic *P. vannamei* imported for cultivation in Asia and that it could be transmitted directly from shrimp to shrimp by the oral route (Tangprasittipap *et al.,* 2013). This differed from the most common microsporidian previously reported from cotton shrimp, where transmission required an intermediate fish host, allowing disruption of transmission by exclusion of fish from the production system. Rajendran *et al.,* (2016) have reported the incidence of EHP in India. Against this background, the study was carried out with an objective to understand the prevalence of WSSV and an EHP disease which is severely affect the shrimp production in the nagapattinam district, which is one of the major shrimp farming districts of Tamil Nadu, India.

Materials and Methods

The experiment was conducted at 57 farms (in and around farms of the ten creeks) located in Nagapattinam district Tamil Nadu, India for the period from October 2016 to September 2017. The sampling sites were shown in figure 1 and Table 1.

Sample collection

Samples of juveniles and adults of *P. vanammei* culture ponds were collected from the shrimp farms. Samples of live shrimp were collected and fixed onsite using 70% ethyl alcohol for PCR.

PCR diagnosis

Genomic DNA was extracted from the gills and hepatopancreas of juveniles (Fig. 2). In case of post larvae the whole larvae was pooled and DNA was extracted using a commercial DNA extraction kit (Qiagen,

Germany). The extracted DNA was then suspended in 50 µl of nuclease free water.

PCR diagnosis of WSSV and EHP were carried out using the published protocols of Van Hulten *et al.*, (2001) and Jaroenlak *et al.*, (2016) respectively. The PCR amplification was carried out in a thermal cycler ((BIO-RAD T100 Thermal cycler, USA) in a total volume of 25 µL reaction mixture containing 2X mastermix RED (Ampliqon Taq DNA polymerase, Denmark) 1.0 µL (10 pmol) of forward and reverse primer each and 1.0 µL (50 ng) of DNA extracted from sample.

PCR products were separated on 1.5 % agarose gel containing 0.2 µg/mL ethidium bromide alongside 3µl 100bp DNA ladder (GeneDirex) and the amplified DNA was visualized under UV illumination using a gel documentation system (BIO-RAD, USA).

Results and Discussion

Among the total number of 171 samples screened for WSSV and EHP by PCR, 84 samples (49.12%) were positive for WSSV samples of *P. vanammei* screened by PCR (Figure 3), 54 samples (hepatopancreas) were found to be positive for EHP in the first step, resulting in the amplification of 514bp product which reveals that the sample is heavily infected (Figure 4).

However in the nested PCR 60 *P. vanammei* samples (66.66%) were positive, showing specific amplification at the 148 bp fragment of EHP (Figure 5). The overall prevalence's of diseases caused by WSSV and *EHP* in the *P. vanammei* farms located at Nagapattinam district were 49.12% and 66.66% respectively (Table 2).

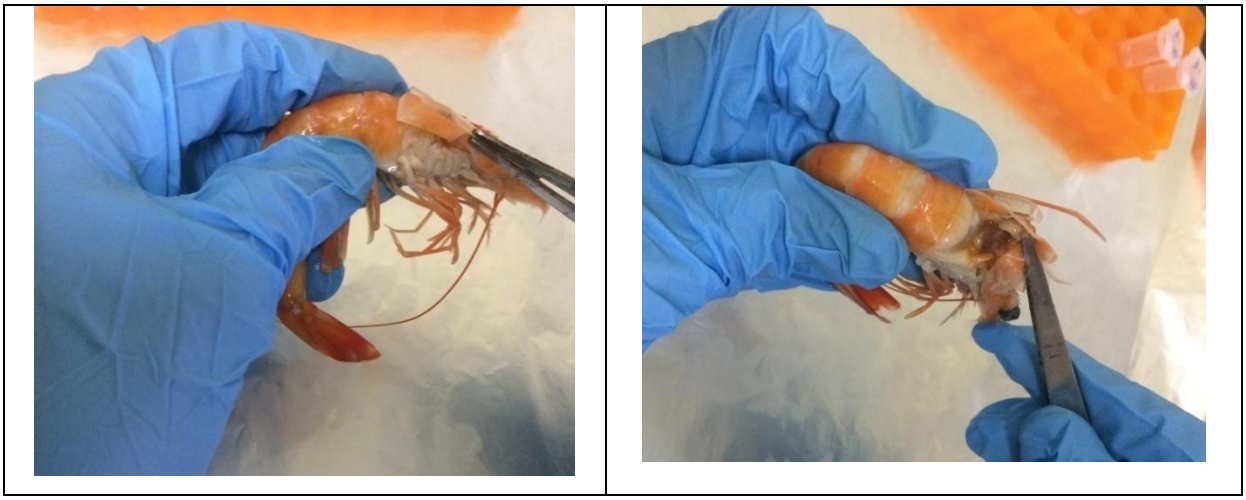
Table.1 Location coordinates of sampling areas

Location	Latitude	Longitude
Nagoore	10° 49'14.5"N	79°49'59.8"E
Kallimedu	10° 29'36.8"N	79°49'23.3"E
Vettaikaran iruppu	10° 33'20.0"N	79°49'48.7"E
Velanganni	10° 40'35.4"N	79°50'04.9"E
Karuvelankadai	10° 44'29.6"N	79°50'08.8"E
Periyathambur	10° 66'83.6"N	79°81'14.6"E
Chinathambur	10° 67'76"N	79°81'64"E
Sembodai	10°46'19° N,	79°82'41° E
Thopputhurai	10° 24'13.5"N	79°51'37.6"E
Vellapallam	10° 32'54.4"N	79°49'26.3"E

Table.2 Prevalence of diseases caused by WSSV and EHP in *P. vanammei* farms at Nagapattinam district, Tamil Nadu

Sl.no	Sample	No. of samples screened	PCR results					Prevalence (%)
			Positive				Negative	
			Disease	I step	Nested Step	Total		
1	Gill of *P.vanammei*	171	WSSV	--------	--------	84	87	49.12%
2	HP of *P. vanammei*	171	EHP	54	60	114	57	66.66%

Figure.1 and 2 DNA was extracted from the gills and hepatopancreas of *P. vanammei*



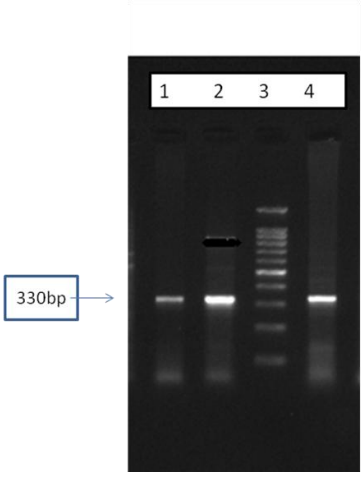


Fig:3
Lane 1,2 & 4,:PCR amplification of WSSV DNA in the PCR (330bp)
Lane 3:100bp molecular weight ladder

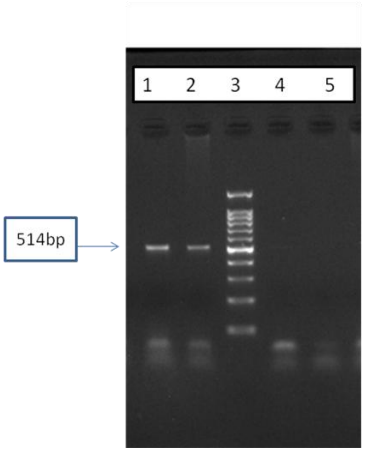


Fig: 4
Lane 1 & 2:PCR amplification of EHP DNA in the 1st step PCR (514bp)
Lane 3:100bp molecular weight ladder

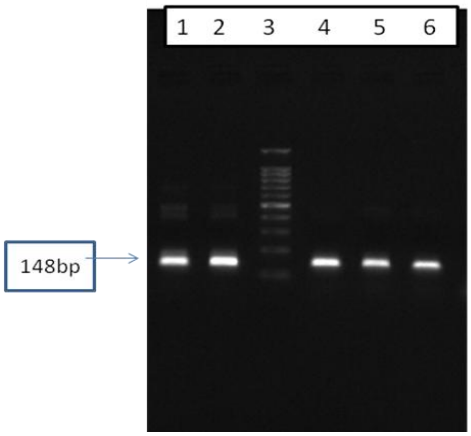


Fig:5
Lane 1,2,4,5 &6:PCR amplification of EHP DNA in the 2nd step (nested) PCR (148bp)
Lane 3:100bp molecular weight ladder

High prevalence of WSSV has been reported in samples collected from *P. vannamei* farms in Nagapattinam district. The About 49.12% prevalence recorded for WSSV pathogens was comparatively lower than the 80% and 75% occurrence reported (Otta *et al.,* 1999 and Otta *et al.,* 2014) from the west coast of India and higher compared to the 39.4% prevalence reported by Uma *et al.,* 2005 from south coast of India.

Tourtip *et al.,* 2009 reported a new microsporidian parasite in *P. monodon. E. hepatopenaei* which was reported in pond reared *P. vannamei* in Vietnam, china Indonesia and Malaysia (Ha *et al.,* 2010 and Tang *et al.,* 2015). In India white feaces and reduced growth associated with EHP infestation has caused severe production losses to the shrimp farmers and the first report on prevalence of EHP causing hepatopancreatic microsporidiosis was reported in 2016 (Rajendran *et al.,* 2016). In the present study, the PCR protocol reported by Jaroenlak *et al.,* (2016) was followed to screen the collected shrimp samples. The prevalence of hepatopancreatic microsporidiosis recorded in the present study was 66.66 % (114/171). An earlier study by Rajendran *et al.,* (2016) has documented a lower prevalence a rate of 63.5% by the nested PCR. However, higher prevalence was documented by Biju *et al.,* (2016) at a rate of 69%. The prevalence of EHP in the hepatopancreatic tissue of *P. vanammei* was recorded around 66.66 % which is comparatively higher that the earlier report (Giridharan and Uma., 2017). EHP spreads through spores which can remain viable up to six months to one year in the aqueous condition like pond water or soil. Higher prevalence rate of EHP (66.66%) compared to WSSV shows the need for the adoption of better management practices for the prevention and control of disease caused by EHP. There is no drug for the control of EHP infection in shrimp. Hepatopancreatic microsporidiosis does not cause any mortality but it is seriously associated with growth retardation in *P. vannamei* (Thitamadee *et al.,* 2016) there by affecting the production and profits in commercial shrimp farming. Application of lime and maintaining soil pH to 12 has been suggested for the disinfection of ponds (CIBA, 2016). The recommended management measure for *E. hepatopenai* is to treat the water with calcium hypochloride at a rate of 18mg/l, treating the hatchery facilities using 2.5% sodium hydroxide solution with a contact time of three hours and rinsing using acidified chlorine and inactivation of EHP spores in shrimp ponds can be done by using quick lime at a rate of 6 tons/hectare and maintain the moist soil at a pH level of 12 for few days Sritunyalucksana *et al.,* (2014). The usage of negative EHP post larvae in farms

and burnt lime application during pre stocking period reduces the incidence of EHP in *P. vanammei* ponds (Sritunyalucksana *et al.*, 2014).

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