

Experimental Transmission of Hepatopancreatic Parvovirus (HPV) Infection in *Penaeus monodon* Postlarvae

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ABSTRACT

Hepatopancreatic parvovirus (HPV) infection in penaeid shrimps was first reported in various countries of the Asia-Pacific region in mid-1980. The virus affects the hepatopancreas of postlarvae and juveniles, usually leading to slow growth and mortality during the early stage of culture. At present, there is no established experimental model of infection in *Penaeus monodon*, a susceptible species, since there has not been any report of successful HPV transmission under laboratory conditions. Therefore, experiments were undertaken to induce HPV infection by feeding *P. monodon* postlarvae (PL) with virus-infected PL. Postlarval *P. monodon* (PL-16), initially examined to be free from HPV, were found HPV-positive 24 hours after they were fed with the infected material. Percentage of infection was from 30% (day 1) to 100% (day 7) based on the examination of wet mounts of hepatopancreas (squashed tissue) stained with malachite green and through histopathology. This is the first report of a successful horizontal transmission of HPV in *P. monodon* PL. This infection model could be used to study the pathogen further and would permit controlled experiments to be undertaken in order to identify methods of prevention and control.

INTRODUCTION

Hepatopancreatic parvovirus (HPV) infection, grossly characterized by nonspecific signs such as poor growth rate, anorexia, decreased preening activity, increased surface fouling, and sporadic opacity of tail musculature, was first reported by Lightner and Redman (1985) in samples of four different penaeid shrimp species obtained from separate aquaculture facilities in China, Singapore, Kuwait and Philippines. In addition to these signs, mortalities during the juvenile stages that could reach 40-100% within 4 to 8 weeks of disease onset were also reported. The main pathological feature is the presence of large, basophilic intranuclear inclusion bodies in E-type epithelial cells of infected hepatopancreatic distal tubules (Lightner *et al.*, 1993).

HPV has been reported to infect most species of penaeid shrimps and is now found in Asia, Australia, Africa, and in some countries in South America (Brock and Lightner, 1990; Lightner, 1996). The significance of HPV infection as a disease has been underestimated since no specific clinical signs could be attributed to HPV and it often occurs in mixed

Catap, E.S. and R.D. Travina. 2005. Experimental transmission of Hepatopancreatic Parvovirus (HPV) infection in *Penaeus monodon* postlarvae. In P. Walker, R. Lester and M.G. Bondad-Reantaso (eds). *Diseases in Asian Aquaculture V*, pp. 415-420. Fish Health Section, Asian Fisheries Society, Manila.

infections with other shrimp viruses (Pantoja and Lightner, 2001). However, a recent study by Flegel *et al.* (1999) indicated that HPV infection in cultured *Penaeus monodon* in Thailand is associated with stunted shrimps, which could result in considerable harvest losses. Thus, HPV is now considered the third most important shrimp virus in Thailand in terms of economic impact, with white spot syndrome virus (WSSV) and yellow head virus (YHV) as first and second, respectively (Flegel, 2002). Consequently, it was recommended that PL must be screened for HPV infection prior to stocking and that PL with moderate to high prevalence of HPV infections be rejected.

The transmission of HPV is not clearly understood since there has been no report of any experimental infection of HPV in any susceptible shrimp species. The main objective of this study is to establish a pathology model of HPV infection in postlarval *P. monodon*. Therefore, this experiment was undertaken to determine the pathogenicity of HPV by feeding uninfected *P. monodon* postlarvae with HPV-infected PL. This paper reports the first successful horizontal transmission of HPV in postlarval *P. monodon*.

MATERIALS AND METHODS

Postlarvae (2,000 pcs PL-16), purchased from a private hatchery in Tigbauan, Iloilo, Philippines were held 4 days in a circular fiberglass tank (200 L capacity) with aerated seawater. In the holding tank, shrimp were fed once daily with 200 ml *Artemia nauplii* (50 nauplii/ml). PLs were then randomly distributed in 5 x 10 L glass tanks (300 PLs per tank) filled with 5 liter, UV-sterilized and well-aerated seawater. Prior to distribution in glass tanks, postlarval samples were examined for the presence of HPV inclusion bodies using the wet-mount technique commonly used to screen PLs for the presence of occlusion bodies indicative of monodon baculovirus (MBV) infection. Briefly, the hepatopancreas was dissected out, squashed on a glass slide with a glass cover slip and stained with 0.5% malachite green (Lightner, 1996). No HPV infection was detected.

Three days after stocking in the glass tanks, the experimental PL were fed with HPV-infected postlarvae (PL-26 stored at -80°C), obtained from a hatchery in Iloilo Province, that were examined previously through histopathology. For the HPV-treated tanks, 2.0 g of frozen PL was added once in each of the 4 tanks. For the control, PLs in one tank were fed with *Artemia* instead of HPV-infected material. After 24 hours, one-half of the water in each tank was changed with UV-sterilized seawater. Sampling of PL for the wet-mount technique (5 PLs from each 5 tanks) and histopathology (10 PLs from each 5 tanks) was undertaken on days 1, 3, 5 and 7 post-infection (after feeding with infected PL). During the experimental period, PLs in each tank were fed daily with 10 ml *Artemia nauplii* (50 nauplii/ml), except on the days before and during the infection treatment. Temperature during the experiment ranged from 25-30°C and UV-sterilized seawater was used during daily water exchange.

Samples for histopathology were fixed whole in Davidson's fixative, then transferred to 70% ethanol and passed through a dehydrating series of ethanol concentrations. Tissues were embedded in paraffin wax, sectioned at 5 µm and stained with hematoxylin and eosin. Sections were examined under an Olympus

BH-2 light microscope for the presence of large, basophilic inclusion bodies in the distal tubules of the hepatopancreas, which signify HPV infection.

RESULTS AND DISCUSSION

Table 1 shows the number of PL (PL 23 at start of infection) examined that were infected with HPV, based on the wet-mount technique and histopathological examination. The wet-mount technique was used as a preliminary procedure to detect the round HPV inclusion bodies. Figure 1 shows the appearance of the HPV inclusion bodies in a squashed preparation of hepatopancreas and observed under a light microscope. Virus infection was already detected in 6 out of 20 samples (30%) at 24 hours after the experimental PLs were fed with dead HPV-infected PLs. The detection of HPV in postlarvae 24 hours post infection (p.i.) conforms to those previously reported for MBV, baculovirus penaei (BP) and WSSV. However, a prevalence of 100% was obtained within 24 hrs p.i. for MBV and 48-72 hrs p.i. for BP (Natividad and Lightner, 1992; Hammer *et al.*, 1998; Soto *et al.*, 2001). These differences could be due to the different detection techniques and the methods of infection used in the experiments. In addition, HPV initially infects the generative E-cells of the distal tubules of the hepatopancreas, as opposed to MBV, which can infect all cells of the hepatopancreas and anterior midgut, thus possibly enabling this virus to establish infection faster.

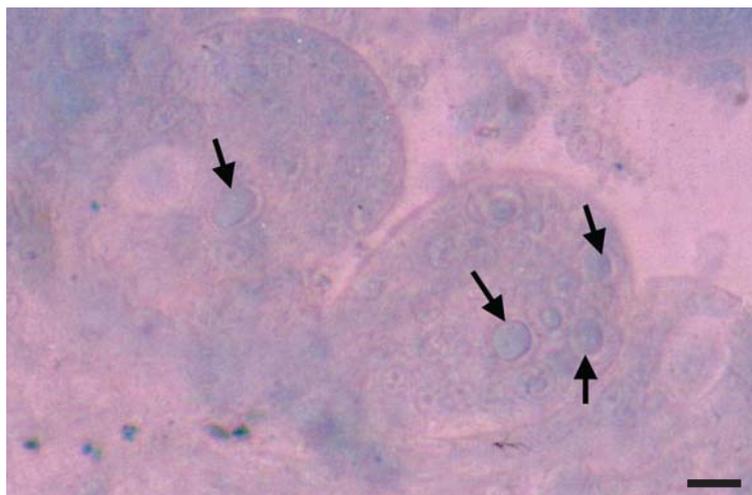


Figure 1. Wet-mount of HPV-infected hepatopancreas of postlarval *P. monodon* with the ovoid to round inclusion bodies (arrows) in the cells of the distal tubules. Malachite green stain; scale bar = 10 μ m.

The percentage of HPV infection increased dramatically to 60% (12/20) at day 3 p.i., 95% (19/20) at day 5 and reached 100% at day 7. Since only half of the water was replaced after 24 hours, the significant increase of HPV at day 3 p.i. could be attributed to the remaining dead HPV-infected PL that were eaten by the experimental PLs. It could also be due to secondary transmission from the PL that were initially infected at 24 hrs p.i. and cannibalized by the experimental PL.

Table 1. Experimentally-induced HPV-infection, based on wet mounts of hepatopancreatic tissues and histopathology, in *P. monodon* postlarval samples (PL-23 at start of experimental infection) fed with virus-infected PLs.

Day p.i.	Wet-mount		Histopathology	
	No. PLs examined*	HPV-(+) PLs**	No. PLs examined*	HPV-(+) PLs**
1	20	6	40	13
3	20	12	40	38
5	20	19	39	38
7	20	20	40	40

* number of samples from 4 tanks infected with HPV

** number of HPV (+) samples from 4 tanks infected with HPV

No inclusion bodies were observed in samples from the control tank (tank 1). However, at day 1 p.i., there was one sample from one tank of the HPV-treated group (tank 5) which exhibited MBV infection manifested by the presence of multiple occlusion bodies in the hepatopancreas. At day 3 p.i., samples from the control tank (tank 1) also had MBV, along with some PLs from HPV-treated tanks 4 and 5. This further spread to some PLs in the other HPV-treated tanks 2 and 3 at days 5 and 7 p.i. It is likely that the experimental PLs had latent MBV infection. At the end of the trial, 146 PL survived in the control tank. For the HPV-treated tanks, 122, 139, 170 and 154 PL survived in tank 2, 3, 4 and 5, respectively. The mortalities which occurred during the experiment, including the PL in the control tank, could be attributed to cannibalism and viral infections.

Histopathology was also used to confirm the preliminary data obtained from the wet mount technique. No remarkable difference was observed in the percentages of HPV infection among the experimental PLs at each sampling day, except at day 3 p.i. when considerably higher HPV prevalence was detected via histopathology. These results show that the wet mount technique could be used to screen PLs for both MBV and HPV; however, histopathology is still more sensitive technique to detect and confirm HPV infections. Moreover, it is suggested that PCR protocol be developed to detect HPV in the Philippines. At present, the existing PCR techniques to diagnose HPV do not give satisfactory results when employed using PL samples from the Philippines. This could be due to differences in the genomes of HPV strains, as reported previously in Thai and Korean HPV, which showed different reactions when DNA detection reagents based on the Korean HPV were used (Phromjai *et al.*, 2001).

Histopathological examination of samples collected at days 1 and 3 p.i. showed the early stage of infection characterized by the presence of both developing and very few large basophilic intranuclear inclusion bodies in few cells of the hepatopancreas while most cells still remained uninfected (Fig.2a). During the advanced stage of infection at days 5 and 7 p.i., almost all of the epithelial cells of the distal tubules had been infected with large, mostly single, HPV inclusion bodies (Fig.2b). The inclusion bodies caused the lateral displacement of the nucleoli and margination of the chromatin. The size of the inclusion bodies ranged from 5 to 11 μm and appeared ovoid to spherical in shape. In some advanced stages of infection, inclusion bodies were observed in the lumen of the damaged hepatopancreas. It is highly possible that these could also serve as source of infection in the culture system. No remarkable inflammatory response or necrosis was observed in the affected hepatopancreatic tissues.

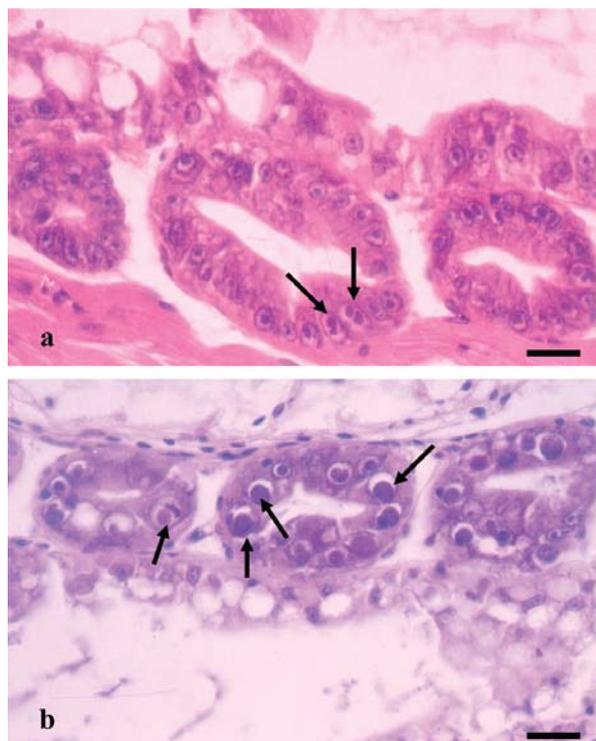


Figure 2. a) Photomicrograph showing the early stage (day 1 post-infection) of HPV infection in the distal tubules of the hepatopancreas. Developing inclusion bodies (arrows) were present in enlarged nuclei of some hepatopancreatic cells. b) Advanced stage (day 7 post-infection) of HPV-infection in the hepatopancreatic distal tubules. Most of the cells exhibited the single, round basophilic inclusion bodies within their nuclei. H & E stain; scale bar = 20 μ m.

Based on the wet mount and histopathology detection techniques, HPV was horizontally transmitted in *P. monodon* PL through oral feeding of HPV-infected material (whole body of infected *P. monodon* PL). This is the first report of experimental HPV transmission in this penaeid shrimp species. Transmission of HPV was earlier reported in *Penaeus chinensis* (Z-3, PL-7, and adults) through immersion and oral feeding methods and mortality was higher in younger stages than in adult *P. chinensis* (Sun and Kusuda, 1997). However, recent experiments undertaken in our laboratory when juvenile *P. monodon* were used did not produce consistently high HPV infection as when PL were used. In addition, no HPV infection was produced in adult *P. monodon*. This could explain the lack of report of an experimental model for HPV in *P. monodon*, considered a very important penaeid species in Asia. Thus, the role of various factors on the pathogenicity of HPV strains, such as shrimp species susceptibility and life stage or age, method of infection, viral concentration and mixed viral infection, still need to be studied to improve this infection model.

ACKNOWLEDGMENTS

The authors are grateful to the financial assistance provided by the Government of Japan - Trust Fund granted to the SEAFDEC-AQD and to Dr. Gilda Lio-Po's help in obtaining HPV-infected postlarval samples.

REFERENCES

- Brock, J.A. and Lightner, D.V. 1990. Diseases caused by microorganisms (Diseases of Crustacea). In Kinne, O. (ed.). Diseases of Marine Animals Vol III. Biologische Anstalt Helgoland. pp 245-349.
- Flegel, T.W. 2002. An overview of PCR techniques for shrimp disease diagnosis in Asia, with emphasis on Thailand. In Inui, Y. and Lacierda, E.C. (eds.). Disease Control in Fish and Shrimp Aquaculture in Southeast Asia - Diagnosis and Husbandry Techniques. Southeast Asia Fisheries Development Center, Iloilo. pp 34-64.
- Flegel, T.W., Thamavit, V., Pasharawipas, T. and Alday-Sanz, V. 1999. Statistical correlation between severity of hepatopancreatic parvovirus infection and stunting of farmed black tiger shrimp (*Penaeus monodon*). Aquaculture 174, 197-206.
- Hammer, H.S., Stuck, K.C. and Overstreet, R.M. 1998. Infectivity and pathogenicity of *Baculovirus penaei* (BP) in cultured larval and postlarval Pacific white shrimp, *Penaeus vannamei*, related to the stage of viral development. Journal of Invertebrate Pathology 72, 38-43.
- Lightner, D.V. 1996. A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp. World Aquaculture Society, Baton Rouge.
- Lightner, D.V. and Redman, R.M. 1985. A parvo-like virus disease of penaeid shrimp. Journal of Invertebrate Pathology 45, 47-53.
- Lightner, D.V., Redman, R.M., Moore, D.W. and Park, M.A. 1993. Development and application of a simple and rapid diagnostic method to studies on hepatopancreatic parvovirus of penaeid shrimp. Aquaculture 116, 15-23.
- Natividad, J.M. and Lightner, D.V. 1992. Susceptibility of the different larval and postlarval stages of black tiger prawn, *Penaeus monodon*, Fabricius, to monodon baculovirus (MBV). In Shariff, M., Subasinghe, R.P. and Arthur, J.R. (eds.). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila. pp 111-125.
- Pantoja, C.R. and Lightner, D.V. 2001. Detection of hepatopancreatic parvovirus (HPV) of penaeid shrimp by *in situ* hybridization at the electron microscopic level. Diseases of Aquatic Organisms 44, 87-96.
- Sun, X. and Kusuda, R. 1997. A study on the pathogenicity of hepatopancreatic parvo-like virus in Chinese Prawn, *Penaeus chinensis*. Suisanzoshoku 45, 55-60 (in Japanese with Abstract in English).