VIRAL PATHOGENS OF THE PENAEID SHRIMP

Philip C. Loh, Lourdes M. Tapay, Yuanan Lu, and E. C. B. Nadala, Jr.

Department of Microbiology
University of Hawaii
Honolulu, Hawaii 96822

I. INTRODUCTION

The first shrimp virus, *Baculovirus penaei* (BP), was isolated from wild penaeid shrimp (*Penaeus duorarum*) in the early 1970s (Couch, 1974a,b). Since then, the penaeid shrimp viruses have assumed great importance because of their effect on the growth and sustenance of the penaeid shrimp aquaculture industry. Several of these viruses have been associated with large epizootics and massive mortality in shrimp farms and hatcheries. Penaeid shrimp viruses isolated and/or observed by electron microscopy at present belong to six families (Table I). This number, however, is expected to rise as additional studies of viral diseases of penaeid shrimp result in more being isolated. In some studies, the viruses independently studied in different laboratories may be different strains belonging to the same family or may even be identical. Until recently, only information on clinical symptoms, histopathology, and electron microscopic thin-section structural morphology was available. For several viruses, there is still no definite association with a specific disease condition. The majority of reported viruses have not been investigated in any detail.

Several reviews describing the clinical picture, histopathology, epidemiology, and etiology of the diseases caused by the penaeid viruses have been published (Lightner and Redman, 1991; Lightner, 1993;

TABLE I

PENAEID SHRIMP VIRUSES

Family	Isolated	Electron microscopy
Parvoviridae	2	1
Baculoviridae	6	1
Rhabdoviridae	2	1
Reoviridae		2
Togaviridae		1
Iridoviridae		1
Ungrouped	4	
Total:	14	7

Sindermann, 1990; Couch, 1991). These reviews and descriptions provide valuable reference sources and background information for investigators interested in viral diseases of the shrimp and other crustaceans. In the present review, we have emphasized the penaeid viral agents which have been isolated and purified and several of their relevant properties characterized. Several of these agents have caused large epizootics and mass mortality and are thus a serious threat to the growth of the shrimp aquaculture industry. Also included in this review are the latest developments in penaeid shrimp cell culture technology and systems. Finally, their use in the growth and titration of the penaeid viruses, as well as early detection and diagnostic procedures, are discussed.

II. THE HOST ANIMAL

The major species for shrimp aquaculture in the world are the penaeid shrimp, of which there are 318 species belonging to the family *Penaeidae*. Of these, some 109 species have present or potential commercial value (Holthius, 1980) and are reared in grow-out ponds and tanks. Depending upon the geographic location, the following eight species have been commercialized on a large scale: *Penaeus monodon, P. japonicus, P. merguiensis, P. vannamei, P. semisulcatus, P. chinensis (orientalis), P. setiferus,* and *P. stylirostris.* The dominant species of cultured shrimp in Southeast Asia is *P. monodon* (black tiger shrimp); in the Americas, *P. vannamei* (white shrimp); in Japan, *P. japonicus (kuruma* shrimp); and in the People's Republic of China, *P. chinensis (orientalis)* (fleshy prawn).

III. Economic Importance

Shrimp aquaculture has today become an established industry in many areas of the world. Over 50 countries have shrimp farms. The penaeid shrimp represents a high-value food commodity. Shrimp culture provides a vital source of income, employment, trade, and economic well-being to the people involved. The world production of cultured shrimp has a total value of several billion U.S. dollars (Subasinghe, 1996). Viral diseases have had a severe impact on production, and the development of new management strategies for handling viruses has cut into profits (Rosenberry, 1995).

IV. The Virus Problem

As seen in Table II, the major penaeid viruses implicated in epizootics belong to four groups. Several members have been associated with massive mortality in cultured shrimp. The susceptibility of the different species of cultured penaeid shrimp to these viruses varies from virus infection of only a few species to infection of all penaeid species (Table III). However, the range of these viruses in wild shrimp has not been fully documented. Several of these viruses have been reported to infect the penaeid shrimp at various stages of its developmental cycle, from protozoea to adult, with the highest mortality occurring at the early postlarval stage. Age has been reported to affect susceptibility, with older shrimp being less susceptible to infection than younger ones (LeBlanc and Overstreet, 1990; Lu and Loh, 1994a). Depending upon

TABLE II

Major Viral Diseases of Cultured Penaeid Shrimp

Disease	Viral agent
Baculovirus penaei disease	BP: occluded baculovirus
Monodon baculovirus disease	MBV: occluded baculovirus
Baculovirus midgut gland necrosis	BMNV: nonoccluded baculovirus
White spot syndrome	WSBV: nonoccluded baculovirus
Infectious hypodermal and hematopoietic necrosis	IHHNV: parvovirus
Hepatopancreatic parvo-like virus disease	HPV: parvo-like virus
Yellow-head disease	YHV: rhabdovirus (provisional)
Taura syndrome	TSV: ungrouped

TABLE III

SUSCEPTIBILITY OF CULTURED PENAEID SPECIES TO PENAEID VIRUSES

Host penaeid species	Virus[a]								
	BP	MBV	BMN	WSB	IHHNV	HPV	YHV	RPS	TSV
P. monodon	++[b]	+++	++	+++	++	++	+++		
P. vannamei	+++	+		+++	++	+	+++	+	+++
P. chinensis			+		+	++			
P. merguiensis		++			−	++	−		
P. japonicus			+++	+++					
P. stylirostris	++			+++	+++	+	+++	+	+
P. duorarum	+++								

[a] BP, *Baculovirus penaei,* MBV, monodon baculovirus; BMN, baculoviral midgut necrosis virus; WSB, white spot baculovirus (also CBV = Chinese baculovirus); IHHNV, infectious hypodermal and hematopoietic necrosis virus; HPV, hepatopancreatic parvovirus; YHV, yellow-head virus; RPS, rhabdovirus of penaeid shrimp; TSV, Taura syndrome virus.

[b] +++, highly susceptible, causing significant mortality; ++, moderately susceptible, causing significant disease; +, weakly susceptible, mortality depending upon life stages of species; −, resistant.

the virus and the species of penaeid shrimp, infected animals exhibit gross physical changes and histopathological aberrations of their organs and tissues. Primary organ and tissue targets may vary with the viral pathogen.

Scant information is available on the natural modes of transmission of these infectious agents. Experimental transmission via the oral route has been demonstrated through feeding of either contaminated foods or infected carcasses and appears to be a dominant route of natural infections. Although it has not been definitively demonstrated, transmission by way of the gills is another possible route. Little is known concerning latent or persistent viral infections in the penaeid host. With a few of the penaeid viruses, asymptomatic infections have been reported (Funge-Smith and Briggs, 1996).

At present, the most effective means of controlling the virus problem is to destroy the infected animals, decontaminate the ponds, and start over with virus-free stocks. Although specific-pathogen-free (SPF) shrimp has been developed (Wyban et al., 1992) as a way to control the disease problem, it represents a partial solution since the animals were tested for only a limited number of viral pathogens. Such SPF

animals have been found to be just as susceptible as wild stock to other pathogenic shrimp viruses.

Until recently, a major hindrance to the development of solutions to the viral problem was the lack of relatively simple, cost-effective systems, such as permissive cell cultures, in which to grow the penaeid virus. As a consequence, there is little information on the replication of these viruses at the cellular and molecular levels. This has also limited the development of detection, diagnostic, and assay technologies. A significant advance involved the recent report of a protocol for the successful preparation of primary cell cultures from the lymphoid (Oka) organ (Nadala et al., 1993; Oka, 1969). Although the primary lymphoid cell lines could not be passaged, they could be maintained for 3 weeks or longer (Fig. 1A). Furthermore, the primary lymphoid cell cultures were found to be highly susceptible to certain shrimp viral pathogens, such as the yellow-head virus (YHV) and the Chinese baculovirus (CBV) (or white spot baculovirus), resulting in the production of definite cytopathology (CP) (Fig. 1B). This then allowed the development of quantal assay protocols [tissue culture infectious dose 50 ($TCID_{50}$)] for the measurement of infectious virus (Lu et al., 1995a; Tapay et al., 1996a). Such primary cell lines have been used in preliminary studies on the synthesis of viral proteins of CBV, viral pathogenesis, and the development of virus detection/diagnostic protocols (Lu et al., 1995a; Tapay et al., 1996a). Until now, no stable, continuous cell lines have been reported for marine invertebrates. The transformation of the primary shrimp lymphoid cells into a stable, continuous cell line with SV-40 oncogene was recently reported (Tapay et al., 1995) and represents a significant breakthrough. The transformed lymphoid cells exhibited all of the properties of stable, continuous cell lines (Fig. 2 and Table IV), and their interactions with shrimp viruses are currently being examined.

Present diagnostic/detection procedures of shrimp viral diseases are dependent upon the clinical history and light and/or electron microscopic examination of affected tissues showing characteristic cytopathology obtained from infected shrimp (Lightner and Redman, 1992; Lightner, 1993). Asymptomatic and latent infections can be detected only through use of either enhancement or bioassay techniques in sensitive indicator shrimp (Lightner et al., 1992). Among the problems associated with these traditional methodologies are limited sensitivity, the long time involved (days to weeks), the need for specialized equipment, the need for highly trained personnel, and the high cost.

In spite of the lack of cell culture systems, marked progress has been made in the development of detection and diagnostic technologies of
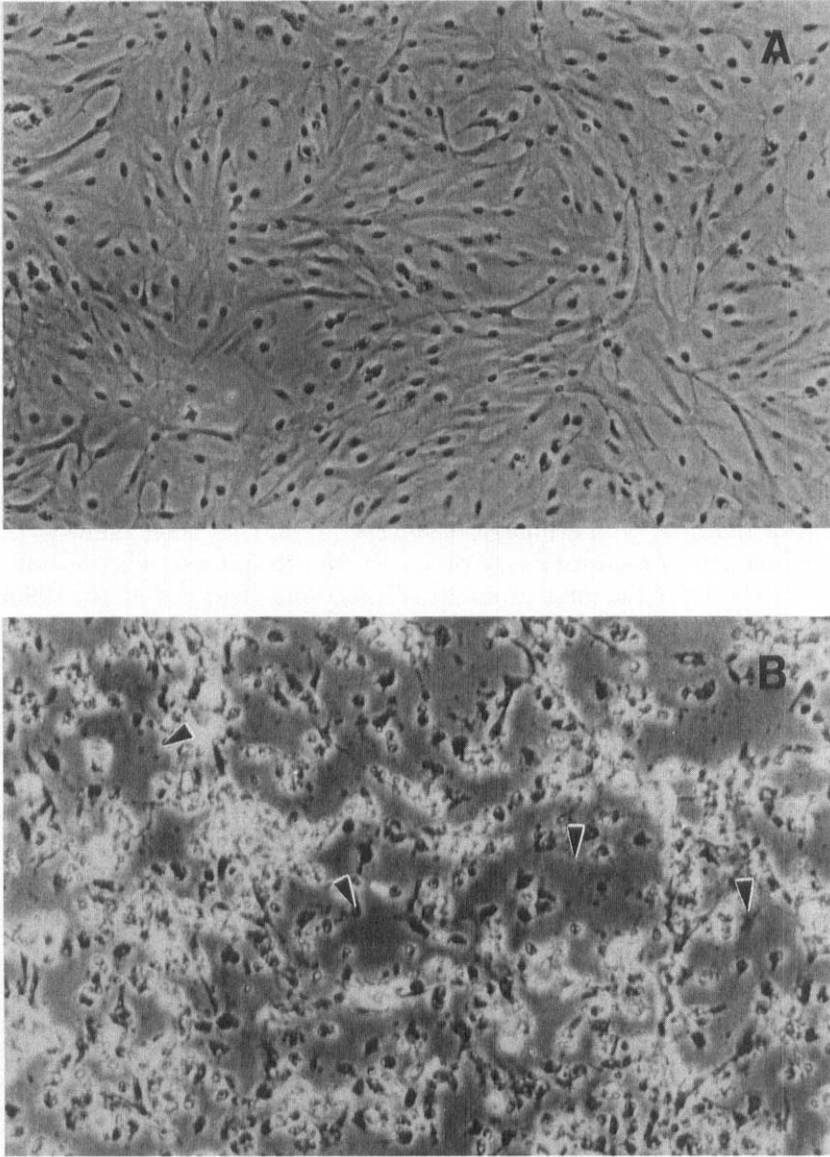
FIG 1. (A) A monolayer of fibroblast-like primary lymphoid cells grown in 2× Leibovitz Medium-15 plus supplements (Tapay *et al.*, 1995). (B) Photomicrograph of primary lymphoid cells experimentally infected with CBV. Note the foci of CP indicated by arrowheads.
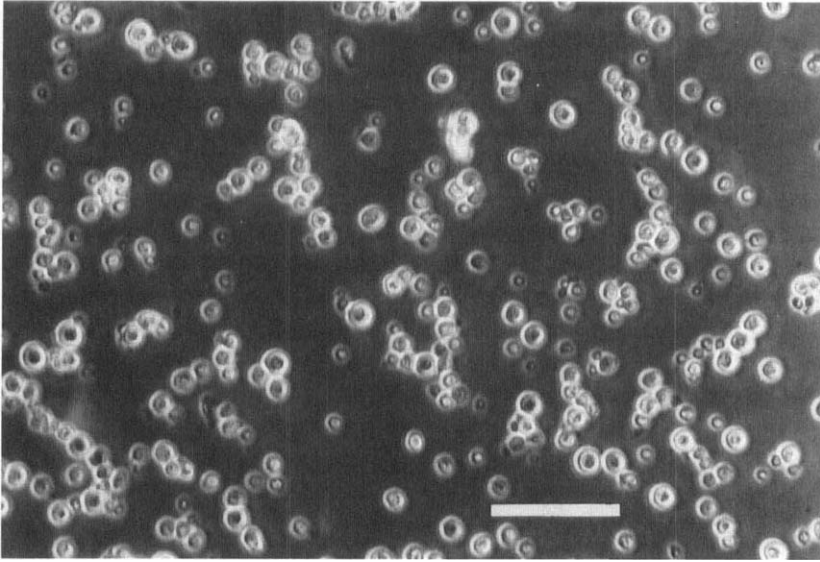
FIG 2. Lymphoid cells transformed with a viral oncogene from SV-40. Bar: 25 μm.

the economically important shrimp virus pathogens. Two approaches have been employed: (1) a solid-phase, immunologically based enzyme assay procedure, the nitrocellulose-enzyme immunoassay (NC-EIA), and, in certain studies, its amplification by streptavidin-biotin (SAB) (Nadala et al., 1992a; Lu et al., 1996a; Tapay et al., 1996b; Owens et al., 1991) and (2) various modifications of the nucleic acid probe (NAP)

TABLE IV

PROPERTIES OF PRIMARY AND TRANSFORMED SHRIMP LYMPHOID CELLS

Property	Primary cells	Transformed cells
Morphology	Spindle-shaped, fibroblast-like	Round
SV-40 T-antigen, G-418 resistance	−	+
Colonies in semisolid medium	−	+
Reduced serum requirement	−	+
Anchorage dependence	+	−
Passage number	Primary	>40

and polymerase chain reaction (PCR) procedures (Lightner *et al.*, 1994a; Lo *et al.*, 1996; Kimura *et al.*, 1996; Takahashi *et al.*, 1996; Wang *et al.*, 1996; Wongteerasupaya *et al.*, 1996). Initial experimental and field evaluation studies strongly indicated that these protocols can contribute to the early diagnosis and detection of the viral pathogens. The procedures appear to be both highly specific and sensitive.

V. THE VIRUSES INVOLVED

In this section, the discussion will be limited to those shrimp viruses which have been isolated and on which there is more information concerning their basic properties (Table V). For a more detailed discussion

TABLE V

PENAEID SHRIMP VIRUSES ASSOCIATED WITH EPIZOOTICS

Family	Name	Acronym	Type of nucleic acid	Shape/size (nm)
Baculoviridae				
Occluded	*Baculovirus panaei*	BP	dsDNA	Rod, 75 × 228
	Monodon baculovirus	MBV	dsDNA	Rod, 69 × 275
Non-occluded	Baculoviral midgut necrosis	BMN (PjNOB)	dsDNA	Rod, 72 × 310
	Bacilliform virus	BV	dsDNA	Rod, 83 × 275
	Chinese baculovirus	CBV	dsDNA	Rod, 120 × 265
	Systemic ectodermal mesodermal baculovirus	SEMBV	dsDNA	Rod, 121 × 276
	White spot baculovirus	WSBV	dsDNA	Rod, 87 × 330
	Penaeid rod-shaped DNA virus	PRDV (formerly RV-PJ)		Ovoid, 84 × 226
Parvoviridae	Infectious hypodermal and hematopoietic necrosis virus	IHHNV	ssDNA	Icosahedron, 22–25
	Hepatopancreatic parvovirus	HPV	ssDNA	Icosahedron, 22–24
Rhabdoviridae	Yellow-head virus (provisionally)	YHV	ssRNA	Bacilliform, 45 × 160
Ungrouped	Taura syndrome virus	TSV	ssRNA	Icosahedron, 31–32

of the clinical picture, histopathology, and epidemiology, the reader is referred to the several reviews mentioned in the introduction.

A. RNA Viruses

1. Rhabdovirus of Penaeid Shrimp (RPS)

Family:	*Rhabdoviridae*
Genus:	*Vesiculovirus*
Morphology:	Bullet-shaped, enveloped with peplomers, and helical nucleocapsid
Dimensions:	115–138 × 65–77 nm
Nucleic acid:	Negative sense, single-stranded RNA (~10.4 kb) $M_w = 3.6 \times 10^6$ Da
Site of replication:	Cytoplasm
Maturation:	Budding from plasma membrane
Host species:	*P. stylirostris, P. vannamei*

The RPS is the first rhabdovirus to be isolated from penaeid shrimp and also to uniquely infect a continuous fish cell heteroploid line, epithelioma papulosum cyprini (EPC). It was originally isolated from infectious hypodermal and hematopoietic necrosis (IHHN)-diseased and healthy *P. stylirostris* and *P. vannamei* obtained from shrimp farms in Hawaii and Ecuador (Lu *et al.*, 1991). However, experimental infection of juvenile (5–6 g) *P. stylirostris* and *P. vannamei* did not induce the histopathological lesions characteristically associated with IHHNV infection, nor were clinical or gross manifestations of disease observed. In such animals, no mortality occurred and virus replication was demonstrated only in the lymphoid (Oka) organs by plaque assay and immunofluorescence. The affected lymphoid organs, which showed gross cellular changes, were significantly larger in size (6–7×) than the corresponding organs from uninfected animals (Nadala *et al.*, 1992b) and appeared to be the primary target organ of RPS infection. Mortality was observed in younger postlarval (PL) shrimp (0.2 g) experimentally infected by three routes of infection: water-borne (12%), oral feeding (21%), and intramuscular injection (43–50%) (Lu and Loh, 1994a). The water-borne and oral feeding routes may represent the natural routes of transmission.

Thin-section electron microscopic studies indicated that RPS replicates in the cytoplasm of infected cells and appears to bud from both cytoplasmic vesicles and the plasma membrane (Lu *et al.*, 1991). Both

thin sections and negative staining studies showed bullet-shaped parti-
cles which are enveloped (typical of animal rhabdoviruses). Emanating
from the envelope are regularly shaped projections with a knob-like
structure at the distal end. Complete virions measured 115–138 ×
65–77 nm (Fig. 3) (Lu et al., 1991).

The biological, biochemical, and serological properties of RPS have
been examined (Lu and Loh, 1992, 1994b; Lu et al., 1994a). In virus
susceptibility studies of several fish cell lines, the heteroploid EPC cell
line was determined to be the most susceptible to RPS and had the
highest yield of virus (Table VI). The EPC was found to be especially
useful for the primary isolation of RPS. While several of the other fish
cell lines were susceptible, their yields of infectious RPS were much
lower (<10%). Single-cycle growth studies of RPS in EPC cells showed
an eclipse period of 3 hr, followed by a period of exponential growth
which was completed by 48 hr postinfection (p.i.). Since the virus
uniquely replicates in a fish cell line (EPC) causing distinct cytopathic
changes, this has enabled the development of a quantitative plaque
assay protocol (Fig. 4) which has greatly facilitated the study of RPS.
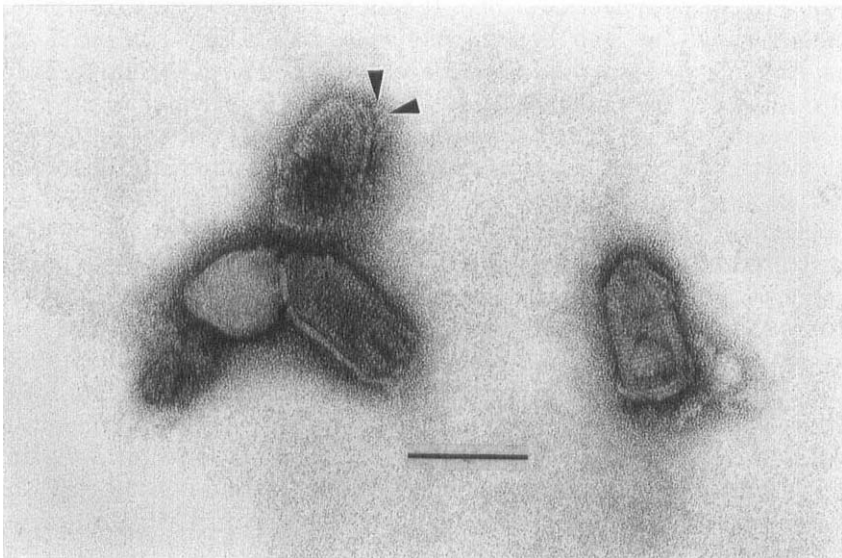The efficiency of plating (EOP) of RPS in EPC was determined to be

FIG 3. Electron micrograph of purified RPS showing knob-like peplomers (indicated
by arrowheads) emanating from the envelope of the virus. Uranyl acetate stain; bar:
100 nm.

TABLE VI

YIELD OF RPS IN VARIOUS FISH CELL LINES

Cell line	Abbreviation	Titer (TCID$_{50}$/ml)[a]
Epithelioma papulosum cyprini	EPC	$10^{8.33}$
Brown bullhead	BB	$10^{7.0}$
Grass carp fin	GCF	$10^{6.67}$
Grass carp swim bladder	GCSB	$10^{7.0}$
Grass carp snout-2	GCS-2	$10^{6.3}$
Chinook salmon embryo	CHSE-214	0
Rainbow trout gonads	RTG-2	0
Fathead minnow	FHM	$10^{5.8}$

[a] Infection period was 2 days at 20°C with 0.01 multiplicity of infection of RPS.
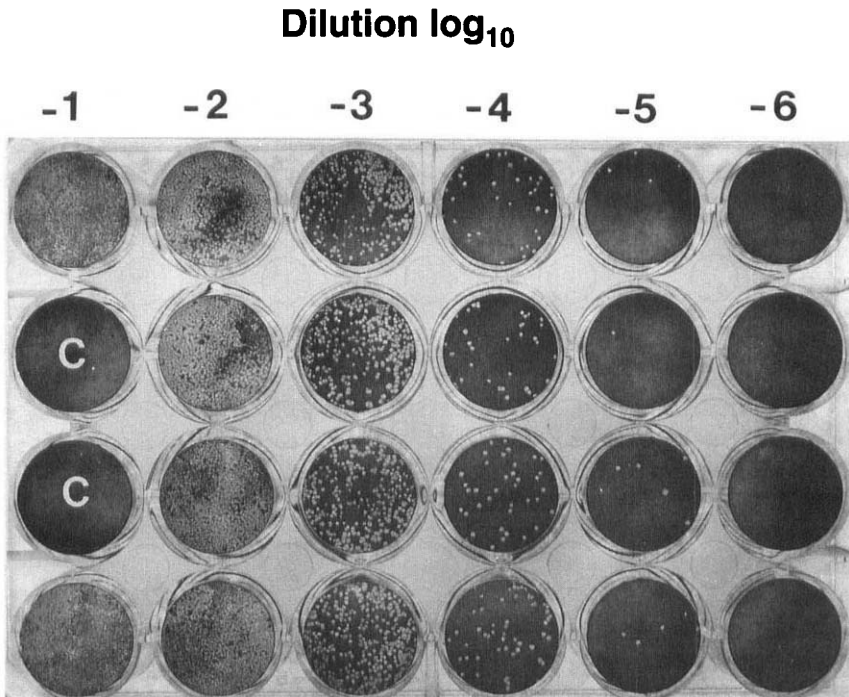
Dilution log$_{10}$

FIG 4. Plaque assay for RPS in EPC cells in a 24-well plate. C = control.

30 virus particles per infectious unit. The virus was found to be highly fragile, being sensitive to 20% ethyl ether, low pH, repeated freezing and thawing (3×), to 37°C (12 hr), and storage at −10°C (4 weeks) but was stable at −70°C for several weeks. The buoyant density of RPS in sucrose gradients is 1.19 g/cm^3.

The molecular weight of the single RNA species of the RPS genome was determined to be 3.6 × 10^6 Da (~10.4 kb), which falls within the genomic range of 3.1 to 4.4 × 10^6 Da reported for other rhabdoviruses (Heyward et al., 1979). The viral RNA is sensitive to ribonuclease, and since it is a rhabdovirus, we can assume that it possesses a negative polarity (Lu and Loh, 1994b). Since the replication of RPS in EPC was not inhibited by the DNA antagonist 5-bromo-2'-deoxyuridine (20 μg/ ml), this confirms the RNA genome of the virus.

Analysis by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of RPS proteins revealed the presence of at least four major structural proteins with the following molecular weights (kDa): 165, 65.7, 45.1, and 27.8. The number of structural proteins and the electrophoretic profile of RPS are very similar to those of the prototype rhabdovirus, vesicular stomatitis virus (VSV), and of the fish rhabdovirus carpio (RC) (also named spring viremia of carp virus, SVCV), both of which belong to the genus Vesiculovirus. In number of structural proteins and electrophoretic profile, they are different from the lyssa-type fish rhabdoviruses, infectious hypodermal necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV). The lyssa variety is easily distinguished from the vesiculo group in that the matrix proteins are composed of two structural polypeptides, M1 and M2, versus a single polypeptide, M, in the vesiculo group (Lu and Loh, 1994b). Western blot analysis of the electrophoretically separated RPS structural proteins with anti-RPS polyclonal serum revealed, in addition to the four major proteins, an extra viral protein with a molecular weight of 38 kDa. Based upon its molecular weight, this polypeptide was presumed to be nonstructural protein which is present in the vesiculo group of rhabdoviruses. The Western blot technique further revealed that the RPS is partly related to VSV, IHNV, and VHSV. The anti-RPS serum identified the G protein of both IHNV and VHSV and the M protein of VSV. Although the anti-RPS serum cross-reacted with the structural proteins of RC, suggesting a close evolutionary relation of these two viruses, the intensity of reaction to RC was much weaker than that observed with RPS. Serologically, RPS is unrelated to IHNV and VHSV and is closely related to but distinguishable from RC using the plaque reduction and neutralization techniques (Lu et al., 1994b).

A solid-phase enzyme-immunoassay protocol, nitrocellulose-enzyme immunoassay/streptavidin-biotin (NC-EIA/SAB), employing a rabbit polyclonal anti-RPS IgG was developed for the diagnosis and detection of RPS (Nadala *et al.*, 1992b). The NC-EIA/SAB was reported to detect as few as 10 plaque-forming units (pfu) or 300 viral particles (400 pg of viral protein) in experimentally infected shrimp. In preliminary studies, the protocol has successfully detected RPS infections in apparently healthy animals at different stages of development in shrimp farms in Hawaii (Tapay *et al.*, 1996b).

2. Yellow-Head Virus (YHV)

Family:	*Rhabdoviridae* (provisionally)
Morphology:	Bacilliform, enveloped with peplomers, and helical nucleocapsid
Dimensions:	150–200 × 40–60 nm
Nucleic acid:	Negative sense, single-stranded RNA $M_w = 8 \times 10^6$ Da (22 kb)
Site of replication:	Cytoplasm
Maturation site:	Budding from cytoplasmic vesicles and plasma membrane
Host species:	*P. monodon*
Other species:	*P. stylirostris, P. vannamei*

Yellow-head disease (YHD) of the black tiger shrimp, *P. monodon,* was first observed in Thailand in 1990. It resulted in significant mortality and adversely affected the mariculture shrimp industry in Thailand (Boonyaratpalin *et al.*, 1993; Chantanachookin *et al.*, 1993). The term yellow-head disease was used to describe the yellow color of the cephalothorax and gills and the pale or bleached appearance of the diseased shrimp. The infection generally resulted in a cumulative mortality of 100% within 3–5 days after the onset of the disease (Boonyaratpalin *et al.*, 1993). The etiological agent was initially identified as a baculolike virus (Boonyaratpalin *et al.*, 1993; Chantanachookin *et al.*, 1993) but subsequently was found to contain RNA and to possess properties not characteristically associated with baculoviruses (Wongteerasupaya *et al.*, 1995). It was suggested that YHV resembled either rhabdoviruses or coronaviruses. Although some differences were noted, our accumulated data strongly suggest that YHV is a member of the rhabdovirus family, and we have provisionally classified it in that family.

In addition to the original host, *P. monodon,* two other penaeid species, *P. stylirostris* and *P. vannamei,* were reported to be highly susceptible to YHV (Lu *et al.*, 1994b). Intramuscular inoculation of a

10% w/v cephalothorax filtrate prepared from naturally infected *P. monodon* Fabricius into subadult (3–10 g) *P. stylirostris* (Stimpson) and *P. vannamei* (Boone) resulted in 100% cumulative mortality within 5–7 days p.i. (Lu *et al.*, 1994b). In such experimentally infected animals, the characteristic light yellowing of the hepatopancreas and gills observed in naturally infected *P. monodon* was not seen. Histopathological examination of naturally and experimentally infected shrimp revealed widespread cellular necrosis in the gills, connective tissues, hemocytes, hematopoietic organs, and lymphoid organ, and strongly indicated a preferential infection of the cells of ectodermal and mesodermal origins (Boonyaratpalin *et al.*, 1993; Chantanachookin et al., 1993; Lu *et al.*, 1994b; Tapay *et al.*, 1996c).

The quantitative titration of infectious YHV in primary cultures of shrimp lymphoid cells was recently reported (Lu *et al.*, 1995a). This has permitted study of the pathogenesis of YHV in the infected shrimp. Infectious YHV was demonstrated in varying amounts in the tissues and organs tested (Table VII). This was also confirmed by bioassay in shrimp indicators. The lymphoid (Oka) organ, gill, and head soft tissues (HST) were found to contain 10- to 800-fold higher titers of infectious virus than the other tissues and organs tested, suggesting that they are apparently the primary targets for YHV infection (Lu *et al.*, 1995b).

In vitro infectivity studies of YHV-containing gill filtrate in four fish cell lines (EPC, fathead minnow or FHM, chinook salmon embryo or CHSE-214, and brown bullhead or BB) and two insect cell lines (SF9

TABLE VII

TITERS ($TCID_{50}$/ML) OF YHV IN SELECTED TISSUES
AND ORGANS OF *P. vannamei* EXPERIMENTALLY INFECTED
WITH YHV

Organ/tissue	$TCID_{50}$/ml
Branchial portion of the gill	8×10^6
Lymphoid organ (Oka)	8×10^6
Head soft tissues (HST)	5×10^6
Midgut	5.6×10^5
Abdominal muscle	3.1×10^5
Heart	1.5×10^5
Nerve cord	5.6×10^4
Hepatopancreas	1.1×10^4
Eyestalk	1.1×10^4

Source: Reprinted with permission from Lu *et al.* (1995b).

from *Spodoptera frugiperda* and CRL 1963 from *Drosophila*) did not result in any observable cytopathogenic effects (Tapay, 1996).

Electron microscopic examination of ultrathin sections of gills and lymphoid tissues from infected animals revealed the presence of numerous enveloped virus particles with bacilliform morphology, measuring 150–200 × 40–50 nm in the cytoplasm of infected cells (Fig. 5). Virions budding out through the cytoplasmic membrane were frequently observed. Cross sections of the complete virions showed an electron-dense nuclear core which measured 20–30 nm in diameter, surrounded by a trilaminar envelope (Boonyaratpalin *et al.*, 1993; Chantanachookin *et al.*, 1993; Lu *et al.*, 1994b).

Among the sources of infected material used to prepare purified virus, the hemolymph was found to yield the cleanest preparation and the largest number of virus particles. Attempts to purify YHV from the gill and HST met with difficulty because of contaminating vesicles
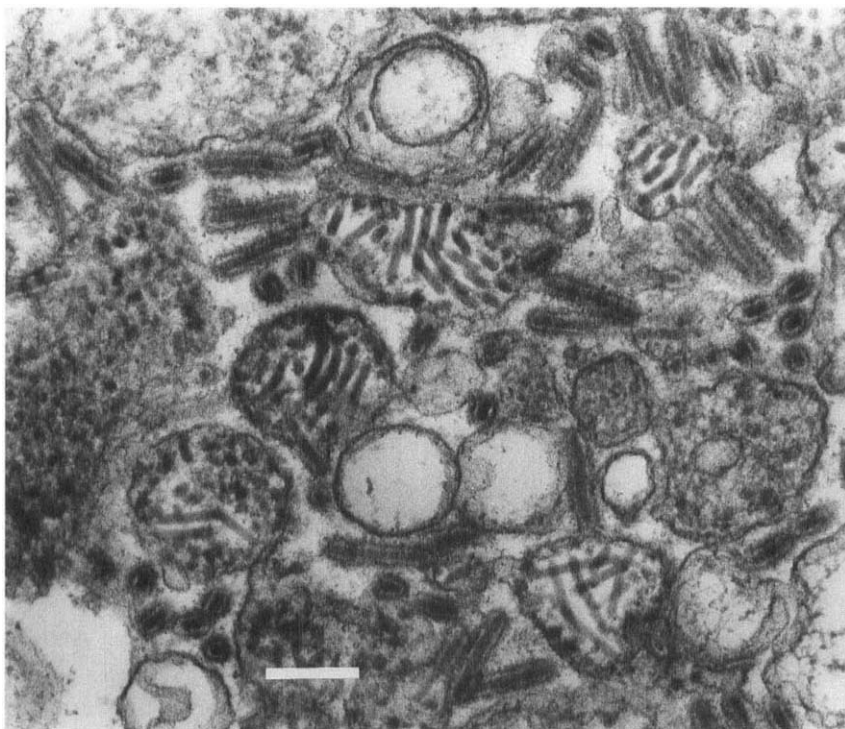

FIG 5. Electron micrograph of thin-sectioned gill tissues showing YHV in the cytoplasm of infected cells. Bar: 200 nm.

and other membranous cellular materials which banded nonspecifically with the virus in sucrose gradients. The buoyant density of purified YHV ranged from 1.18 to 1.20 g/cm³. This is comparable to the buoyant densities of known rhabdoviruses (Jackson *et al.*, 1987; Payment and Trudel, 1993).

Electron microscopic examination of uranyl acetate-stained, purified YHV revealed peplomer-containing, enveloped, rod-shaped particles measuring 190–200 × 50–60 nm (Fig. 6A) and resembling the plant rhabdoviruses. As with plant rhabdoviruses, the virus particle is fragile and flexible, often assuming pleomorphic forms (Fig. 6B). The nucleocapsid is arranged in an orderly helical fashion. The helix consists of a coiled tubular structure layered in a regular fashion at right angles to the long axis of the complete virus particle. YHV does not exhibit the bullet-shaped morphology typical of rhabdoviruses infecting vertebrates. However, it closely resembles the bacilliform (with rounded ends) rhabdo-like viruses infecting the blue crab, *Callinectes sapidus* (Yudin and Clark, 1979), and the helical, rod-shaped plant rhabdoviruses (Jackson *et al.*, 1987). In terms of their dimensions and ultrastructure, these rhabdoviruses are very much alike.

Some of the biochemical properties of YHV have been reported (Tapay, 1996; Nadala *et al.*, 1996). Genome analysis of YHV by agarose gel electrophoresis showed a single band of RNA of M_w 8 × 10⁶ Da (~22 kb) (Fig. 7) (Nadala *et al.*, 1996a). The size of the RNA genome is approximately twice that of the rhabdoviruses. The viral RNA is susceptible to ribonuclease (RNAse) and is therefore a single-stranded structure (Wongteerasupaya *et al.*, 1995; Nadala *et al.*, 1996). The failure of YHV RNA to be translated in an *in vitro* rabbit reticulocyte translation system (Amersham) strongly indicates that the viral genome possesses a negative polarity (E. C. B. Nadala, Jr., L. M. Tapay, and P. C. Loh, unpublished, 1997).

Structural protein analysis of purified YHV preparations by SDS-PAGE revealed four major bands with the following estimated molecular sizes (kDa): 170, 135, 67, and 22 (Fig. 8A). These bands probably correspond to the large (L), glycoprotein (G), nucleocapsid (N), and matrix (M) proteins of rhabdoviruses. The putative G protein of YHV was determined to be glycosylated with the use of a glycoprotein detection kit (Amersham) (E. C. B. Nadala, Jr., L. M. Tapay, and P. C. Loh, unpublished, 1997).

Western blot analysis of the bands using rabbit polyclonal anti-YHV immunoglobulin G (IgG) showed strong reactivity by the antibody with the putative G protein. The proteins of VSV, RPS, and control shrimp tissues did not cross-react with the same antibody preparation (Fig. 8B).
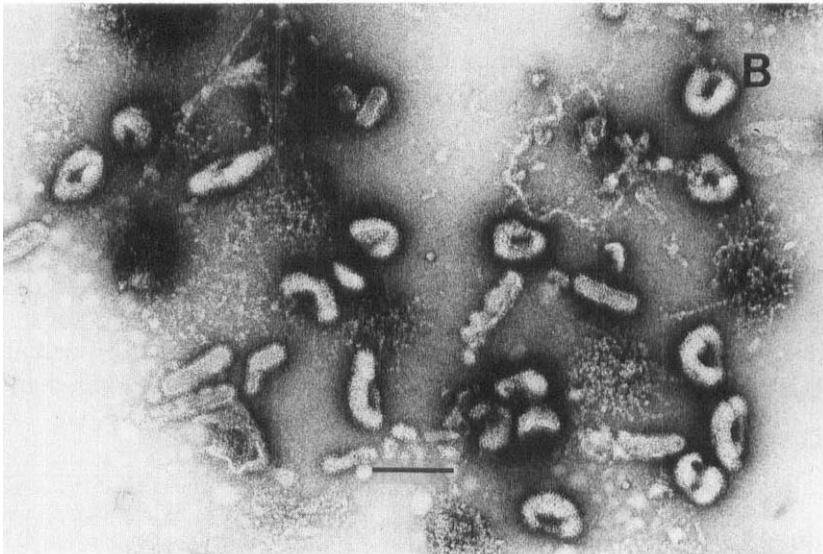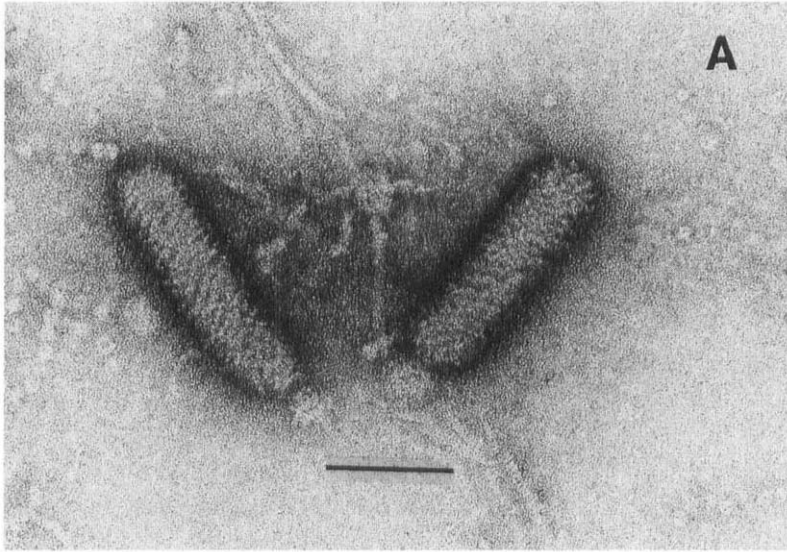
FIG 6. (A) Purified complete virions of YHV. Note the prominent peplomers and helical nucleocapsid. (B) Pleomorphic forms of YHV. Uranyl acetate stain; bar: 200 nm.
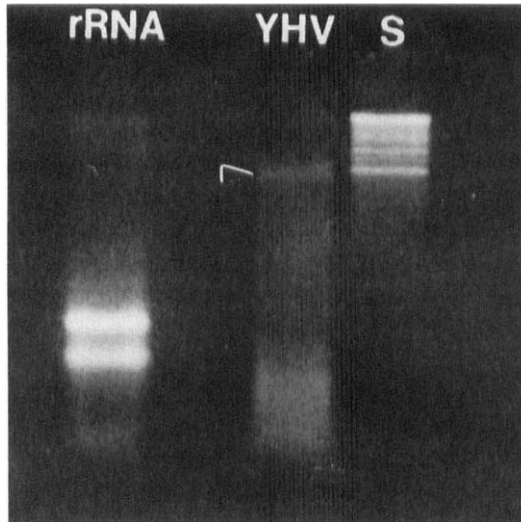
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Fig 7. Agarose gel showing the genome of YHV. Arrowhead indicates the YHV RNA, which was also sensitive to RNase. Cellular ribosomal RNA (rRNA) and high molecular weight DNA markers (S) were also run for comparison.

Although the structural protein profile of YHV is similar to that of RPS and other members of the family *Rhabdoviridae* (Wagner, 1987), the molecular sizes of YHV proteins are different. The G protein of YHV is larger (135 kDa versus 90 kDa) and probably accounts for the very prominent peplomers. Furthermore, YHV has a smaller M protein (22 kDa versus 30 kDa), which may account for the high flexibility and fragility of the virus.

In hemagglutination studies, both YHV and VSV were found to agglutinate chicken red blood cells with endpoints of 1:256 and 1:64, respectively. Neither virus was eluted even after 24 hr of incubation at room temperature, suggesting the formation of a stable complex

Fig 8. Analysis of YHV proteins. (A) SDS-PAGE (12%) of high molecular weight markers (HMw) (lane 1); YHV proteins (lane 2): low molecular weight markers (LMw) (lane 3); and VSV (lane 4). Arrowheads correspond to the large (L), glycoprotein (G), nucleocapsid (N), and matrix (M) proteins of rhabdoviruses. (B) Western blot showing the putative G protein of YHV (arrowhead) strongly reacting with the polyclonal anti-YHV serum. The proteins of VSV, RPS, and control shrimp tissues did not cross-react with the same antibody. VSV (lane 1); YHV (lane 2); RPS (lane 3); 30% shrimp tissue homogenate (1:1000) (lane 4); 30% shrimp tissue homogenate (1:100) (lane 5).
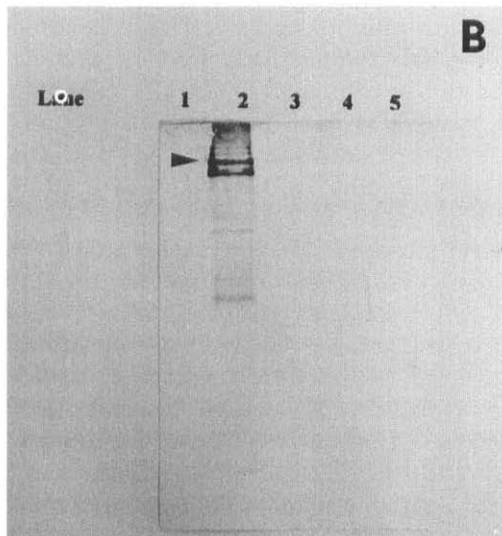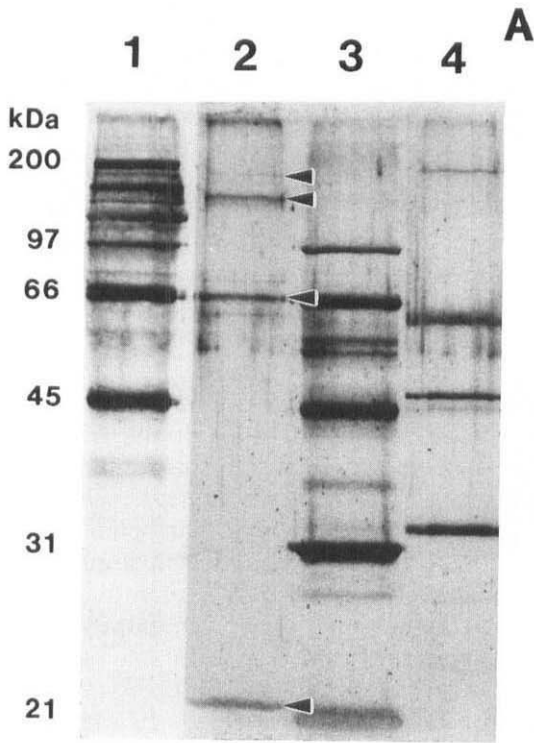
which lacked receptor-destroying enzymes similar to those of members of the orthomyxovirus family (Tapay, 1996).

For the rapid, specific diagnosis and detection of acute and asymptomatic YHV infection of the penaeid shrimp, an indirect NC-EIA method employing a rabbit polyclonal anti-YHV IgG was developed. The solid-phase enzyme immunoassay procedure was capable of detecting as few as 100 $TCID_{50}$ units of virus or an equivalent of 0.4 ng of viral protein (Lu et al., 1996b). In experimentally infected animals, the gill tissues were found to be a highly satisfactory source of YHV antigen for the NC-EIA test. In addition, gill tissues were more easily prepared for sampling compared to the lymphoid (Oka) organ. Both tissues are primary targets for YHV in the whole animal (Lu et al., 1995b). Another convenient, economical, and less invasive way of monitoring YHV infection in cultured shrimp, particularly invaluable broodstock animals, involves sampling the hemolymph, which was found to contain a considerable amount of virus, as shown by electron microscopy and infectivity assay in primary lymphoid cell cultures. The hemolymph sample may be collected from the hemocoel without sacrificing the animal.

Western blot analysis has been used to monitor the appearance of YHV proteins in the hemolymph of experimentally infected animals. In time-course experiments, viral G protein was detected as early as 48 hr p.i. in the hemolymph (Tapay, 1996) (Fig. 9A). Also, an extra band corresponding to a ~92-kDa protein was observed in the infected hemolymph sample but was not detected in purified virus preparations. Whether this protein is a viral nonstructural protein, a precursor of a viral structural protein, or a host protein whose production was induced by the viral infection remains to be clarified. A similar phenomenon was reported for a fish rhabdovirus (IHNV), where a nonstructural protein was detected in cells experimentally infected with IHNV but was missing in mature virions (Kurath et al., 1985; Schutze et al., 1995). In primary lymphoid cell cultures, the putative G protein was detected 72 hr p.i. (Fig. 9B) (L. M. Tapay, E. C. B. Nadala, Jr., and P. C. Loh, unpublished, 1997).

Some recent preliminary studies on the detection of YHV in P. monodon employed the reverse transcriptase-polymerase chain reaction (RT-

FIG 9. Detection of YHV proteins by Western blot analysis in the (A) hemolymph of experimentally infected P. vannamei during a time-course experiment and in (B) primary cell cultures infected with YHV. The putative G protein (arrowheads) was detected as early as 2 days and 3 days p.i. in the hemolymph and primary cell cultures, respectively. S, prestained high molecular weight protein standards.
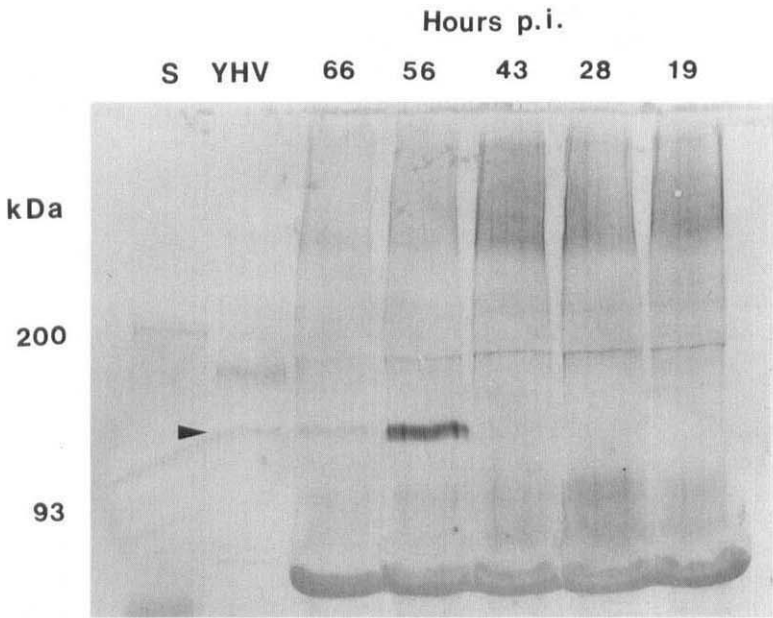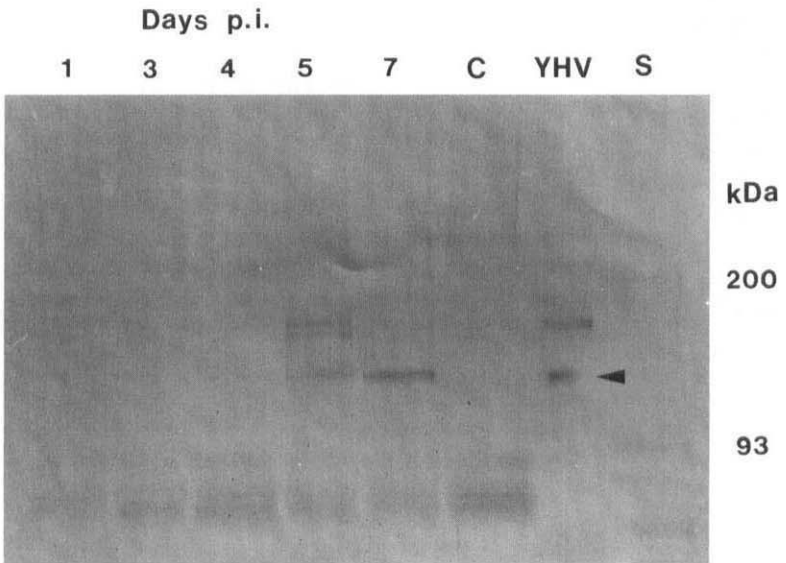
PCR) protocols. Two of 45 recombinant clones which gave positive hybridization with YHV RNA were negative with the DNA of *P. monodon* and that of the white spot baculovirus (Wongteerasupaya *et al.*, 1996). The problem with the probes is that we do not know from which regions of the viral genome they originate. Also, the family of genes making up the YHV genome has not been identified.

3. Taura Syndrome Virus (TSV)

Family:	*Picornaviridae* (provisionally)
Morphology:	Nonenveloped, icosahedron
Dimensions:	30–32 nm
Nucleic acid:	Positive sense, linear, single-stranded RNA (~9 kb)
Site of replication:	Cytoplasm
Host species:	*P. vannamei*
Other species:	*P. chinensis, P. aztecus, P. duorarum*

Taura syndrome (TS) was first recognized in penaeid shrimp farms located near the Taura River in the province of Guayas, Ecuador, in 1992 (Jimenez, 1992; Lightner *et al.*, 1994a; Wigglesworth, 1994; Brock *et al.*, 1995). In the Western hemisphere and Hawaii, TS has emerged as a major epizootic disease of *P. vannamei*. Initially, fungicide toxicity was considered the cause, but subsequent studies demonstrated the infectious nature of the agent and a virus was eventually isolated from carcasses of moribund and dead shrimp (Brock *et al.*, 1995; Hasson *et al.*, 1995a). Nevertheless, there are still laboratories which maintain that TS is a toxic pathological condition (Barniol, 1995) and that an infectious agent, a bacterium, may also be involved (Jimenez *et al.*, 1995). These claims have yet to be confirmed.

Several penaeid species have been reported to be susceptible to TSV: *P. chinensis, P. aztecus,* and *P. duorarum* (Overstreet *et al.*, 1996). In contrast, *P. stylirostris* and *P. setiferus* appeared to be resistant following experimental challenge with TSV. There are some experimental results indicating that both *P. monodon* and *P. japonicus* are also relatively resistant (Brock *et al.*, 1995).

There is scant information available concerning the presence or distribution of TSV in wild shrimp populations. The occurrence of TS in wild shrimp recovered from Ecuador and from broodstock captured in Mexican waters has been reported (Lightner *et al.*, 1995; Lightner, 1995). Several attempts to demonstrate TSV in captured wild broodstock, spawned eggs, or nauplii from El Salvador were unsuccessful (Brock *et al.*, 1996).

The buoyant density of TSV in CsCl gradients is 1.337 g/ml. Electron microscopic examination of 2% phosphotungstic acid-stained TSV revealed an icosahedral morphology and a diameter of 31–32 nm. Based upon its morphology, cytoplasmic site of replication, linear single-stranded RNA genome (~9 kb), and composition of three major (49, 36.8, and 23 kDa) and two minor (51.5 and 52.5 kDa) viral structural proteins, the TSV has been provisionally classified as a picornavirus (Lightner, 1996).

Little is known about the natural transmission routes of TSV. Experimental transmission through the oral route was achieved through feeding of minced TS-infected tissues to specific pathogen-free *P. vannamei* (Brock *et al.*, 1995). The water-borne–oral feeding route may represent the natural route of transmission. Transmission of TSV is further complicated by a recent report that an aquatic insect may be involved (Lightner, 1995). The virus was determined to be present in the water boatman, *Trichocorixa reticulata*, by bioassay in indicator shrimp.

Taura syndrome generally occurs in juvenile (0.1 to 5 g) *P. vannamei*, and its clinical effect is mortality (>90%). As a rule, older animals are more resistant (Lightner *et al.*, 1995).

The histopathological changes associated with TS have been described in detail (Jimenez, 1992; Lightner *et al.*, 1994a, 1995; Hasson *et al.*, 1995a; Brock *et al.*, 1996). These characteristic changes have been used for the diagnosis of TS. Shrimps with acute natural and experimentally induced TSV infections show a distinctive histopathology that consists of multifocal areas of necrosis in the cuticular epithelium and often in the subcuticular connective tissue. Present in these tissues are numerous variably sized eosinophilic to basophilic cytoplasmic inclusion bodies that give TS lesions a "buckshot" pattern of necrosis which is typical of the disease.

Recently, a complementary DNA (cDNA) probe was developed as a diagnostic tool for the detection of TSV. The probe has been successfully used in *in situ* hybridization assays with fixed tissue preparations (Hasson *et al.*, 1995b). The efficacy of such probes remains to be tested in field studies.

B. DNA Viruses

1. Baculovirus penaei (BP) Occluded Type A

Other names: *Penaeus vannamei* single nuclear polyhedrosis virus (PvSNPV)
Family: *Baculoviridae*

Morphology: Free and occluded, rod-shaped, enveloped vi-
                       rions; occlusion bodies are tetrahedral and of
                       varying sizes
Dimensions: Enveloped virion, 312–320 × 75–87 nm
                       Nucleocapsid, 306–312 × 62–68 nm
Nucleic acid: Circular double-stranded DNA
                       $M_w = 75 \times 10^6$ Da
Site of replication: Nucleus
Maturation: Nucleus — formation of tetrahedral occlusion
                       bodies.
Host species: *P. duorarum*
Other species: *P. stylirostris, P. vannamei, P. monodon, P.
                       aztecus, P. penicillatus, P. marginatus*

The BP was originally described by Couch (1974a), who employed light and electron microscopy in naturally infected penaeid *P. duorarum* (pink shrimp). Since the time of this report, BP has been found in several wild and cultured penaeid species: *P. aztecus, P. setiferus, P. vannamei, P. stylirostris, P. penicillatus, P. schmitti, P. paulensis, P. subtilis,* and several others (Lightner *et al.*, 1994b). The virus thus far has been limited to the Western hemisphere and Hawaii. To date, BP is the only noninsect baculovirus accepted by the International Committee on Taxonomy of Viruses (ICTV). Recently, a new designation based upon the guidelines for virus nomenclature set forth by the ICTV was proposed. The BP from *P. vannamei* is named *Penaeus vannamei* single nuclear polyhedrosis virus (PvSNPV) (Francki *et al.*, 1991).

Epizootics of BP can result in high mortality of larval and early postlarval shrimp, particularly in intensive culture systems which facilitate the development and transmission of the disease (LeBlanc and Overstreet, 1990; Stuck and Overstreet, 1994). However, in larval or late postlarval penaeid shrimp, the effects of BP infection are minimal. Experimentally infected postlarvae have exhibited depressed growth rates (Stuck and Overstreet, 1994). Age appears to play a role in susceptibility of the penaeid shrimp to BP.

In the infected animal, BP affects primarily cells of the hepatopancreatic and midgut epithelium (Couch, 1974a, 1989; Johnson and Lightner, 1988). In the hypertrophied nuclei of infected cells in these affected tissues, the newly synthesized progeny may be either free or occluded, with characteristic tetrahedral crystalloid bodies termed occlusion bodies (OBs). The polyhedra are easily seen by light microscopy and may be as large as 17 μm on a side (Lightner, 1983). From one to several

polyhedra may occupy a nucleus (Overstreet *et al.,* 1988). The OBs, which are composed primarily of the protein polyhedrin, are used as a diagnostic feature of patent BP infections (Summers, 1977; Rohrmann, 1986).

Little is known concerning persistent BP infections in the natural environment. Attempts to demonstrate this phenomenon experimentally in the larvae and postlarvae of *P. vannamei* have not been successful (Stuck and Wang, 1996). While the BP could be detected at 72 hr after infection, the amount of virus in shrimp decreased with time and was not detected even by molecular methods such as PCR in shrimp examined 120 days after infection.

A mixed infection involving BP and a reo-like agent has been described (Krol and Hawkins, 1990). Each shrimp with a reo-like infection also had a BP infection, but the reverse was not always true. Both viruses were observed in the same tissue and occasionally in the same cell.

There is sparse information concerning the biochemical and cellular events involved in the infection cycle of BP. The ultrastructural aspects of virus replication and cytopathology of BP-infected hepatopancreatic cells in the shrimp have been well described (Couch, 1974a,b, 1989, 1991; Johnson and Lightner, 1988). Accordingly, the BP virion, presumably after attachment and viropexis or fusion, uncoats or injects its DNA into the host cell nucleus at the nuclear pore. After integration of the virion into the host cell genome, a series of morphologically recognizable sequences of events occur, leading to the eventual production of mature BP and tetrahedral OBs. A distinct intracellular morphological change observed is the appearance of extensive membranous labyrinths (ML) adjacent to the endoplasmic reticulum. The ML appeared to originate from dilated Golgi and endoplasmic reticulum vesicles and from the outer nuclear envelope.

The BP virion is a rod-shaped nucleocapsid surrounded by a trilaminar envelope. Unlike some nuclear polyhedrosis viruses with multiple nucleocapsids per envelope, BP has only one per envelope. The intact, enveloped virions, when banded in CsCl gradient, exhibit a buoyant density of 1.265 g/cm^3. On the basis of negative staining, the enveloped virion is 312–320 × 75–87 nm and the nucleocapsid is approximately 306–312 × 62–68 nm (Lightner, 1993). The complete enveloped virions appear to possess appendage-like structures at both extremities that are assumed to be loose envelope extensions. Similar structures have also been seen among other shrimp baculoviruses, such as the nonoccluded virions. Thin section measurements of the virion reveal a smaller

particle of approximately 270–296 × 54–59 nm with nucleocapsid dimensions of 260 × 44.2 nm (Couch, 1974a).

Sucrose-banded OBs, when analyzed by SDS-PAGE using 12% polyacrylamide gels, revealed one major polypeptide with a molecular weight of 52 kDa (Bonami et al., 1995). Nothing is known concerning the structural proteins and glycoproteins that compose the complete enveloped virus.

The BP genome is a double-stranded, circular DNA with a molecular weight of 75 × 10⁶ Da (Summers and Anderson, 1973; Summers, 1977). Analysis by electrophoresis in a 1% agarose gel of extracted BP DNA digested with the restriction endonuclease BamHI revealed seven bands with estimated sizes: ≥23, 11.7, 8.2, 4.8, 4.0, 2.9, and 1.1 kbp. The largest band probably contained two or more high molecular weight bands (Bonami et al., 1995).

The BP virion is sensitive to a number of physical and chemical conditions (LeBlanc and Overstreet, 1991a,b). It is completely inactivated under the following conditions: within 30 min at pH 3 but not at pH 11; after 10 min at 60–90°C; after ultraviolet (UV) inactivation for 40 min at a wavelength of 254 nm; and after desiccation for 48 hr. On the other hand, it survives 32 ppt sea water at 22°C for 7 days and at 5°C for at least 14 days.

Diagnosis of BP infections has been accomplished in a number of ways: (a) by light microscopic observation of characteristic tetrahedral OBs in wet mount squash preparations of the hepatopancreas, midgut, or feces or on histological sections (Lightner, 1992) and (b) by the use of the recently developed gene probes that detect BP nucleic acid in infected cells by in situ hybridization assay (Bruce et al., 1993). The latter procedure, which was found to be specific, can also detect BP infections even before the appearance of OBs in wet mount squashes (Bruce et al., 1994). The probes can detect BP in various species of shrimp from different geographical areas.

More recently, a PCR-based detection procedure was developed for BP. However, this procedure still has, among several caveats, the potential problem of the presence of compounds in shrimp tissues that inhibit the DNA polymerase used in the PCR procedure (Wang et al., 1996). It should be added that these recently developed molecular procedures have not been comprehensively evaluated in field studies.

2. Penaeus monodon Baculovirus (MBV) Occluded Type A

Family:	Baculoviridae
Other names:	Penaeus monodon single nuclear polyhedrosis virus (PmSNPV)

Morphology:	Occluded, rod-shaped, enveloped virion; occlusion bodies are spherical and of varying sizes
Dimensions:	Enveloped virion, 265–282 × 68–77 nm
	Nucleocapsid, 250–269 × 62–68 nm
Nucleic acid:	Double-stranded DNA
	M_w = 58–110 × 10^6 Da (160 kbp)
Site of replication:	Nucleus
Maturation:	Nucleus–formation of spherical occlusion bodies.
Host species:	*P. monodon*
Other species:	*P. merguiensis, P. penicillatus, P. plebejus, P. esculentus, P. semisulcatus, P. vannamei, P. kerathurus*

The MBV is the second occluded baculovirus type A isolated from the penaeid shrimp (*P. monodon*) (Lightner and Redman, 1981). Since its initial isolation, the virus has been found in a wide variety of both cultured and wild penaeid shrimp species from Asia, Australia, Africa, southern Europe, and the Middle East (Lightner, 1993). It has also been reported in shrimp stock in the Americas, but the animals were originally imported from Asia. The MBV has recently been designated *P. monodon* singly enveloped nuclear polyhedrosis virus (PmSNPV) (Mari *et al.*, 1993a; Francki *et al.*, 1991). The virus has been linked to serious diseases and major economic losses in shrimp farms in Southeast Asia and Asia (Anderson, 1988; Baticados, 1988; Chen *et al.*, 1990; Fukuda *et al.*, 1988; Lightner *et al.*, 1992; Lin, 1989).

A similar agent was found in cultured *P. plebejus* in Australia and was called *Plebejus* baculovirus (PBV) (Lester and Atherton, 1987). However, on the basis of virus-induced host cell cytopathology and virus morphology, PBV is believed to be a strain of the MBV type of viruses rather than a distinct virus type (Doubrovsky *et al.*, 1988).

The MBV is a highly infectious virus that spreads very quickly and causes high larval and juvenile mortality. In adult shrimp the infection is less severe, with the animals showing no significant external signs of disease (Liao *et al.*, 1992). In the Indo-Pacific region, MBV has been reported to be a ubiquitous pathogen of cultured *P. monodon*. However, despite its high prevalence and wide distribution, the virion does not appear to be a highly virulent pathogen for *P. monodon*. In disease epizootics, the penaeid shrimp has been frequently found to have mixed infections of MBV and other pathogens (Lightner, 1993). Transmission of MBV is believed to be primarily oral, e.g., from cannibalism (Lightner, 1993). However, other routes of horizontal transmission may oc-

cur, such as through contamination of spawned eggs with virus-contaminated feces.

As with all occluded baculoviruses, a principal histopathological and diagnostic feature of MBV infections is the presence of single and multiple, generally spherical OBs in the hepatopancreas and, less often, in midgut epithelial cells. The OBs, which have diameters ranging from 0.1 to 20 μm, may be demonstrated in squash preparations of hepatopancreas, midgut, or feces by phase or bright-field microscopy. Different kinds of stains, such as 0.05% aqueous Malachite Green, Acridine Orange, or Phloxine, can be used to enhance visualization of MBV occlusions (Lightner, 1988; Lightner and Redman, 1991; Thurman et al., 1990; Fegan et al., 1991).

Very little information is available concerning the replication of MBV. A limited ultrastructural study on the morphogenesis of the virions in hepatopancreatic cells revealed certain cytopathic alterations occurring late in the infection such as nuclear hypertrophy, chromatin diminution, loss of nucleolus, formation of virogenic stromata, appearance of many enveloped virions, and appearance and formation of OBs. Another distinct change was the appearance of ML membranes, as was observed in BP infection. Again, the ML was postulated to play two roles in the virion replication cycle: first, as a conduit or transport system for viral structural precursors from the cytoplasm to the nucleoplasm, and second, after this function is completed, as a mechanism for release of virus and OBs (Couch, 1991).

The replication of MBV in primary lymphoid cell cultures has been reported (Chen et al., 1989). As the result of viral replication, cytopathogenic effects occurred as early as 2–3 days p.i. and became more extensive as the infection progressed. The virus was successfully passaged in primary lymphoid cell cultures at least six times. Unfortunately, no further studies were done until the recent report on the use of primary shrimp lymphoid cells for the growth and assay of YHV and the Chinese baculovirus (or white spot baculovirus) (Lu et al., 1995a,b; Tapay, 1996). This is a major step toward facilitating shrimp viral studies, particularly at the cellular, molecular, and genetic levels.

Electron microscopic examination of uranyl acetate-stained MBV revealed enveloped, rod-shaped particles measuring 265–282 × 68–77 nm and nucleocapsids measuring 250–269 × 62–68 nm. The envelope surface appeared to consist of small, uniformly sized granular structures interspersed with small spikes which were more apparent at the vertices. At the extremities of the envelope were appendage-like structures which were believed to be envelope extensions. Each

extremity of the nucleocapsid was enclosed with a double-layered structure, or cap, 16 to 18 nm thick (Mari et al., 1993a).

When banded in 30–50% CsCl, complete MBV has a buoyant density of 1.28–1.29 g/ml, and the OBs have a buoyant density of 1.32–1.33 g/ml (Chang et al., 1993).

The polyhedrin subunits of the spherical MBV polyhedron were icosahedral-like structures measuring 22–23 nm in diameter (Mari et al., 1993a). Analysis of purified MBV OBs by SDS-PAGE and Western blot protocols revealed a single protein band of 62 kDa. The molecular size of the MBV polyhedrin appears to be slightly larger than that of BP (53 kDa), the other occluded baculovirus of penaeid shrimp (Chang et al., 1992; Summers, 1977).

Visualization of MBV DNA by electron microscopy revealed large, supercoiled molecules which were not sufficiently relaxed to allow measurement of the total M_w of the genome. However, the viral DNA, after digestion with BamHI endonuclease and electrophoresis in 1% agarose gel yielded five bands with the following estimated sizes: ≥ 21, 9, 6.5, 3.5, and 2.8 kbp. From these studies, the M_w of the MBV DNA was estimated to be $58–110 \times 10^6$ kDa (80–160 kbp), which falls within the DNA size range of insect baculoviruses (Mari et al., 1993a; Matthews, 1982). In another study, the molecular size of MBV DNA based on EcoRI-cleaved fragments was estimated to be 100–200 kbp (Chang et al., 1993).

Traditional diagnosis/detection of MBV infection is accomplished by histological examination for the presence of characteristic spherical OBs in hypertrophied nuclei of the hepatopancreas and anterior midgut of the infected animal. Still another source of OBs is shrimp feces. However, these methods do not detect MBV infection at early stages, nor are they adequately sensitive. Currently, several laboratories are developing molecular-based methods for the early and specific detection of MBV infections. The PCR procedure and the DNA probes that were developed were used in either in situ or with dot-blot hybridization techniques. The preliminary results suggest that these techniques can be used for accurate and early diagnosis or detection of MBV infection (Chang et al., 1993; Poulos et al., 1994).

3. Baculoviral Midgut Gland Necrosis Virus (BMNV) Nonoccluded Type C

Other names:	Penaeus japonicus nonoccluded baculovirus (PjNOB)
Family:	Baculoviridae

Morphology:	Nonoccluded, rod-shaped virion with two envelopes; mature virion has appendage-like structures at extremities
Dimensions:	Enveloped virion, 330 × 70 nm
	Nucleocapsid, 260 × 50 nm
Nucleic acid:	Double-stranded DNA
	$M_w = 85.1 \times 10^6$ Da
Site of replication:	Nucleus
Maturation:	Nucleus
Host species:	*P. japonicus*
Other species:	*P. monodon, P. vannamei*

The BMNV is a nonoccluded, gut-infecting virus first isolated in *kuruma* shrimp, *P. japonicus,* larvae (Sano *et al.,* 1981). It is highly pathogenic in the early life stages of the shrimp, causing heavy mortality in larval production (Momoyama and Sano, 1989). Although *P. japonicus* is the natural host for BMNV, other penaeid species, such as *P. monodon, P. chinensis,* and *P. semisulcatus,* were found to be experimentally susceptible. While *P. monodon* was found to be highly susceptible, both *P. chinensis* and *P. semisulcatus* showed great resistance to the virus (Momoyama and Sano, 1996). As with the other shrimp viral pathogens, the water-borne–oral feeding route may represent the natural route of transmission.

Histological examinations of BMNV-infected animals indicate that the midgut and the intestine are the target organs. Infected cells show characteristic nuclear hypertrophy and chromatolysis, as well as the absence of OBs which characterize infections by type A baculoviruses (Momoyama, 1981; Sano *et al.,* 1981).

Thin section electron micrographs of the infected nuclei and the midgut lumen reveal numerous rod-shaped, enveloped viral particles, many of which have outer and inner envelopes. The average dimension of the virion was 310 × 72 nm (Sano *et al.,* 1981, 1984). Purified inner rod-like nucleocapsid structures had capped ends and measured approximately 260 × 50 nm (Arimoto *et al.,* 1995).

No information is available concerning the replication of BMNV at the cellular level.

Viral DNA extracted from purified nucleocapsids was sensitive to digestion with restriction endonucleases *Bam*HI and *Sau*3AI, but not with *Eco*RI, *Pst*I, *Xho*I, and *Sal*I. Electrophoretic analysis in agarose gels of the enzyme-digested viral DNA revealed 13 fragments with relative molecular sizes ranging from 2.2 to 27.0 kbp. From these results, the M_w of viral DNA was estimated to be 85.1×10^6 Da (Arimoto *et al.,* 1995).

Structural protein analysis of nucleocapsid preparation by SDS-PAGE revealed two major proteins with molecular weights of 35 and 14 kDa and three minor bands (M_w = 72, 65, and 12 kDa) (Arimoto *et al.*, 1995).

Several methods are available for the diagnosis of BMNV infections. Both stained preparations and dark-field microscopic diagnostic methods are used to detect infected, hypertrophied nuclei in squashed preparations of affected tissues such as midgut and intestine (Momoyama, 1983). The dark-field microscopic method, because of its simplicity, rapidity, precision, and low cost, is the method of choice in shrimp hatcheries in Japan (Momoyama, 1992). An immunofluorescent antibody (IFA) procedure has been successfully used to detect BMN-specific virus antigen in smears or sectioned preparations of affected tissues, such as midgut epithelial cells (Sano *et al.*, 1985; Momoyama, 1988).

*4. White Spot Baculovirus (WSBV) Nonoccluded Type C*

Other names:	Systemic ectodermal and mesodermal baculovirus (SEMBV), bacilliform virus (BV), rod-shaped nuclear virus of *Penaeus japonicus* (RV-PJ), penaeid rod-shaped DNA virus (PRDV), penaeid hemocytic rod-shaped virus (PHRV), and Chinese baculovirus (CBV)
Family:	*Baculoviridae*
Subfamily:	Nudibaculovirinae
Morphology:	Nonoccluded, rod-shaped virion with two envelopes; mature virion has appendage-like structures at extremities
Dimensions:	Enveloped virion: 276 × 121 nm; 330 ± 20 × 87 ± 7 nm; 350 × 144 nm
	Nucleocapsid: 220 × 70 nm; 328 × 65 nm
Nucleic acid:	Double-stranded DNA
	Molecular size: 163, 150, 171.3, 168 kbp
Site of replication:	Nucleus
Maturation site:	Nucleus
Host species:	*P. monodon, P. japonicus, P. penicillatus, P. chinensis*
Other species:	*P. vannamei, P. stylirostris*

Several isolates of WSBV, a currently important shrimp pathogen, have been made from different species of cultured penaeid shrimp in different parts of Asia and have been given different labels: bacilliform

virus (BV) or rod-shaped nuclear virus of *Penaeus japonicus* (RV-PJ) or penaeid rod-shaped DNA virus (PRDV) from *P. japonicus,* Japan (Takahashi *et al.,* 1994; Inouye *et al.,* 1994; Kimura *et al.,* 1996); penaeid hemocytic, rod-shaped virus (PHRV) from hybrids of *P. esculentus* × *P. monodon,* Australia (Owens, 1993); systemic ectodermal and meso-dermal baculovirus (SEMBV) from *P. monodon,* Thailand (Wongteera-supaya *et al.,* 1995); white spot syndrome baculovirus (WSBV) from *P. japonicus, P. monodon,* and *P. penicillatus* (Chou *et al.,* 1995); and Chinese baculovirus (CBV) from *P. japonicus,* Hawaii, originally ob-tained from the People's Republic of China (Lu *et al.,* 1997). Until further characterizations of their serological, biochemical, and genomic properties are made, the isolates may be either related strains of the same virus or identical. A recent report employing an *in situ* hybridiza-tion procedure has presented evidence strongly suggesting that these nonoccluded baculovirus isolates may be closely related variants (Wong-teerasupaya *et al.,* 1996). All of these isolates cause epizootics and mass mortality in cultured penaeid shrimp. Diseased shrimp show a characteristically abnormal reddish color together with white spots on the inside surface of the carapace (Takahashi *et al.,* 1994; Nakano *et al.,* 1994; Chou *et al.,* 1995). However, with two experimentally infected penaeid species, *P. stylirostris* and *P. vannamei,* the characteristic white spots were not seen and the reddish color was seen only in the extremities of the appendages (Tapay *et al.,* 1996c). These gross distinctive changes have been used in the diagnosis of WSBV infection. At the cellular level, infected cells showed markedly hypertrophied nuclei. In certain cases, histopathological examination of infected gill tissues may show Cowdry type A nuclear inclusions in hypertrophied nuclei (Wongteerasupaya *et al.,* 1995). The natural route of transmis-sion for these virions appears to be the water-borne–oral feeding routes.

For optimal purification of CBV particles and nucleocapsids from infected shrimp tissues, isopycnic centrifugation in CsCl was found to be superior to sucrose gradients. The complete virons banded at 1.23 g/ml and the nucleocapsid particles at 1.31 g/ml in CsCl (Nadala *et al.,* 1996b).

FIG 10.   Thin section electron micrographs of gill tissues from CBV-infected *P. vanna-mei.* Uranyl acetate stain. (A) Note the nuclear localization of the virus and the hypertro-phied nucleus (N) of the infected cell; bar: 10 μm. The adjacent uninfected cell had a smaller nucleus (Nu). (B) Complete virions budding out from the nucleus of an infected gill cell. Uranyl acetate stain; bar: 100 nm.
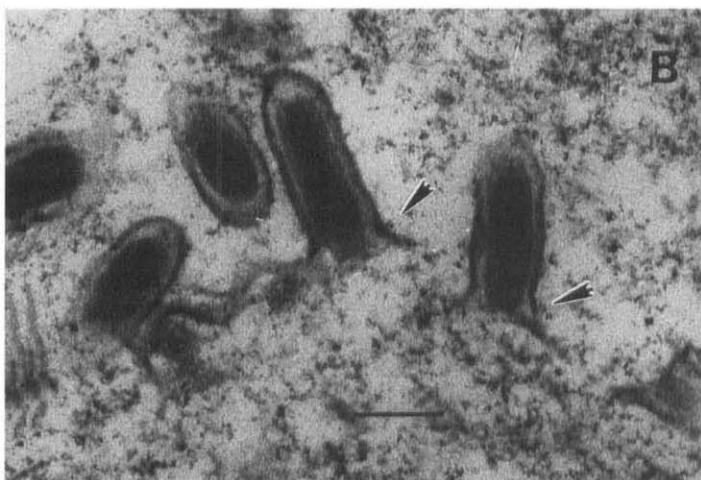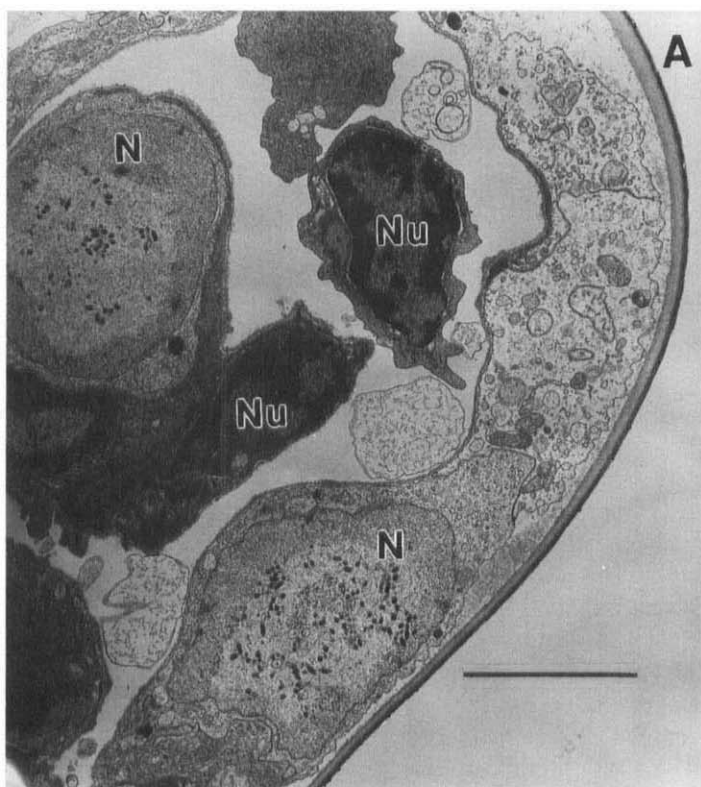
Electron microscopic examination of thin sections of affected tissues revealed numerous rod-shaped, enveloped, nonoccluded virions in the hypertrophied nuclei of infected cells (Fig. 10A). A large number of virions were found to contain double envelopes (Figs. 10B and 11A) (Tapay et al., 1996b). Little is known concerning the functional activities of the envelopes. The dimensions of thin section and negatively stained, purified virions for the different viral isolates are shown in Table VIII.

Ultrastructural analysis of negatively stained CBV nucleocapsids reveals 14 or 15 conspicuous vertical striations located periodically along the long axis of the nucleocapsid and about 22 nm apart (Fig. 11B). Between the striations are two rows of six or seven essentially globular-like structures, each approximately 10 nm in diameter (Fig. 12A). These globular structures appear to be tightly attached to each other like beads on a string (Fig. 12B). the total length of the nucleocapsid is 316–350 × 65–66 nm. Similar subunit structures arranged in a stacked series aligned perpendicular to the longitudinal axis of the nucleocapsid have been described for WSBV (Wang et al., 1995).

Genomic analysis of virion isolates revealed that the genome is an RNAse-resistant, nonsegmented, double-stranded DNA molecule. Digestion with restriction endonucleases such as EcoRI, BamHI, or HindIII and electrophoresis in agarose gels yielded fragments with a total molecular size of 163 kbp for BV (Inouye et al., 1996), 168 kbp for SEMBV (Wongteerasupaya et al., 1995), 150 kbp for WSBV (Wang et al., 1995), and 171.3 kbp for CBV (Tapay et al., 1996c).

Analysis of the structural proteins of purified CBV by SDS-PAGE revealed several polypeptide bands whose molecular sizes ranged from 200+ to 19 kDa. The most prominent and consistent proteins measured 19, 23.5, and 27.5 kDa (Fig. 13A) (Nadala et al., 1996b). The low molecular weight proteins were found not to be glycosylated and, by Western blot analysis, were present only in purified nucleocapsid preparations (Fig. 13B). In contrast, glycosylation was demonstrated with two of the higher molecular weight proteins (~220 and 50 kDa). These results strongly imply that the glycosylated proteins are envelope-associated components and are present only in the complete virion (E. C. B. Nadala, Jr., L. M. Tapay, and P. C. Loh, unpublished, 1997).

For the rapid, sensitive, and specific detection of WSBV infections, diagnostic probes for in situ hybridization and primers for detection by PCR technology have been developed (Wongteerasupaya et al., 1996; Durand et al., 1996; Chang et al., 1996; Lo et al., 1996). Preliminary results employing the in situ DNA hybridization procedure have indicated that the gut and the gills of P. monodon were the primary routes
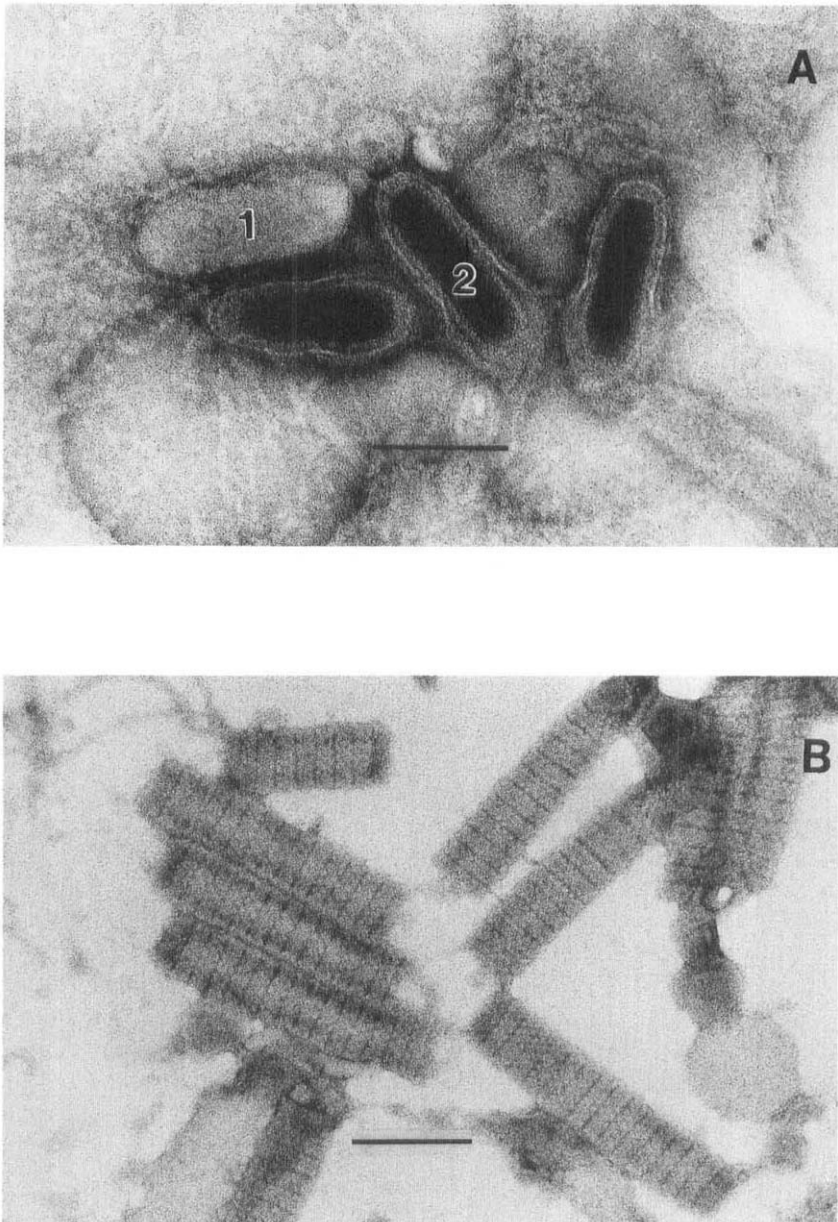
FIG 11.   Electron micrograph of purified CBV. (A) Complete virions: 1, virion stained on external surface; 2, virion penetrated by negative stain showing double envelope. Bar: 200 nm. (B) Nucleocapsids showing distinct striations. Uranyl acetate stain; bar: 300 nm.

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TABLE VIII

DIMENSIONS OF THE VARIOUS WSBV ISOLATES

| Virus | Enveloped virions | |
	Thin sections (nm)	Negative staining (nm)
BV (PRDV)[a,b]	275 × 83	404 ± 25.6 × 152 ± 16.8
SEMBV[c]	292 ± 29 × 111 ± 8	276 × 121
WSBV[d]		330 ± 20 × 87 ± 7
CBV[e]	265 × 120	350 ± 28 × 144 ± 14

| | Nucleocapsids | |
	Thin sections (nm)	Negative staining (nm)
BV (PRDV)[a,b]	216 × 54	
SEMBV[c]	244 × 80	
WSBV[d]		220 × 70
CBV[e]	205 × 78	328 × 65

[a] Takahashi et al. (1994).
[b] Inouye et al. (1994).
[c] Wongteerasupaya et al. (1995).
[d] Chou et al. (1995).
[e] Nadala et al. (1996b); Tapay (1996); Tapay et al. (1996c).

of WSBV entry and that the lymphoid organ and gills were primary targets for viral replication (Chang et al., 1996). If PCR is employed with specific primer sets, WSBV infection could be detected as early as 12 hr p.i. in water-borne, infected shrimp (Chou et al., 1996). The nested PCR technology has also been used to detect PRDV from experimentally infected P. japonicus (Kimura et al., 1996).

A combined SDS-PAGE/Western blot/EIA protocol has been developed for the early detection of CBV (and for YHV) in experimentally infected animals before the appearance of clinical symptoms (Tapay, 1996). Detection of infectious CBV was also examined in infected primary shrimp lymphoid cell cultures. This combination technology de-

FIG 12. Ultrastructure of CBV nucleocapsids. (A) A computer-enhanced image of the boxed part of the nucleocapsid shows a detailed illustration of the arrangement of the globular subunits. (B) Nucleocapsid with the globular subunits partially unwound showing a "beads-on-a-string" arrangement. Uranyl acetate stain; bar: 100 nm.
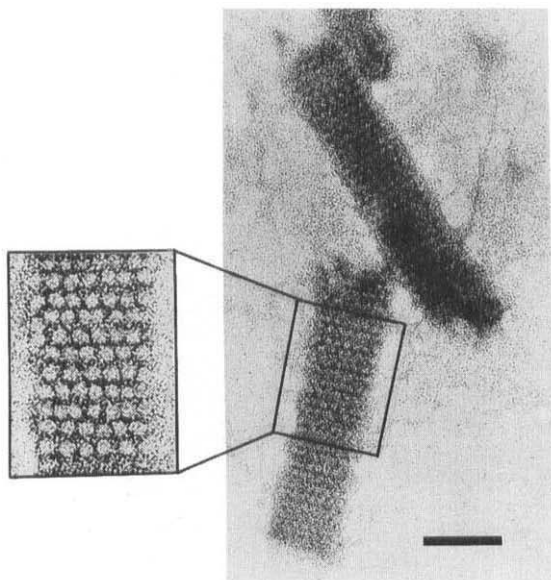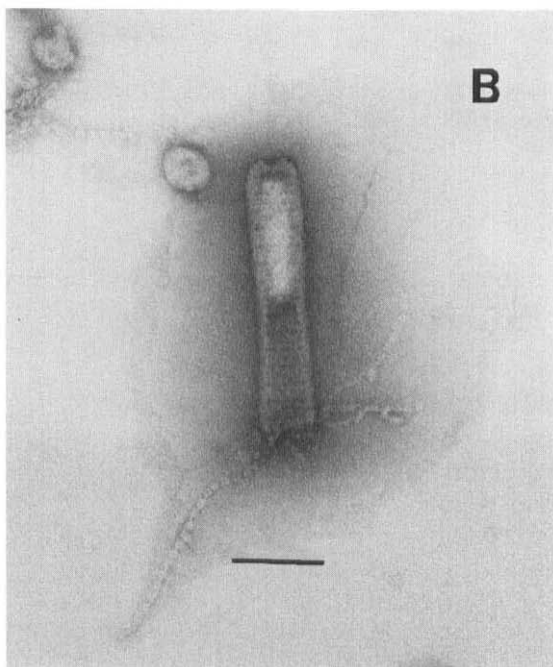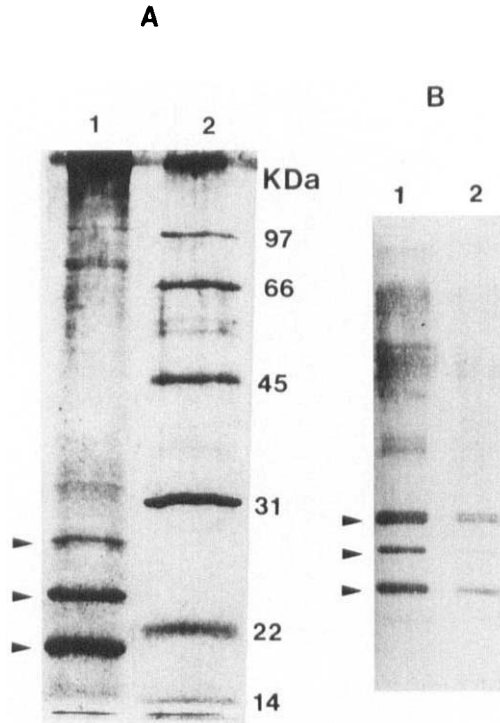
FIG 13. Analysis of CBV proteins. (A) SDS-PAGE of CBV from hemolymph (lane 1) and low molecular weight markers (LMw) (lane 2). Note the three prominent viral proteins (arrowheads). (B) Western blot of CBV proteins (arrowheads) from CBV-infected hemolymph (lane 1) and CBV-infected HST (lane 2).

tected viral proteins in the hemolymph as early as 36 hr p.i. (Fig. 14A) and infected cell cultures as early as 5.5 days p.i. (Fig. 14B). This highly specific combination protocol has several advantages for the monitoring and surveillance of shrimp viral infections. Furthermore, the sampling of hemolymph is relatively simple and less invasive, particularly for the monitoring of invaluable shrimp broodstock populations.

An *in vitro* quantal assay (TCID$_{50}$) for CBV has been developed using primary shrimp lymphoid cell cultures (Tapay *et al.*, 1996a). Despite limitations associated with primary cell cultures, this assay provides a simple, convenient, and quantitative method for the study of shrimp viruses and the diseases they cause (Lu *et al.*, 1995b).

In vitro infectivity studies of WSBV filtrates from diseased shrimp in four fish cell lines [epithelioma papulosum cyprini (EPC); chinook
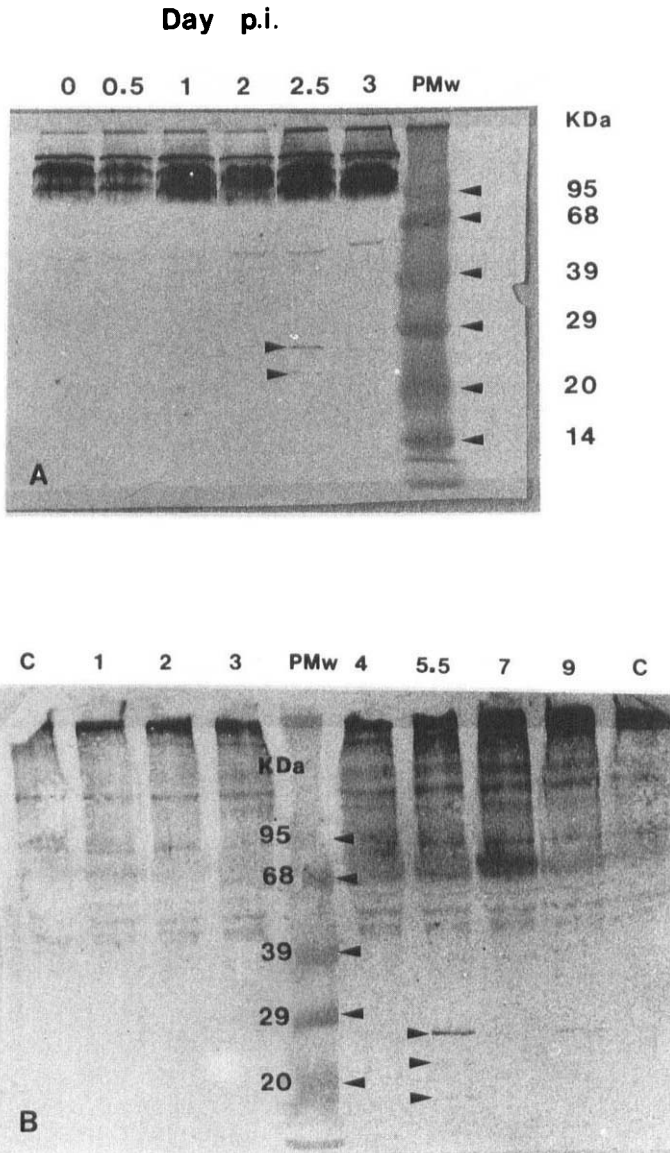
FIG 14. Detection of CBV nucleocapsid proteins by Western blot immunoassay in time-course infectivity experiments. (A) Hemolymph sampled at various days p.i. showing the appearance of the nucleocapsid proteins as early as 2.5 days (36 hr) p.i. (B) The CBV-specific proteins (arrowheads) appeared 5.5 days p.i. in infected primary lymphoid cell cultures.

salmon embryo (CHSE-214); fathead minnow (FHM); and sockeye salmon embryo (SSE-5)] (Chou *et al.*, 1995), EPC, and CRL1963 (a *Drosophila* cell line) (Tapay, 1996) did not induce any cytopathogenic effect.

5. Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV)

Family:	*Parvoviridae*
Morphology:	Nonenveloped, icosahedral virion
Dimensions:	20–22 nm
Nucleic acid:	Single-stranded, DNA-positive and negative polarities
	Molecular size: 4.1 kb
Site of replication:	Nucleus
Maturation:	Nucleus
Host species:	*P. stylirostris, P. vannamei, P. monodon, P. japonicus, P. semisulcatus,*
Other species:	*P. chinensis, P. aztecus, P. duorarum, P. setiferus* (?)

The IHHNV is widely distributed, causing severe epizootics and massive mortality in cultured penaeid shrimp, particularly the juveniles of *P. stylirostris* (Lightner and Redman, 1991). In other penaeid species, the disease is somewhat less severe. With certain cultured penaeid species, such as *P. vannamei*, a "runt-deformity syndrome" (RPS) may be the consequence (Kalagayan *et al.*, 1991). The affected animals characteristically exhibit greatly reduced growth rates and a variety of cuticular deformities, all of which lessen their market value.

Natural infections by IHHNV have been reported in a number of penaeid species, such as *P. stylirostris, P. semisulcatus,* and *P. japonicus*. As with the other shrimp viral pathogens, the water-borne–oral feeding route may represent the natural route of transmission. Survivors of IHHN epizootics apparently harbor the virion for life and transmit it to their progeny by vertical and horizontal routes (Lightner and Redman, 1991).

Gross clinical symptoms of acute IHHNV infections are not specific. In the infected animal, certain distinguishable histopathological changes occur. Present in affected cells are intranuclear Cowdry type A inclusion bodies (CAI) contained in hypertrophied nuclei in tissues of ectodermal (epidermis, hypodermal epithelium of foregut and hindgut, nerve cord, and nerve ganglia) and mesodermal (hematopoietic organ, antennal gland, gonads, lymphoid organ, connective tissues, and striated muscle) origin (Lightner, 1988).

Purified IHHNV has been prepared from infected penaeid shrimp and banded in CsCl gradients at a buoyant density of 1.40 g/ml. Negatively stained, purified virions are nonenveloped icosahedrons with a diameter of 20–22 nm. The virions possess a linear, single-stranded DNA genome of either negative or positive polarity with an estimated size of 4.1 kb (Bonami *et al.,* 1990; Mari *et al.,* 1993b). The purified virion is made up of at least four structural polypeptides, VP1 to VP4, with molecular weights of 74, 47, 39, and 37.5 kDa (Bonami *et al.,* 1990).

Since no cell lines exist to grow the IHHNV, there is no information regarding viral replication at the cellular and molecular levels.

Several methods are available for the diagnosis of IHHNV infections. The histopathological examination of affected tissues for the presence or absence of intranuclear CAI has provided a fairly reliable diagnosis. However, the formation of CAI may be induced by rather general types of cell injury not involving viruses. Under certain conditions, both DNA- and RNA-containing viruses have been reported to cause CAI (Hirst, 1959; Norrby and Oxman, 1990). Another method involves enhancement procedures in which the suspected animals are kept under stressful conditions for 2 to 3 weeks prior to sampling for histologic examination (Lightner, 1988; Lightner and Redman, 1991).

For the detection of asymptomatic IHHNV infections, susceptible small juveniles of *P. stylirostris* have been used as indicator shrimp for the presence or absence of the virus.

Recently, gene probes were developed for IHHNV (Mari *et al.,* 1993b). One of the probes, in dot-blot experiments, had a sensitivity level of 0.5 pg of viral DNA. The probe reacted specifically with IHHNV DNA, purified IHHNV, and homogenized tissues from known IHHNV-infected animals. It did not react with homogenates from healthy animals or with purified hepatopancreatic parvo-like virus (HPV) and tissues from known HPV-infected shrimp. *In situ* hybridization using labeled probe on paraffin-embedded sections from IHHNV-infected and healthy animals showed a positive reaction only with virus-infected tissue sections. Furthermore, when these sections were tested against DNAs from four insect parvoviruses, no hybridization was observed. The probe is presently commercialized as a kit.

6. Hepatopancreatic Parvo-Like Virus (HPV)

Family:	*Parvoviridae*
Morphology:	Nonenveloped, icosahedral virion
Dimension:	22 nm
Nucleic acid:	Single-stranded DNA, positive and negative polarities

Molecular size: ~5 kb
Site of replication: Nucleus
Maturation: Nucleus
Host species: *P. chinensis, P. merguiensis, P. semisulcatus,*
                        P. monodon, P. indicus, P. penicillatus, P. escu-
                        lentus

The HPV is another parvo-like virus that infects a number of cultured and wild *Penaeus* species (*P. chinensis, P. merguiensis, P. semisulcatus, P. monodon, P. indicus, P. penicillatus, P. esculentus*). It has a wide geographic distribution, including the Indo-Pacific area (China, Korea, the Philippines, Malaysia, Singapore, Australia, Indonesia, and Thailand), Africa, the Middle East (Israel and Kuwait), and the Americas (Hawaii, Ecuador, Mexico, and Brazil) (Lightner and Redman, 1992; Lightner, 1993). The relationship between all of these reported HPV-type viruses is not known since identification of these virions was based solely on microscopic or histopathological examinations.

Although HPV has been circumstantially implicated in the cause of major disease epizootics, its role as a serious pathogen remains to be clearly defined. This is because of the relative difficulty of diagnosing HPV infections and also because these infections are often accompanied by other viral pathogens that may obscure its importance. Little is known concerning the natural mode of transmission of HPV, although the water-borne–oral route is the most likely route. No cell lines are currently available that support the replication of HPV.

In the infected animal, the principal lesion of the disease is characterized by the necrosis and atrophy of the hepatopancreas, which is common to all the penaeid species (Lightner, 1985). Large, prominent, basophilic, Feulgen-positive intranuclear inclusion bodies were often observed in hypertrophied nuclei of hepatopancreatic tubule epithelial cells. These histological changes were used in the diagnosis of HPV infections (Lightner and Redman, 1991, 1992; Lightner, 1988, 1993).

The HPV and the IHHNV are both parvoviruses, but in the permissive host animal they infect different target tissues: the hepatopancreatic epithelial cells for HPV and all nonenteric tissues for IHHNV (Lightner and Redman, 1985; Lightner *et al.*, 1983).

Electron microscopic analysis of thin sections of HPV-infected cells revealed intranuclear inclusion bodies containing granular virogenic stroma and viral particles 22–24 nm in diameter (Lightner and Redman, 1985; Roubal *et al.*, 1989; Bonami and Lightner, 1991).

Purified HPV prepared from infected penaeid shrimp and banded in CsCl gradients had a buoyant density of 1.41 g/ml (Bonami *et al.*,

1995). Negatively stained, purified virions were nonenveloped, icosahedral particles with a diameter of 22 nm. The virions contained a linear, single-stranded DNA genome of either negative or positive polarity with an estimated molecular size of 5 kb, which, surprisingly, encoded a single protein of 54 kDa (Bonami et al., 1995).

Gene probes were recently developed for the diagnosis of HPV infections in shrimp (Mari et al., 1995). The probes, labeled with digoxigenin, were used for in situ hybridization assays for HPV in fixed tissues from infected P. chinensis and healthy shrimp. Only the HPV-infected epithelial cells of the anterior midgut and hepatopancreas showed a positive reaction. These are the target tissues of the virus based on histological studies (Lightner, 1993). Although this gene probe prepared from HPV isolated from P. chinensis reacted positively with most of the HPV-infected penaeids tested, regardless of the host species or geographic source, it did not react with the intranuclear inclusions in several species of HPV-infected penaeid shrimp from the Indo-Pacific region, and the Americas (Lightner et al., 1994b). These findings may indicate that there are several different strains of HPV and that they are not sufficiently related to yield a positive reaction in an in situ hybridization assay. The HPV probe did not cross-react with the DNA isolated from the other shrimp parvovirus, with IHHNV, or with IHHNV-infected tissues in in situ hybridization. As with the IHHNV, the HPV probe is commercially available as a kit.

C. Other Shrimp Viruses

The following penaeid shrimp viruses have been reported. Most of them remain to be isolated and their relevant properties characterized:

Lymphoidal parvo-like virus (LOV) (Owens et al., 1991);
Penaeid hemocyte-infecting, nonoccluded baculovirus (PHRV) (Owens, 1993);
Shrimp iridovirus (IRIDO) (Lightner and Redman, 1993);
Type III reo-like virus (REO-III) (Tsing and Bonami, 1987);
Type-IV reo-like virus (REO-IV) (Nash and Nash, 1988);
Lymphoid organ vacuolization virus (LOVV) (Spann et al., 1995); and
Naked star-shaped virus (NSV) (Lu et al., 1989, 1996a).

VI. CONCLUDING REMARKS AND FUTURE DIRECTIONS

While significant advances have been made in determining the role of viruses involved in several of the epizootics occurring in penaeid

shrimp aquaculture, viral diseases will continue to plague the industry. A major obstacle to the study of these diseases is the lack of convenient and quantitative methodologies, such as *in vitro* cell culture systems to grow and study (characterize) the virus. A beginning has been made with the recent development of protocols for the consistent preparation of primary shrimp lymphoid cells, which were employed for the quantal assay of some of the shrimp viral pathogens. The primary cell lines have also been used to analyze the synthesis of viral proteins at the cellular level and to study viral pathogenesis. With the further success- ful development of additional primary cell lines from other shrimp tissues and the establishment of continuous diploid and transformed shrimp cell lines, this problem is being solved. The value of cell culture systems is becoming increasingly clear. They present several obvious advantages: they are more cost effective, sensitive, and convenient than whole animals, particularly for rapid monitoring of infectivity; they yield quantitatively reproducible results; and viral growth kinetics, biochemical and genetic characteristics, and so on can be studied more easily. Their biggest potential use is in future molecular biology and genetic studies of shrimp viruses. These aspects are currently inade- quately investigated. It should be emphasized that a major advantage of the cell culture systems is their sensitivity in detecting the viable virion and, consequently, asymptomatic and latent infections, particu- larly in broodstock populations. This is currently accomplished by em- ploying sensitive indicator animals, a procedure that is both expensive and laborious. Finally, infectivity assays provide a crucial measure of the number of infectious, transmissible virus particles present in the sample.

An important advance in the study of shrimp viral diseases is in the area of detection/diagnostic technologies. A number of molecular and immunologically based technologies have been developed which have facilitated specific, sensitive, and early detection and diagnosis of the shrimp viral pathogen in infected animals. Unfortunately, methods such as PCR and nucleic acid probes and their modifications will be of limited use because of their high cost and the requirement of both skilled personnel and sophisticated equipment. Although they lack the sensitivity of the nucleic acid-based methodologies, the immunologi- cally based methods such as the solid-phase enzyme immunoassay protocols (NC-EIA), with their enhancement modifications, are highly specific and cost effective. Furthermore, when available in kit form, they will require neither highly skilled personnel nor sophisticated equipment. It should be noted that a major disadvantage of all of these new technologies is that they detect only a component of the virus, not

the viable infectious agent. Other practical problems, particularly in relation to NA-based technologies, such as specimen collection and processing, carryover contamination, interfering or inhibitory substances, and quality assurance for the test system, correct interpretation of the results, have yet to be fully solved. However, they can serve as early warning indicators of the potential presence of the infectious virus in the cultured shrimp population and enable proper control and preventive measures to be taken.

Another problem associated with shrimp viral diseases which needs to be further investigated is mixed infections. There is little or no information regarding the dynamics in mixed infections between viruses and between viruses and bacteria. Here again the cell culture systems would aid immensely in analyzing the roles of the infectious agents in the disease process.

An area not included in this review on penaeid shrimp viral pathogens is a discussion of the defense mechanisms found in the penaeid shrimp in relation to disease control and prevention. Information on these mechanisms is still sparse. What is currently known is that the penaeid shrimp has an open circulatory system with a nonspecific, nonrecall defense mechanism consisting of a lymphoid organ, effector cells, and humoral effectors. There is lack of information on the antiviral activities of these defense components. Additional studies on the defense mechanisms of the commercially important penaeid shrimp will be required if future objectives include the selection of pathogen-resistant strains of penaeid shrimp.

Lastly, this review provides an update on what has been accomplished in the study of penaeid shrimp viruses and the diseases they cause. As the accumulated data indicate, continued studies on these infectious agents are necessary for their eventual control and eradication.

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