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

D.V. LIGHTNER[1], R.M. REDMAN[1], B.T. POULOS[1],
J.L. MARI[1], J.R. BONAMI[2] and M. SHARIFF[3]

[1]*Department of Veterinary Science*
University of Arizona
Tucson, AZ 85721 USA

[2]*Laboratorie de Pathologie Comparee*
Universite de Montpellier II
Montpellier, France

[3]*Faculty of Fisheries and Marine Science*
Universiti Pertanian Malaysia
43400 UPM Serdang
Selangor Darul Ehsan, Malaysia

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 Ko-rea. 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 miscroscopical 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 IHHNV, 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 nonradioactive Genius I Kit (Boehringer Mannheim, Inc.), which contains digoxygenin-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 pr6be 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 IHHNV (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 µm.

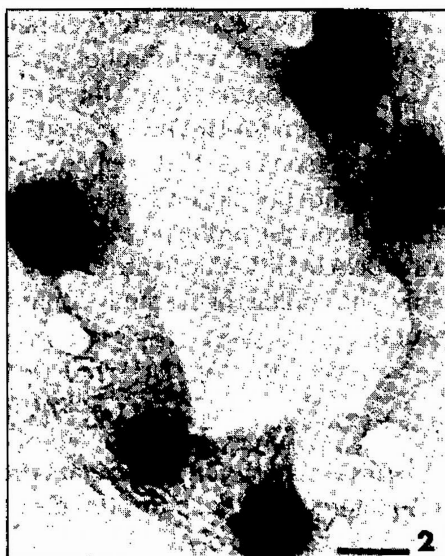

Fig. 2. A histological section of the hepatopancreas from a *Penaeus chinensis* infected with HPV-Chin. The section has been reacted with the DIG-labeled probe S2.0 using *in situ* hybridization. Nuclei of several hepatopancreatic tubule epithelial cells display intense positive reactions for HPV-Chin.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

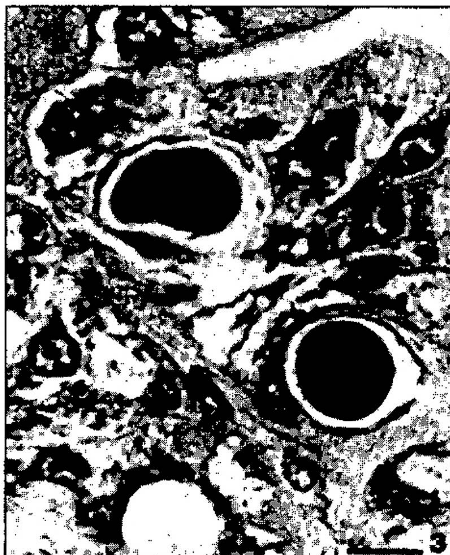

Fig. 3. Histological section of an HPV-Mac infected tubule in the hepatopancreas of a juvenile *Macrobrachium rosenbergii* showing prominent basophilic intranuclear inclusion bodies (I) diagnostic of infection by an HPV-type virus are shown in a number of hepatopancreatic tubule epithelial cells. Unlike 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 µm.

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 µm.

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.

Acknowledgements

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References

Adams, J.R. and J.R. Bonami, Editors. 1991. Atlas of invertebrate viruses. CRC Press, Boca Raton. 53 pp.

Anderson, I.G., A.T. Law, M. Shariff and G. Nash. 1990. A parvo-like virus in the giant freshwater prawn, *Macrobrachium rosenbergii*. Journal of Invertebrate Pathology 55:447-449.

Bell, T.A. and D.V. Lightner. 1988. A handbook of normal shrimp histology. Special Publication No. 1, World Aquaculture Society, Baton Rouge. 114 pp.

Bonami, J.R., B. Trumper, J. Mari, M. Brehelin and D.V. Lightner. 1990. Purification and characterization of the infectious hypodermal and hematopoietic necrosis virus of penaeid shrimps. Journal of General Virology 71:2657-2664.

Bonami, J.R. and D.V. Lightner. 1991. Unclassified viruses of crustacea. In: Atlas of invertebrate viruses (eds. J.R. Adams and J.R. Bonami), pp. 593-622. CRC Press, Boca Raton.

Brock, J.A. 1992. Current diagnostic methods for agents and diseases of farmed marine shrimp. In: Diseases of cultured penaeid shrimp in Asia and the United States (eds. W. Fulks and K. Main), pp. 209-231. The Oceanic Institute, Makapuu Point, Honolulu.

Brock, J.A. and D.V. Lightner. 1990. Diseases of Crustacea. Diseases caused by microorganisms. In: Diseases of marine animals, Vol. 3 (ed. O. Kinne), pp. 245-349. Biologische Anstalt Helgoland, Hamburg.

Bruce, L.D., R.M. Redman, D.V. Lightner and J.R. Bonami. 1993. Application of gene probes to detect a penaeid shrimp baculovirus in fixed tissue using *in situ* hybridization. Diseases of Aquatic Organisms 17:215-221.

Bruce, L.D., R.M. Redman and D.V. Lightner. 1994. Application of gene probes to determine target organs of a penaeid shrimp baculovirus using *in situ* hybridization. Aquaculture 120:45-51.

Chong, Y.C. and H. Loh. 1984. Hepatopancreas chlamydial and parvoviral infections of farmed marine prawns in Singapore. Singapore Veterinary Journal 9:51-56.

Colorni, A., T. Samocha and B. Colorni. 1987. Pathogenic viruses introduced into mariculture systems by imported penaeid shrimp. Bamidgeh 39:21-28.

Flegel, T.W., D.F. Fegan, S. Kongsom, S. Vuhikomudomkit, S. Sriurairatana, S. Boonyaratpalin, C. Chantanachookin, J.E. Vickers and O.D. MacDonald. 1992. Diseases in Thailand. In: Diseases of cultured penaeid shrimp in Asia and the United States (eds. W. Fulks and K. Main), pp. 57-112. The Oceanic Institute, Makapuu Point, Honolulu.

Fulks, W. and K. Main, Editors. 1992. Diseases of cultured penaeid shrimp in Asia and the United States. The Oceanic Institute, Makapuu Point, Honolulu. 392 pp.

Lightner, D.R. 1993. Diseases of penaeid shrimp. In: CRC handbook of mariculture. Second edition, Volume 1. Crustacean aquaculture (ed. J.P. McVey), pp. 393-486. CRC Press, Boca Raton.

Lightner, D.V. and R.M. Redman. 1985. A parvo-like virus disease of penaeid shrimp. Journal of Invertebrate Pathology 45:47-53.

Lightner, D.V. and R.M. Redman. 1992. Geographic distribution, hosts, and diagnostic procedures for the penaeid virus diseases of concern to shrimp culturists in the Americas. In: Culture of marine shrimp: principles and practices (eds. A.W. Fast and L.J. Lester), pp. 573-592. Elsevier, Amsterdam.

Lightner, D.V., B.T. Poulos, L. Bruce, R.M. Redman, J. Mari and J.R. Bonami. 1992. New developments in penaeid virology: application of biotechnology in research and disease diagnosis for shrimp viruses of concern in the Americas. In: Diseases of cultured penaeid shrimp in Asia and the United States (eds. W. Fulks and K. Main), pp. 233-253. The Oceanic Institute, Makapuu Point, Honolulu.

Lightner, D.V., R.M. Redman, D.W. Moore and M.A. Park. 1993. Development and application of a simple and rapid diagnostic method to studies on hepatopancreatic parvovirus of penaeid shrimp. Aquaculture 116:15-23.

Mari, J., J.R. Bonami, B. Poulos and D. Lightner. 1993a. Preliminary characterization and partial cloning of the genome of a baculovirus from *Penaeus monodon* (PmSNPV = MBV). Diseases of Aquatic Organisms 16:207-215.

Mari, J., J.R. Bonami and D.V. Lightner. 1993b. Partial cloning of the genome of infectious hypodermal and hematopoietic necrosis virus, an unusual parvovirus pathogenic for penaeid shrimps; diagnosis of the disease using a specific probe. Journal of General Virology 74:2637-2643.

Mari, J., J.R. Bonami, B.T. Poulos and D.V. Lightner. Partial cloning of the genome of an unusual shrimp parvovirus (HPV); the use of gene probes in disease diagnosis. Journal of Virology. (In press).

Poulos, B.T., J. Mari, J.R. Bonami, R. Redman and D.V. Lightner. 1994. Use of non-radioactively labeled DNA probes for the detection of a baculovirus from *Penaeus monodon* (PmSNPV = MBV) by *in situ* hybridization on fixed tissue. Journal of Virological Methods 49:187-194.

Paynter, J.L., D.V. Lightner and R.J.G. Lester. 1985. Prawn virus from juvenile *Penaeus esculentus*. The Second Australian National Prawn Seminar, NPS2, Cleveland: 61-64.

Roubal, F.R., J.L. Paynter and R.J.G. Lester. 1989. Electron microscopic observation of hepatopancreatic parvo-like virus (HPV) in the penaeid prawn, *Penaeus merguiensis* de Man, from Australia. Journal of Fish Diseases 12:199-201.

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