Asian Fisheries Science **19**(2006):113-116
ISSN: 0116-6514
https://doi.org/10.33997/j.afs.2006.19.2.003

Asian Fisheries Society, Manila, Philippines

Prevalence of Hepatopancreatic Parvovirus (HPV) in *Penaeus monodon* Postlarvae from Commercial Shrimp Hatcheries in Tamilnadu, Southeast Coast of India

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Abstract

The prevalence of hepatopancreatic parvovirus (HPV) in *Penaeus monodon* postlarvae produced from commercial shrimp hatcheries in Tamilnadu, located along the southeast coast of India was studied during the period July 2002 to July 2003. A total of 1020 hatchery tanks rearing postlarvae were screened by microscopic method and polymerase chain reaction (PCR). Microscopic observation of Giemsa stained smear by rapid method or wet mount observation of squash preparation of hepatopancreas from postlarvae samples from the different tanks revealed a prevalence of 7.74% for HPV and 2.75% for dual infection with HPV and monodon baculovirus (MBV). PCR analysis of samples during the period of study showed a prevalence of 9.3% for HPV and 4.5% for dual infection with HPV and MBV.

Short Communication

Hepatopancreatic parvovirus (HPV) is known to cause disease in penaeid shrimp (Lightner and Redman 1985, Lightner 1988, Sukhumsirichart et al.1999). HPV in shrimp was first reported from *Penaeus merguiensis* and *P. indicus* by Chong and Loh (1984) and from *P. chinensis* by Lightner and Redman (1985). HPV has been reported to cause mortalities in

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early larval and postlarval stages of shrimp (Lightner et al. 1993, Spann et al. 1997), stunted growth in juveniles (Flegel et al. 1999, Limsuwan 1999), production and economic losses in shrimp farming (Flegel et al. 1999). HPV infection has also been reported from Indian hatcheries (Manivannan et al. 2002, Umesha et al. 2003) and in wild shrimp samples in India (Manjanaik et al. 2005). Recent reports show that HPV infection and dual infection with HPV and monodon baculovirus (MBV) is associated with stunted growth syndrome in Thailand (Chayaburakul et al. 2004). We report here the prevalence of HPV infection in the postlarvae from the commercial shrimp hatcheries in Tamilnadu, located along the southeast coast of India.

A total of 1020 larval tanks from several shrimp hatcheries located in Tamilnadu, southeast coast of India, rearing postlarvae (PL 8-20) of *P. monodon* were sampled. The samples were brought to the laboratory in live condition in oxygenated plastic bags. Each bag contained 400 postlarvae selected randomly from individual tanks of the hatchery for the study by Polymerase chain reaction (PCR) for HPV and MBV, rapid Giemsa stained preparation and wet mount squashes of hepatopancreas for microscopic observation of MBV and HPV. A subsample of 25-30 postlarvae from each bag was used for the DNA extraction for PCR analysis. Live samples were used for the preparation of rapid Giemsa stained smears and wet mount squashes of hepatopancreas for microscopic observation of HPV and MBV respectively.

A commercial PCR diagnostic kit (Mangalore Biotech Laboratory, Mangalore, India) was used for the PCR amplification of HPV and MBV in postlarvae samples. Extraction of viral DNA was carried out as per manufacturer's instructions. An aliquot (2 µl) of the DNA extract was used as template for PCR amplification along with HPV positive and negative controls, MBV positive and negative controls. The programme protocol followed for PCR amplification of HPV DNA was as follows: an initial delay of 94^{o}C for 5 min, 30 cycles at 94^{o}C for 30s, 54^{o}C for 30s, 72^{o}C for 30s and a final extension at 72^{o}C for 5 min. The programme protocol followed for PCR amplification of MBV DNA included an initial delay of 94^{o}C for 5 min, 30 cycles at 94^{o}C for 30s, 60^{o}C for 30s, 72^{o}C for 30s and a final extension at 72^{o}C for 5 min. PCR amplification was carried out in an Eppendorf Personal Master Cycler (Eppendorf, USA) and the amplified PCR products resolved in 1.2% agarose gel containing 0.5 µg·ml^{-1} ethidium bromide and observed in a DNA transilluminator. The expected product size for HPV and MBV was 441 and 361 bp respectively.

Rapid Giemsa stained smears of hepatopancreas of the postlarvae samples were prepared following the method described by Lightner et al. (1993) with slight modification. The hepatopancreas of live postlarvae were dissected and dabbed on to clean glass slides. The slides were air dried and fixed immediately in methanol for 3 min followed by staining with Giemsa stain for 30 min. The stained slides were observed under the microscope. Wet mount squashes were prepared by dissecting out the hepatopancreas of postlarvae and staining with 0.05% malachite green. The wet mount preparations were observed under the microscope.

Of 1020 tanks with postlarvae screened by microscopy, 79 (7.74%) were positive for HPV (Table 1). Microscopic observation of the rapid Giemsa stained smears of hepatopancreas showed small eosinophilic inclusions associated with the nucleolus or large basophilic inclusions in the hypertrophied nuclei with the compressed and crescent shaped nucleolus as described by Lightner et al. (1993). When the postlarval samples were screened for HPV by PCR, 95 (9.3%) were found to be HPV positive, yielding a 441 bp HPV specific band. Observation of the wet mount squashes/ rapid Giemsa stained smears showed both HPV inclusions and MBV occlusion bodies in 28 (2.75%) samples (Table 1). PCR analysis of DNA from

HPV positive samples using MBV specific PCR primers showed that 46 (4.5%) samples were positive for MBV yielding a MBV specific band at 361bp.

Table 1. The prevalence of HPV and MBV in *P. monodon* postlarvae produced from the commercial shrimp hatcheries in Tamilnadu South east coast of India, during July 2002 to July 2003.

Total number of PL tanks samples	Number of HPV positive samples (%)		Number of samples with dual infection with HPV and MBV (%)	
	Rapid Giemsa stained smear/wet mount squash	PCR	Rapid smear/wet mount squash	PCR
1020	79 (7.74%)	95 (9.3%)	28 (2.75%)	46 (4.5%)

Although there are many reports of HPV infection in *P.monodon* in growout ponds (Lightner 1996, Flegel et al. 1999), there are very few reports on HPV infection in hatchery reared *P.monodon* larvae (Manivannan et al. 2002, Umesha et al. 2003). HPV infections in postlarvae have been reported to accompany infections with other hepatopancreatic pathogens (Flegel et al. 1992, Lightner 1988, Lightner et al. 1993). Infection with pathogens like Yellow head virus and MBV in the HPV infected shrimp has been reported (Chantanachookin et al. 1993). Multiple infection in *P.monodon* postlarvae (PL8-10) has been reported from an Indian hatchery indicating that HPV could be a major problem in shrimp hatcheries when present with other viruses like MBV and WSSV (Manivannan et al. 2002). Owing to the high diagnostic sensitivity of PCR, HPV prevalence of 9.3% was recorded in the postlarvae samples that are comparatively higher than 7.74% recorded by the rapid Giemsa stained smear method.

The prevalence of HPV infection and dual infection with other viruses in the postlarvae produced from the commercial hatcheries reported in this study stresses the need for screening of brooders for HPV as they could transmit the disease to the larvae by water contamination. Stocking of HPV infected postlarvae could cause economic losses due to growth stunting in shrimp (Flegel et al. 1999). Moreover it is suggested that the modified rapid Giemsa stained smear method adopted in this study can be followed in the hatcheries and diagnostic labs as its sensitivity is adequate to carry out preliminary screening of post larvae and brooders for HPV.

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