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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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.

Keywords
P. vannamei, Shrimp farming, Hepatopancreatic microsporidiosis, Better management practices

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 caused disease and mortality reaching up to 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 up to 100% within 3-10 days in P. monodon (Cai et al., 1995) and P. vannamei. The major targets of 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. vannamei 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).

| 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.1 Location coordinates of sampling areas**

**Table.2 Prevalence of diseases caused by WSSV and EHP in *P. vanammei* farms at Nagapattinam district, Tamil Nadu**
Figure 1 and 2 DNA was extracted from the gills and hepatopancreas of *P. vanammei*
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. vannamei* 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. hepatopenaei* 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. vannamei ponds (Sritunyalucksana et al., 2014).

References

Biju, N., Sathiyaraj, G., Raj, M., Shanmugam, V., Baskaran, B., Govindan, U., and Yohannan, R, 2016. High prevalence of Enterocytozoon hepatopenaei in shrimps Penaeus monodon and LitoPenaeus vannamei sampled from slow growth ponds in India. Diseases of Aquatic Organisms, 120, 225–230.

Cai, S., Huang, T., Wang, C., Song, X., Sun, X., Yu, J., Zhang Y., and Yang C., 1995. Epidemiological studies on the explosive epidemic disease of prawn in 1993–94. J. Fish China, 19, 112–117.

Chang, P. S., Lo, C. F., Wang, Y. C., and Kou, G. H, 1996. Identification of white spot syndrome associated baculovirus (WSBV) target organs in the shrimp Penaeus monodon by in situ hybridization. Dis. Aquat. Org, 27, 131–139.

Chavadej, J., and Withyachumnarnkul, B, 2009. Enterocytozoon hepatopenaei sp. nov. (Microsporida: Enterocytozooidae), a parasite of the black tiger shrimp Penaeus monodon (Decapoda: Penaeidae): Fine structure and phylogenetic relationships. J. Inv. Pathol. 102(1), 21–29.

Chou, H.Y., Huang, C.Y., Wang, C.H., Chiang H.C., and Lo, C.F, 1995. Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. Dis Aquat Org, 23,165–173.

Ha, N.T., Ha, D.T., Thuy N.T., and Lien, V.T.K, 2010. Enterocytozoon hepatopenaei parasitizing on tiger shrimp (Penaeus monodon) infected by white feces culture in Vietnam has been detected (In Vietnamese with English abstract). Agriculture and rural development: science and technology (translation from Vietnamese), 12, 45–50.

Inouye, K. S., Miwa, N., oseka, H., Nakano, T., Kimura, K., Momoyama and Hiroka M, 1994. Mass mortality of cultured kuruma shrimp P. japonicus in Japan in 1993: electron microscopic evidence of the causative virus. Fish Pathol, 29, 149–158.

Jaroenlak, P., Sanguanrut, P., Williams, B. A. P., Stentiford, G. D., Flegel, T. W., Sritunyalucksana K., and Itsathiphaisarn, O, 2016. A Nested PCR Assay to Avoid False Positive Detection of the Microsporidian Enterocytozoon hepatopenaei (EHP) in Environmental Samples in Shrimp Farms. PLOSONE| DOI:10.1371/journal.pone.0166320.

Karunasagar, I., Otta, S. K., and Karunasagar, I, 1997. Histopathological and bacteriological study of white spot syndrome of Penaeus monodon along west coast of India. Aquaculture, 153, 9–13.

Lightner, D. V., 1996. In A Handbook of Shrimp Pathology and Diagnostic Procedures for Disease of Cultured Penaeid Shrimp, The World Aquaculture Society, Baton Rouge Louisana, USA, 305p.

Lo, C.F., Ho, C.H., Peng, S.E., Chen, C.H., Hsu, H.C., Chiu, Y.L., Chang, C.F., Liu, K.F., Su, M.S., Wang, C.H., and Kou, G.H, 1996. White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimps, crabs and other arthropods. Dis. Aquat. Org, 27, 215 – 225.

Nakano, H., Koube, H., Umezawa, S., Momoyama, K., Hiraoka, M., Inouye, K., and Oseka, N, 1994. Mass mortalities of cultured kuruma shrimp, Penaeus japonicus, in Japan in 1993: epizootiological survey and infection trials. Fish Pathol, 29:135-139 (in Japanese).

Otta, S. K., Shuba, G., Joseph, B., Chakraborthy, A., Karunasagar, I., and Karunasagar, I, 1999. Polymerase chain reaction (PCR) detection of white spot syndrome virus (WSSV) in cultured and wild crustaceans in India. Dis. Aquat. Org, 38, 67–70.

Otta, S.K., Arulraj, R., Praveena, P.E., Manivel, R., Panigrahi, A., Bhuvaneswari, T., Ravichandran, P., Jithendran, P.K., and Ponniah, A.G, 2014. Association of dual viral infection with mortality of Pacific
white shrimp (Litopenaeus vannamei) in culture ponds in India. Virus Dis. 25(1), 63–68.

Rajendran, K. V., Shivam, S., Ezhil Praveena, P., Joseph Sahaya Rajan, J., Sathish Kumar, T. Avunje, S., and Vijayan, K. K, 2016. Emergence of Enterocytozoon hepatopenaei (EHP) in farmed Penaeus (Litopenaeus) vannamei in India. Aquaculture. 454, 272–280.

Sriurairatana, S., Boonyawiwat, V., Gangnonngiw W., and Laosutthipong, C, 2014. White feces syndrome of shrimp arises from transformation, sloughing and aggregation of hepatopancreatic microvilli into vermiform bodies superficially resembling gregarines. PLoS ONE 9(6): e99170

Tang, K. F. J., Pantoja, C. R., Redman, R. M., Han, J. E., Tran, L. H., and Lightner, D. V,2015. Development of in situ hybridization and PCR assays for the detection of Enterocytozoon hepatopenaei (EHP), a microsporidian parasite infecting penaeid shrimp. J. Inv. Pathol, 130, 37–41.

Tangprasittipap, A., Srisala, J., Chouwdee, S., Somboon, M.; Chuchird, N.; Limsuwan, C., and Sritunyalucksana, K, 2013. The microsporidian Enterocytozoon hepatopenaei is not the cause of white feces syndrome in white leg shrimp Penaeus (Litopenaeus) vannamei. BMC. Vet. Res, 9(1), 139.

Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Salachan, P. V.; Sritunyalucksana, K., and Itsathiphaisarn, O, 2016. Review of current disease threats for cultivated penaeid shrimp in Asia. Aquaculture, 452, 69–87.

Tourtip, S., Wongtripop, S., Stentiford, G. D., Bateman, K. S., Sriurairatana, S., Uma, A., Koteeswaran, A., and Indrani Iddy, K, 2005. Prevalence of white spot syndrome virus and monodon baculovirus in Penaeus monodon broodstock and postlarvae from hatcheries in southeast coast of India. Curr sci, 89, 1619-1622.

Van Hulten, M.C., Witteveldt, J., Snippe, M., and Vlak, J.M, 2001. White spot syndrome virus envelope protein VP28 is involved in the systemic infection of shrimp. Virology 285, 228-233.

Wang, C.H., Lo, C.F., Leu, J.H., Chou, C.M., Yeh, P.Y., Chou, H.Y., Tung, M.C., Chang, C.F., Su, M.S., and Kou, G.H, 1995. Purification and genomic analysis of baculovirus associated with white spot syndrome (WSBV) of Penaeus monodon. Dis Aquat Org, 23,239-242.

Wongteerasupaya, C., Vickers, J.E., Sriurairatana, S., Nash, G.L., Akarajamorn, A., Boonsaeng, V., Panyim, S., Tassanakajon, A., Withyachumnarnkul, B., and Flegel, T.W, 1995. A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn Penaeus monodon. Dis. Aquat. Org, 21, 69 – 77.

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