

# Distinction of HPV-Type Viruses in *Penaeus chinensis* and *Macrobrachium rosenbergii* Using a DNA Probe

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## Abstract

Histological sections of hepatopancreas tissue from postlarval *Macrobrachium rosenbergii* from Malaysia infected with an HPV-type (HPV-Mac) virus were tested for possible virus homology with a DNA probe developed from an HPV-type virus (HPV-Chin) of *Penaeus chinensis* from Korea. No reaction of the HPV-Chin probe was observed with HPV-Mac intranuclear inclusion bodies or infected cells. These findings, along with distinctive cytopathological differences between cells infected with HPV-Chin and HPV-Mac, indicate that these are distinctly different viruses within the parvovirus family.

## Introduction

The hepatopancreatic parvo-like viruses (HPV), as a group, are one of at least 15 different virus pathogens known to infect cultured and wild penaeid shrimp (Brock and Lightner 1990; Adams and Bonami 1991; Lightner 1993). HPV-type viruses are apparently cosmopolitan in distribution, having been reported from wild and/or cultured penaeid shrimp in Asia, Africa, Australia and North and South America (Chong and Loh 1984; Lightner and Redman 1985, 1992; Paynter et al. 1985; Colorni et al. 1987; Roubal et al. 1989; Brock and Lightner 1990; Fulks and Main 1992; Lightner et al. 1993) and from *Macrobrachium rosenbergii* from Malaysia (Anderson et al. 1990).

The importance of HPV-type viruses as a pathogen remains poorly understood, possibly due in part to the relative difficulty of diagnosis of HPV infections and to

the possibility that HPV infections may be masked in multiple infections with other pathogens (Flegel et al. 1992; Lightner 1993; Lightner et al. 1993). Until now diagnosis of HPV has been dependent upon microscopical or histological demonstration of pathognomonic Feulgen positive, basophilic intranuclear inclusion bodies in hepatopancreatic epithelial cells (Brock 1992; Lightner and Redman 1992; Lightner et al. 1993). Other than differences in the interpretation of subtle morphological differences, no methods have been available to compare HPV-type viruses from different geographic regions or from different hosts for relatedness. Genomic probes for several of the penaeid shrimp viruses have been developed recently (Lightner et al. 1992; Bruce et al. 1993, 1994; Mari et al. 1993a, 1993b; Poulos et al. 1994). Recently, Mari et al. (in press) cloned portions of the genome of the penaeid shrimp parvovirus from *Penaeus chinensis* (HPV-Chin). Genomic probes prepared to HPV-Chin were found to react intensively with infected nuclei and with necrotic HPV-infected cells, and to provide far greater diagnostic sensitivity than that provided by routine H&E histology (Mari et al., in press).

Reported here are findings from the application of the HPV-Chin probe developed by Mari et al. (in press) to determine the relatedness of an HPV-type virus in Malaysian *M. rosenbergii*.

## Materials and Methods

### *Prawns and Shrimp*

Postlarval *M. rosenbergii* (obtained from a private prawn hatchery in Peninsular Malaysia) were sampled, preserved in Davidson's AFA (Bell and Lightner 1988), and processed for histological study as described by Anderson et al. (1990). For gene probe *in situ* hybridization assay, histological materials in paraffin blocks from the earlier study of Anderson et al. (1990) were obtained from the archived sample collection at the Universiti Pertanian Malaysia. Samples of postlarval and juvenile *P. chinensis* (obtained from three hatcheries in Korea) were processed in a similar manner as was previously described (Anderson et al. 1990; Lightner et al. 1993).

### *Gene Probe*

The methods used to purify HPV virus (= HPV-Chin) from infected *P. chinensis*, to extract and clone its DNA, and to prepare the labeled gene probe used in this study were essentially identical to those developed for IHNV, except that infected hepatopancreata were excised and used as the source of HPV virus for purification and nucleic acid extraction (Bonami et al. 1990; Lightner et al. 1992; Mari et al. 1993b; Mari et al., in press). The HPV probe (called S2.0) used is approximately 2.0 Kbp in length. For the work reported here, the probe was labeled using the non-radioactive Genius I Kit (Boehringer Mannheim, Inc.), which contains digoxigenin-11-dUTP (= DIG) as the DNA label, and uses an ELISA-based system for final detection. Details on the characterization of HPV-Chin and on the development of the HPV-Chin probe have been described elsewhere (Mari et al., in press).

### Sample Preparation

Consecutive histological sections for routine histological processing and for *in situ* hybridization with labeled probe were obtained from a rotary microtome and mounted on positively charged silanized glass slides (Fisher Scientific). One section of each consecutive pair was stained with hemotoxylin and eosin (H&E) using routine methods (Bell and Lightner 1988). The other slide of the pair was reacted with the DIG-labeled gene probe made to HPV-Chin, and then counterstained with bismark brown as described previously for IHNV (Lightner et al. 1992; Mari et al., in press).

### Results and Discussion

Intense reactions of the DIG-labeled S2.0 probe from *P. chinensis* were readily apparent (and consistent with routine H&E stained histological findings) in sections of hepatopancreata of all HPV-Chin infected shrimp probed and examined (Figs. 1-2). In marked contrast, the S2.0 probe did not react with HPV-Mac intranuclear inclusion bodies in infected hepatopancreatic cells of *M. rosenbergii* (Figs. 3-4).



Fig. 1. Histological section of an HPV-Chin infected tubule in the hepatopancreas of a juvenile *Penaeus chinensis*. Prominent basophilic intranuclear inclusion bodies (I) diagnostic of HPV infection are shown in a number of HP tubule epithelial cells. A prominent feature of HPV-Chin infected nuclei in penaeid shrimp is the lateral displacement of the nucleolus (arrows) by the developing viral inclusion body. H&E stain. Bar = 10  $\mu$ m.



Fig. 2. A histological section of the hepatopancreas from a *Penaeus chinensis* infected with HPV-Chin. The section has been reacted with the DIG-labeled probe S2.0 using *in situ* hybridization. Nuclei of several hepatopancreatic tubule epithelial cells display intense positive reactions for HPV-Chin. Probe and bismark brown. Bar = 10  $\mu$ m.

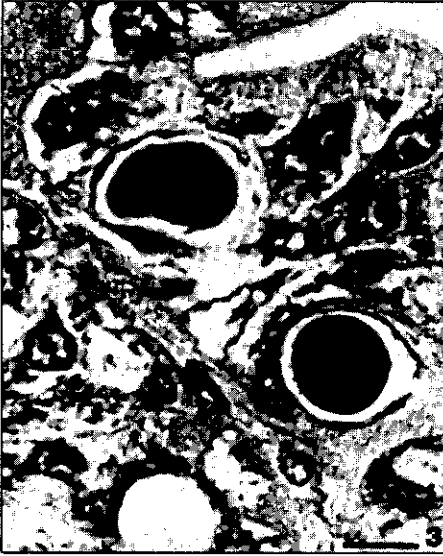


Fig. 3. Histological section of an HPV-Mac infected tubule in the hepatopancreas of a juvenile *Macrobrachium rosenbergii* showing prominent basophilic intranuclear inclusion bodies (I) diagnostic of infection by an HPV-type virus are shown in a number of hepatopancreatic tubule epithelial cells. Unlike infections by HPV-Chin in penaeid shrimp, HPV-Mac infected cell nuclei do not possess a laterally displaced nor prominent nucleolus associated with the developing viral inclusion body. H&E stain. Bar = 10  $\mu$ m.

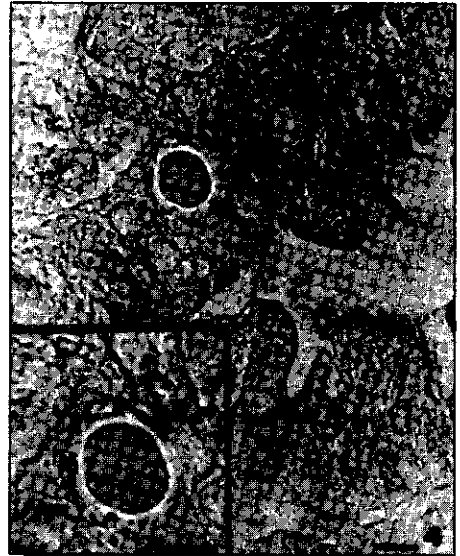


Fig. 4. A parallel histological section from the same *Macrobrachium rosenbergii* shown in Fig. 3, but reacted with the DIG-labeled probe S2.0 using *in situ* hybridization. The HPV-Mac infected cell shown (arrow and inset) displays no reaction for HPV-Chin with the probe. Probe and bismarck brown. Bars = 10  $\mu$ m.

Close inspection of HPV-Chin infected cells of *P. chinensis* and HPV-Mac in *M. rosenbergii* in H&E and probed sections reveals distinct morphological differences. In HPV-Chin infected cell nuclei, the host cell nucleolus is always somewhat hypertrophied and intimately associated with the HPV inclusion body (Figs. 1-2), whereas in HPV-Mac infected cell nuclei, the nucleolus is not so prominent (Figs. 3-4). These distinctive morphological differences visible in histological preparations, when considered with the size differences reported for HPV-Chin and HPV-Mac (22-24 nm for HPV-Chin versus 29 nm for HPV-Mac) (Lightner and Redman 1985; Anderson et al. 1990; Bonami and Lightner 1991), suggest that HPV-Chin and HPV-Mac are not closely related HPV-type viruses. Hence, it was not unexpected that the genomic probe prepared from HPV-Chin DNA lacked sufficient homology to the DNA of HPV-Mac to provide a positive reaction using the *in situ* hybridization assay. The findings of this study confirm that HPV-Chin and HPV-Mac are not closely related.

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