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NOTE

Hepatopancreatic parvo-like virus (HPV) of *Penaeus japonicus* cultured in Australia

K. M. Spann[1,*], R. D. Adlard[2], D. A. Hudson[2], S. B. Pyecroft[2], T. C. Jones[2], M. O. C. Voigt[2]

[1]Department of Parasitology, The University of Queensland, Brisbane, 4072, Australia
[2]Aquatic Diagnostic Services International, Pty, Ltd, PO Box 132, Toowong, Brisbane, 4066, Australia

ABSTRACT: A hepatopancreatic parvo-like virus (HPV) infection was identified in the hepatopancreata of moribund *Penaeus japonicus* postlarvae from a hatchery in Queensland. The virus formed basophilic, fine granular intranuclear inclusion bodies within the hepatopancreocytes. These caused nuclear hypertrophy and displacement of the nucleolus. Subspherical viral particles, 17–20 nm in diameter, were observed embedded within the inclusions. We will refer to this virus as HPV to avoid confusion within the literature. However, molecular data is required to definitively identify this virus as HPV.

KEY WORDS: HPV · *Penaeus japonicus*

Of the 13 virus types which have been described from penaeid shrimp worldwide, 3 of these are parvo, or parvo-like viruses (Lightner 1996). Hepatopancreatic parvo-like virus (HPV; Lightner & Redman 1985) has been documented from 7 penaeid species, including *Penaeus esculentus* (Paynter et al. 1985) and *P. merguiensis* (Roubal et al. 1989) from Australia. HPV infections have been linked to disease. However, they are often accompanied by other hepatopancreatic pathogens. Infectious hypodermal and hematopoietic necrosis virus (IHHNV; Lightner et al. 1983) can infect 10 penaeid species in Asia and the Americas (Brock & Lightner 1990, Lightner 1996). An IHHNV-like virus has been reported from a hybrid penaeid shrimp bred in Australia (Owens et al. 1992). IHHNV infects cells of ectodermal and mesodermal origin (Lightner et al. 1983) and causes disease and mortality in juveniles. Lymphoidal parvo-like virus (LPV; Owens et al. 1991) has only been observed in cultured *P. monodon*, *P. merguiensis* and *P. esculentus* from Australia and is not associated with disease.

*Present address: CSIRO, Tropical Agriculture, PB3, Indooroopilly 4068, Australia. E-mail: kirsten.spann@tag.csiro.au

Materials and methods. Forty moribund *Penaeus japonicus* postlarvae (PL1) were collected from a hatchery in Queensland, Australia, and processed for light and transmission electron microscopy (TEM). Some postlarvae were fixed whole in Davidson's fixative (20% formaldehyde, 30% ethanol, 10% glacial acetic acid, 40% tap water) overnight, then transferred to 70% ethanol and passed through a dehydrating series of ethanol concentrations. Tissues were cleared in methyl salicylate, wax embedded and sectioned at 5 µm. Sections were stained with haematoxylin and eosin (H&E).

The gnathothoraces (cephalothoraces minus the carapace, walking legs and mouthparts) of 10 postlarvae were fixed in 2.5% gluteraldehyde/2% paraformaldehyde in cacodylate buffer. Tissues were post-fixed in 1% osmium tetroxide, dehydrated through a graded series of ethanol and mounted in Spurr's resin (Spurr 1969). Semi-thin sections were cut at 0.9 µm, stained with 0.5% Toluidine blue in Borax and the area of interest identified using a compound microscope. Ultrathin sections were then cut at 50 nm, mounted on Cu-200 copper grids, stained with uranyl acetate/70% methanol and Reynold's lead citrate and viewed at 80 kV under a Jeol 1010 TEM.

Results. Infected postlarvae had atrophied hepatopancreata and the abdominal musculature was opaque. Histologically, singular, prominent, basophilic inclusion bodies (IBs) were observed in the nuclei of 2 to 10% of hepatopancreatic epithelial cells for all 30 postlarvae sampled (Fig. 1). Gills, heart, midgut and muscle appeared normal. By TEM the intranuclear IBs appeared ovoid to spherical, varied in diameter from 5 to 12 µm and were composed of fine granular material (Fig. 2). They caused hypertrophy of infected nuclei and lateral displacement and compression of the host cell nucleolus (Fig. 2). Margination of host chromatin

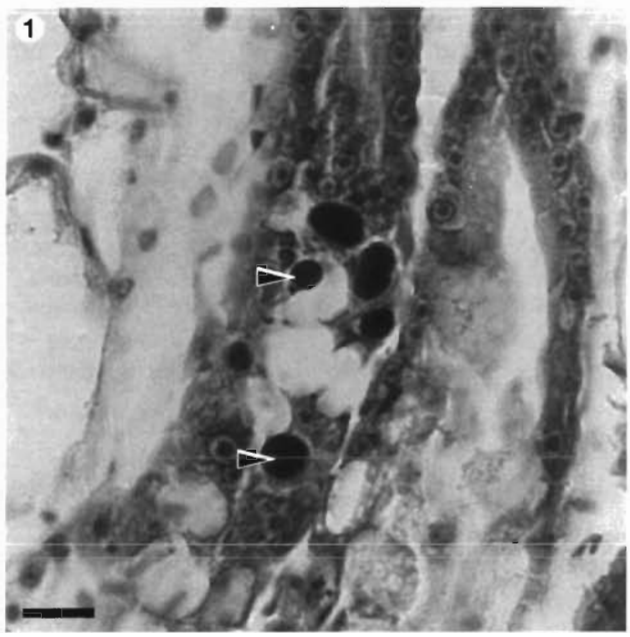

Fig. 1. *Penaeus japonicus*. Basophilic inclusion bodies (arrows) formed by HPV within the hepatopancreocytes of a diseased PL1 postlarvae. H&E stain. Scale bar = 5 µm

was common. Viral particles, 17 to 20 nm, were embedded in the IB and appeared to be unenveloped and roughly spherical (Fig. 3).

Discussion. The presence of roughly spherical viral particles within the nucleus of the host cells is indica-

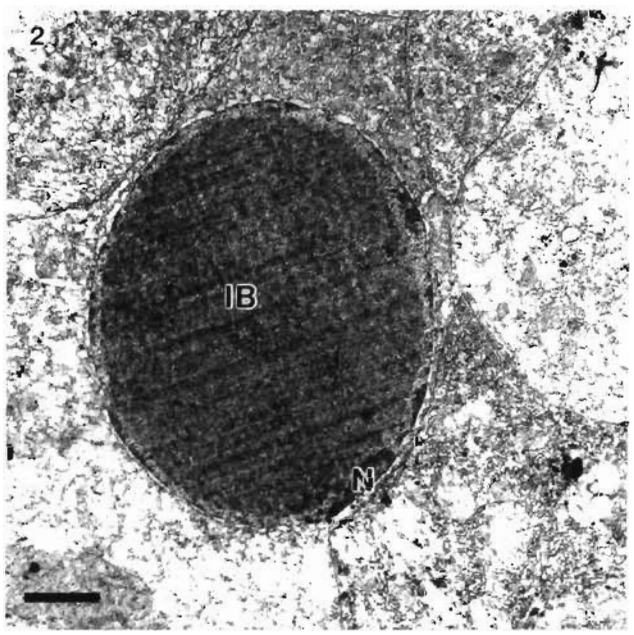

Fig. 2. *Penaeus japonicus*. An intranuclear HPV inclusion body (IB) causing hypertrophy of the nucleus and lateral displacement and compression of the nucleolus (N) within a hepatopancreatic cell of *P. japonicus* postlarvae. Scale bar = 1 µm

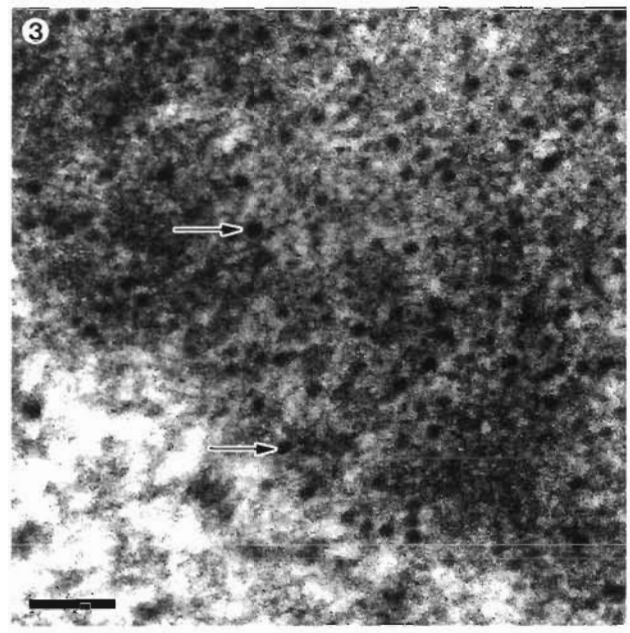

Fig. 3. *Penaeus japonicus*. HPV within the nucleus of a hepatopancreocyte. Unenveloped, roughly spherical P-PJ virus particles (arrows), 17 to 20 nm in diameter, embedded in an inclusion body which is composed of fine granular material. Scale bar = 100 nm

tive of the family *Parvoviridae*. The morphology of the virus particles observed in the moribund *Penaeus japonicus* postlarvae closely resembles that of other parvoviruses and they overlap the size range for viruses within this family, which is 18 to 26 nm (Berns et al. 1995). The virus described here is most similar to the Densovirinae subfamily whose members infect invertebrates (Berns et al. 1995).

The virus observed in the moribund postlarvae is closely related to HPV as they are identical in morphology and cytopathology. Natural infections of HPV have been reported from many species worldwide including *Penaeus japonicus* (Lightner 1996). Signs of HPV such as an atrophied hepatopancreas and opaque abdominal muscles (Lightner & Redman 1985), were observed for the diseased *P. japonicus* investigated here. HPV and HPV-like viruses infect the epithelial cells of the hepatopancreas and form distinct, singular, basophilic IBs, composed of electron-dense, finely granular material (Lightner & Redman 1985, Paynter et al. 1985, Roubal et al. 1989). HPV IBs, when fully formed, cause nuclear hypertrophy and compression and displacement of the host cell nucleolus. The virus particles described here are smaller than those of HPV from *P. merguiensis* (21 to 22 nm; Roubal et al. 1989) and other species (22 to 24 nm; Lightner 1988). However, this may be due to differences in treatment of the tissues.

HPV has been linked to disease and mortality in juvenile prawns, but is seldom observed alone in epizootics with high mortality rates. This differs from the strain of HPV described here, which did not appear to be associated with other pathogens and is a serious pathogen of postlarvae. Lightner et al. (1993) have suggested that HPV may be a serious primary pathogen of younger life stages and that past difficulties in diagnosis may have caused it to be overlooked.

IHHNV has been documented from *Penaeus japonicus* and the viral particles reported here are similar in size (Brock & Lightner 1990). However, IHHNV replication occurs in the cytoplasm, not the nucleus and it rarely infects the hepatopancreas (Lightner et al. 1983). LPV is an intranuclear virus and particles are the same size as those reported here (Owens et al. 1991). However, LPV does not infect the hepatopancreas and has not been recorded from *P. japonicus*.

The virus reported here may be regarded as HPV. However, molecular data is required to determine the appropriate taxonomic classification of the virus.

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