Purification and characterization of the infectious hypodermal and haematopoietic necrosis virus of penaeid shrimps

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Infectious hypodermal and haematopoietic necrosis (IHHN) is one of the most important viral diseases of cultured penaeid shrimps and is potentially a limiting factor in the development of farming projects for some species of these shrimps. Although the IHHN agent was recognized early as being viral in origin, attempts to characterize it were inconclusive because of difficulties in obtaining sufficient amounts of purified virions to permit its characterization. Recent improvements of purification procedures have allowed the physicochemical characterization of this virus. Purified IHHNV is a non-enveloped icosahedral particle averaging 22 nm in diameter, exhibiting a mean buoyant density of 1.40 g/ml in CsCl. The genome is a single molecule of ssDNA with an estimated size of 4.1 kb by molecule length measurement in transmission electron microscopy. As determined by SDS-PAGE, the particle contains four polypeptides with Mr's of 74K, 47K, 39K and 37.5K, respectively. From its characteristics, this virus could be a member of the Parvoviridae family.

Introduction

Infectious hypodermal and haematopoietic necrosis (IHHN) disease (Lightner et al., 1983a) has been found in cultured penaeid shrimps in the Americas and Asia (Lightner, 1985, 1988) and in French Polynesia (Lightner et al., 1983b, 1990a). The gross signs of the disease and the histological diagnostic procedures for it have been extensively described (Lightner et al., 1983a, 1990a; Lightner, 1985, 1988). The international spread of IHHN disease into new regions with asymptomatic carriers underscores the importance of IHHN as one of the major diseases of cultured penaeid shrimps (Lightner et al., 1983b, 1990b). The demonstration of virus-like particles in sections of infected tissue by electron microscopy (Lightner et al., 1983a) and the ability to reproduce the disease experimentally using bioassays indicated that the aetiological agent was a virus (Bell & Lightner, 1984); the agent was named infectious hypodermal and haematopoietic necrosis virus (IHHNV). However, attempts to isolate an IHHNV have been unsuccessful or have yielded amounts of viral particles insufficient to permit its characterization. Recently, purification procedures have been improved, allowing us to obtain the higher concentrations of purified virions necessary to complete the characterization of the IHHN agent.

In the discipline of marine invertebrate virology, very few viruses have been characterized. They are two reoviruses (Bonami, 1980; Mari, 1987; Mari & Bonami, 1988a) and a parvovirus (Mari & Bonami, 1988b) from the crabs Macropipus depurator and Carcinus mediterraneus, one reovirus from the oyster Crassostrea virginica (Meyers, 1979; Winton et al., 1987), one iridovirus (Devauchelle et al., 1977) from the annelid worm Nereis diversicolor and one baculovirus (Summers, 1977) from the shrimp Penaeus duorarum. However, only some characteristics of the marine shrimp baculovirus have been reported, and a report about the isolation, purification and characterization of IHHNV (directly contradictory with our findings) has been recently published (Lu et al., 1989).

We report here our results on the purification, ultrastructure and the physicochemical properties of IHHNV.

Methods

Experimental animals. Although the small juvenile stages of P. stylirostris are most severely affected by IHHN disease, we used as a source of IHHNV a single 10 kg batch of large size (30 to 50 g) P. vannamei obtained from the Ocean Institute (Oahu, Hawaii) in December 1986. These shrimps were stored at −70 °C until processed. Infection by IHHNV in this batch of shrimps was confirmed by direct
histological examination of Davidson's preserved specimens and by bioassay tests for the virus using 0-05 to 0-1 g P. stylirostris as the indicator for virus presence (Lightner et al., 1983b).

Bioassay studies. A sample of eight frozen gnatthoracae ('heads') from the adult P. carnanei population from the Oceanic Institute were bioassayed with 0-05 to 0-1 g juvenile P. stylirostris (the indicator shrimp for presence or absence of IHHNV). In the bioassay, 50 to 55 indicator shrimps were stocked into each of six separate 40 litre glass aquariums with P. stylirostris. The remaining three tanks, which were physically isolated from the other tanks, served as negative control tanks. Shrimps in all six tanks were fed twice daily on an artificial dry pelleted ration at approximately 10% of their total biomass. Water temperature was maintained between 24°C and 28°C with a room air conditioner. Salinity was maintained at 20 to 25%.

Sampling for histopathological diagnosis of IHHNV was scheduled for days 0, 14, 21, 28, 42 and 60 of the bioassay and as morbund individuals were observed. However, actual sampling was done on days 0, 17, 21, 23, 25, 26 and 28 of the bioassay (Table 1). Two or three shrimps per replicate tank were taken at each scheduled sampling time. The bioassay was terminated on day 28 because IHHNV-positive indicator shrimps were found in day 17 and day 21 samples, and because all the indicator shrimps had died by day 26.

 Infectivity studies. Using similar bioassay procedures, semi-purified and purified preparations of IHHNV from sucrose and CsCl gradients were tested for infectivity and for their ability to produce IHHNV disease in juvenile P. stylirostris. In these studies various fractions that were found to contain virus according to their absorption spectra at 260 nm (sucrose gradients), or because they were found to contain 20 to 22 nm diameter virus-like particles (from CsCl gradients) when examined by transmission electron microscopy (TEM), were injected into the tail muscle of 0-5 to 2 g juvenile P. stylirostris in five separate experiments (Table 2). Samples of obviously ill shrimps were taken as they were observed in these studies for histopathological confirmation of IHHNV, or samples were taken according to scheduled sampling schemes (Table 2).

Virus purification. Only heads (gnatthorax with the carapace removed) were used for virus extraction and all buffers used were filtered through 0-22 μm membranes to remove particulate contaminants and bacteria. After homogenization in TN buffer (0-02 M-Tris-HCl, 0-01 M-NaCl, pH 7-4-7-6) the suspension was clarified in three steps at 20 000, 5000 and 15 000 r.p.m., in runs of 10 min, 10 min and 30 min, respectively. The resulting supernatant was pelleted for 3-5 h at 145 000 g then resuspended in TN buffer, shaken with activated charcoal, filtered on a Celite-235 bed and extracted three or four times in freon (1,1,2-trichloro-1,2,2-trifluoroethane). The final suspension was pelleted at 145 000 g for 3-5 h and then resuspended in a small volume of buffer before being layered on a 15 to 40% (w/w) sucrose gradient and centrifuged for 3 h at 140 000 g. Fractions (1 ml) from the sucrose gradient were collected with an Autodensiflow fraction collector and the absorbance at 254 nm was determined and recorded using an Isco UA5 u.v. monitor. Fractions containing virions were pooled, diluted in TN buffer and centrifuged for 3 h at 140 000 g. Pellets, resuspended in TN buffer, were layered on a preformed 25 to 45% (w/w) CsCl gradient in TN buffer and isopycnically centrifuged for 16 h at 145 000 g. The band of virus was collected using the same method as indicated for the sucrose gradient, diluted in TN buffer, pelleted for 1-5 h at 250 000 g and resuspended in a small volume of buffer.

Virus density. Because of the refractive index of TN buffer, we used CsCl in 0-1 × TE (10 mM-Tris-HCl, 1 mM-EDTA pH 7-4) buffer which does not interfere with the refractive index values obtained. Ultrafiltered (0-22 μm) CsCl preformed gradients (20 to 40% w/w and 28 to 48% w/w in 0-1 × TE) were used for isopycnic centrifugations of empty and full particles, respectively, in runs of 17-5 h. Fractions (0-5 ml) were collected, absorbance at 254 nm was recorded with an Isco UA5 monitor and the refractive index of each fraction was measured using an Abbé refractometer.

Electron microscopy. Purified virus suspensions were negatively contrasted on carbon—colloidal—coated 200-μm copper—palladium grids using ultralifted (0-22 μm) 2% sodium phosphotungstate (PTA) in doubly distilled water pH 7. Molecules of extracted nucleic acid were spread on carbon-coated grids according to the procedure of Delain & Brack (1974) and contrasted with platinum-palladium rotational shadowing. Observations were performed using an Hitachi HU11-C electron microscope.

Virus density. Because of the refractive index of TN buffer, we used CsCl in 0-1 × TE (10 mM-Tris-HCl, 1 mM-EDTA pH 7-4) buffer which does not interfere with the refractive index values obtained. Ultrafiltered (0-22 μm) CsCl preformed gradients (20 to 40% w/w and 28 to 48% w/w in 0-1 × TE) were used for isopycnic centrifugations of empty and full particles, respectively, in runs of 17-5 h. Fractions (0-5 ml) were collected, absorbance at 254 nm was recorded with an Isco UA5 monitor and the refractive index of each fraction was measured using an Abbé refractometer.

Spectrophotometry. Spectra from 200 to 320 nm were recorded for both purified virus suspensions and extracted nucleic acid; concentrations of proteins and nucleic acid were estimated according to the procedures of Layne (1957) and Maniatis et al. (1982).

SDS–PAGE. 10% polyacrylamide vertical gel slabs were run using a phosphate buffer (0-1 M-NaHPO₄—Na₂HPO₄, pH 7) containing 0-1% SDS. The run duration was 3 h at a constant 150 V. Samples were treated with a dissociating medium (1% SDS, 5 mM-urea, 0-1% 2-mercaptoethanol) and heated to 100 °C for 3 min. M, markers were phosphorylase B, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor and a-lactalbumin, which have M, of 94K, 67K, 43K, 30K, 20K and 14K, respectively. Bromophenol blue and sucrose were added to the samples and M, markers before electrophoresis. After washing in fixative solution the gels were stained with Coomasie blue or with silver stain (Ag-stain 'Daichi'; Daichii Pure Chemicals). The M, of the polypeptides was estimated by measurement of the electrophoretic mobilities according to the method of Weber & Osborn (1969).

Nucleic acid extraction. Purified virus suspensions were treated with pre-activated proteinase K at a final concentration of 50 μg/ml for 0-5 h at 37 °C, followed with Sarkosyl (final concentration 0-5%) for 1 h at 65 °C. Nucleic acid was extracted twice with buffer-equilibrated phenol (Maniatis et al., 1982), once with a 1:1 mix of equilibrated phenol and chloroform and twice with chloroform—isoamyl alcohol (24:1). The aqueous phase was then adjusted to 0-3 M-sodium acetate pH 5-2, two volumes of absolute ethanol were added and the nucleic acid was allowed to precipitate overnight at −30 °C. The nucleic acid was then pelleted, dried and resuspended in TNE buffer (10 mM-Tris—HCl, 100 mM-NaCl, 1 mM-EDTA, pH 8).

Agarose gel electrophoresis. Electrophoretic separation of nucleic acid segments was performed in 0-7% agarose gels in TAE buffer (0-04 M-Tris—acetate, 0-001 M-EDTA pH 8) run at 100 V for 2 h. Ethidium bromide, sometimes incorporated into the gel (0-5 μg/ml) or used as a stain after the electrophoresis, was used to stain nucleic acid bands. Phage λ HindIII and EcoRI–HindIII DNA digests were used as M, markers. Before each run, samples and markers were heated to 60 °C but separate samples were treated by heating (3 min at 100 °C) with RNase, DNase I or nuclease S1, according to the method of Maniatis et al. (1982).

Gels containing methylmercuric hydroxide (Maniatis et al., 1982) were used to determine, in association with RNase treatment, the nature of the nucleic acid. For these experiments all nucleic acid extractions were performed using RNase-free conditions.
Results

Bioassay studies

The results of the bioassay run on the adult *P. vannamei* using juvenile *P. stylirostris* as the indicator for the presence or absence of IHHNV confirmed that the *P. vannamei* were infected with IHHNV. Severe IHHN infections were present in the indicator shrimps by day 17 of a planned 60 day study and no exposed indicator shrimps survived to day 26 of the bioassay. In contrast, IHHN was not observed in samples of control (unexposed) indicator shrimps, nor were significant mortalities observed in the control groups (Table 1).

Isolation and purification of IHHN virions

The quantity of purified virions obtained from between five and seven gnathothoraces from 30 to 50 g adult shrimps was estimated by measuring the absorbance at 260 and 280 nm (Layne, 1957); virus yield from this quantity of shrimp tissue was estimated at 75 µg to 200 µg, depending on the degree of infection in the animals sampled. The degree of purity of the isolated IHHNV preparations was determined by TEM and gel electrophoresis. TEM of negatively contrasted purified IHHNV preparations showed the absence of cell contaminants (Fig. 1a). Likewise, SDS–PAGE of the virus preparations showed no bands other than those expected to be present in small viruses (Fig. 3).

Infectivity studies

IHHN disease was induced experimentally using semi-purified (from sucrose gradients) and purified IHHNV (from CsCl gradients) in artificially exposed juvenile *P. stylirostris*. IHHN disease was confirmed histologically in those shrimps indicated as positive for the disease in Table 2.

Table 1. Results of the bioassay run on adult *Penaeus vannamei* with juvenile *P. stylirostris* serving as the indicator shrimp

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>17</th>
<th>21</th>
<th>23</th>
<th>25</th>
<th>26</th>
<th>28</th>
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<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td></td>
<td></td>
<td></td>
<td>0/3</td>
</tr>
<tr>
<td>Control 2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td></td>
<td></td>
<td></td>
<td>0/3</td>
</tr>
<tr>
<td>Control 3</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td></td>
<td></td>
<td></td>
<td>0/3</td>
</tr>
<tr>
<td>Exposed 1</td>
<td>0/2</td>
<td>2/2</td>
<td>1/1</td>
<td>1/1</td>
<td></td>
<td></td>
<td>3/3 NS</td>
</tr>
<tr>
<td>Exposed 2</td>
<td>0/2</td>
<td>3/3</td>
<td>1/2</td>
<td></td>
<td>1/1</td>
<td>3/3</td>
<td>NS</td>
</tr>
<tr>
<td>Exposed 3</td>
<td>0/2</td>
<td>2/2</td>
<td>2/2</td>
<td>1/1</td>
<td></td>
<td>1/1</td>
<td>NS</td>
</tr>
</tbody>
</table>

* NS, No sample was taken because no indicator shrimp survived to day 28 of the bioassay.

Fig. 1. Purified IHHNV. (a) Large amount of full particles; bar marker represents 200 nm. (b) Full and empty particles at higher magnification from a non-fully purified virus suspension, small cell debris is associated with the particles. Note the angular shape of virions. Bar marker represents 40 nm. Negative stain (PTA).

Fig. 2. CsCl equilibrium centrifugation of full IHHNV particles. A is given at 254 nm.
Buoyant density of virions

For full particles, using a 28 to 48% (w/w) preformed CsCl gradient in 0.1 x TE buffer, the density of IHHNV was found to be between 1.390 and 1.420, with a mean value of 1.405 at the summit of the recorded peak (Fig. 2). For empty particles, using a preformed 20 to 40% (w/w) CsCl gradient, the density had a mean value of 1.298 (not shown).

Size and structure of purified IHHNV

In negatively stained virus suspensions, full and empty particles were clearly observed (Fig. 1 a and b). Using TMV (diameter 18 nm) as an internal size marker, the virions exhibited a mean diameter of 22 nm, with an average of 20 and 23 nm for side to side and point to point, respectively. The inner component of empty particles showed an average diameter of 13 nm. Due to the presence of five- and six-sided particles in the preparations, we conclude that this virus is icosahedral in shape (Fig. 1 b).

Polypeptides of IHHNV

IHHNV polypeptides were investigated by SDS-PAGE in a 10% gel. A total of four polypeptides were observed both in Coomassie blue- and silver-stained gels (Fig. 3). From different gel electrophoresis preparations, we have estimated the Mr of the four polypeptides to be 74K, 47K, 39K and 37-5K, respectively (Fig. 4).

Nature and structure of the IHHNV nucleic acid

Using purified virions in dissociating medium heated at 60 °C for 3 min and run in an SDS-0.7% agarose gel, only one nucleic acid band was present. Likewise, only a single band was found from phenol-extracted viral nucleic acid run in a regular 0.7% agarose gel or in methylmercuric hydroxide conditions.

Attempts to characterize the nucleic acid show that it is RNase-resistant in the conditions where this enzyme is active against ribosomal RNA (not shown) and it is

<table>
<thead>
<tr>
<th>Study</th>
<th>Gradient</th>
<th>Fraction/band</th>
<th>Days post-injection (number IHHNV-positive/number examined)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1  2  3  4  6  7  9  11 12 16</td>
</tr>
<tr>
<td>1</td>
<td>Sucrose</td>
<td>Control</td>
<td>0/1 0/1 0/1 – – 0/1 0/1 0/1 – –</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peak 1</td>
<td>1/2 0/2 2/2 1/1 3/3 2/2 2/2 1/1 – –</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peak 2</td>
<td>0/2 1/2 0/2 – – 2/2 2/2 1/1 – –</td>
</tr>
<tr>
<td>2</td>
<td>Sucrose</td>
<td>Saline control</td>
<td>– – – – – – – – 0/11 – – – – – –</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Band</td>
<td>– – – 1/2 1/1 2/2 5/5 – – – – – – –</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pellet</td>
<td>– – – – – – – – 4/6 – – – – – – –</td>
</tr>
<tr>
<td>3</td>
<td>Sucrose</td>
<td>Control</td>
<td>0/3 – 0/4 – – 0/4 – 0/4 – – – –</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peak</td>
<td>0/3 – 1/4 – – 2/4 – 3/4 – – – –</td>
</tr>
<tr>
<td>4</td>
<td>CsCl</td>
<td>Diffuse band</td>
<td>– – – – 3/3 – – – – – – – – – –</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sharp band</td>
<td>1/3 – 0/1 0/1 1/1 – 1/1 3/3 – – – – – – – –</td>
</tr>
<tr>
<td>5</td>
<td>CsCl</td>
<td>Sharp band</td>
<td>– – – – 2/2 – – – – – – – – – – – – – – – – – – – – – –</td>
</tr>
</tbody>
</table>
sensitive to nuclease S1, suggesting that the nucleic acid is a ssDNA (Fig. 5). Moreover, the characteristics of the nucleic acid when run in non-denaturing gels, and the absence of hyperchromicity at 260 nm when it is gently heated, confirm that the nucleic acid of IHHNV is ssDNA. When co-electrophoresed with dsDNA markers in a 0.7% gel, the size of the ssDNA in the band can be estimated at about 3.75 kb (Fig. 5). Moreover, a smear was clearly shown along the migration area of the gels in all our nucleic acid preparations. We initially hypothesized that this smear was due to freezing the virions before extraction, underlining the fragility of this ssDNA, giving numerous small pieces of different sizes. But as the material making up the smear is sensitive to RNase I and nuclease S1, the results suggest strongly the presence of ssRNA molecules in our preparations. This is confirmed by the absorbance spectrum of the total extracted nucleic acid which shows a 280/260 ratio of close to 2, indicating the presence of RNA.

TEM examination of total nucleic acid extract spread on a carbon-coated grid and rotationally shadowed, demonstrated supercoiled molecules associated with circular-like and different sized linear molecules. The 3.75 kb band (Fig. 5) was removed from the gel, electroeluted, the ethidium bromide was extracted and the DNA recovered by cold alcohol precipitation before being spread on a carbon-coated grid and shadowed (Fig. 6). Compared with ΦX174 replicative form (RF) DNA circular molecules which are double-stranded, the pictures confirm the single-stranded nature of the IHHNV DNA, which shows a smaller diameter. By length measurement, an average value of 1.36 μm (with a range from 1.26 to 1.40 μm) was obtained for IHHNV ssDNA molecules; the average value for ΦX174 RF DNA was 1.78 μm (from 1.75 to 1.81 μm). As ΦX174 RF DNA has a size of 5.386 kbp, we can estimate the molecular size of the IHHNV ssDNA at about 4.1 kb. Since one base is
negatively stained particles in different laboratories compared to single-stranded viral DNA.

Although the presence of virions in IHHNV-diseased shrimps was suggested by the observation in TEM of intranuclear inclusions in affected tissues of Penaeus vannamei (or of other penaeids) with IHHN infections have not been done. Rather, cytoplasmic masses of virions, sometimes in paracrystalline arrays, have been found in affected tissues of *P. stylirostris* with IHHN (Lightner et al., 1983a; Lightner, 1985, 1988). Comparable ultrastructural studies of *P. vannamei* (or of other penaeids) with IHHN infections have not been done. Hence, because of its small size, the absence of Feulgen-positive intranuclear inclusion bodies and the presence of virus masses and arrays in the cytoplasm, IHHNV was tentatively placed with the picornaviruses.

It was only after several nucleic acid extractions from purified virions isolated from different batches of *P. stylirostris* and *P. vannamei* that a more accurate characterization of IHHNV was achieved. Demonstration of the DNA nature of the IHHNV nucleic acid indicates that IHHNV is more closely related to the *Paroviridae* than to the *Picornaviridae*. However, the significance of the presence of ssRNA in our preparations along with larger amounts of ssDNA has not yet been elucidated. However, because a smear and not a band is equally in evidence using fully denaturing gels (containing methylmercuric hydroxide) and RNase-free extraction protocols, we do not believe that this ssRNA can be attributed to a contaminating picorna-like virus. It appears to be more probable that they are different size fragments of messenger RNA adsorbed or shared by the IHHNV particles.

Finally, the infectivity tests using semi-purified and purified virions demonstrate, by successfully reproducing the histopathological symptoms, mortalities and similarly sized particles, that the isolated virus is the causative agent of IHHN disease.

A report about isolation and characterization of IHHNV has been recently published (Lu et al., 1989). However, the reported results are directly contradictory to our findings. We believe that Lu et al. (1989) did not isolate IHHNV, but characterized a normal shrimp macromolecule. We base this opinion on the following: (i) Lu et al. (1989) report the ‘virus’ particles shown in Fig. 2 of their paper to be 19 nm in diameter yet, according to the magnification bars in Fig. 1, the ‘virus’ particles are closer to 12 nm in diameter; (ii) Lu et al. (1989) provide no evidence of empty capsids in Fig. 2 or elsewhere in the manuscript, this is noteworthy as they are found in most virus preparations; (iii) the shape of...
the particles in Fig. 2 is more star-like than icosahedral; (iv) the diameter (12 nm) and star-like morphology of the 'virus' particles are consistent with that of hexameric haemocyanin molecules (respiratory pigment in crustacea) as reported by van Bruggen (1986) and Bijlholt & van Bruggen (1986).

Concerning the presence of RNA in the preparations of Lu et al. (1989), it should be pointed out that haemocyanin is a glycoprotein which gives a positive periodic acid–Schiff (PAS) reaction and thus it may give a false positive reaction for RNA with the orcinol method. Only SDS–PAGE analyses of these isolates would aid the characterization of these 'virus' particles. Moreover, in contrast to our own results, the authors report 'inconsistent' attempts to infect shrimps with the 'CsCl-banded virus preparation' obtained.

In invertebrate virology the Paroviridae family, and particularly the genus Densovirus, is very well represented, especially in insects (Matthews, 1982) in which extensive studies have been done. Conversely, in marine invertebrates and more particularly in marine crustacea, small-sized non-enveloped viruses have been described only on the basis of TEM observations of infected tissues and/or negatively stained isolated particles, and these have been called picorna-like (Bonami, 1977, 1980; Johnson, 1978; Kuris et al., 1979; Foster et al., 1981; Lightner et al., 1983a) or parvo-like viruses (Lightner & Redman, 1985), depending on their location in the cell. Except for the PC84 virus of the crab Carcinus mediterraneus, which was demonstrated to be a DNA-containing virus (Mari & Bonami, 1988b), no other small DNA-containing viruses have been isolated, purified and characterized in the crustacea.

The findings of the study reported here indicate that IHHNV has cubic symmetry with an icosahedral shape, an average diameter of 22 nm in purified preparations and a density of 1.40 for whole virions and 1.28 to 1.30 for empty particles. The nucleic acid of IHHNV is ssDNA with an $M_r$ of approximately 1350K and a size of 4·1 kb as determined by length measurement of molecules. Its capsid is composed of four polypeptides with $M_r$s of 74K, 47K, 39K and 37·5K, respectively. These characteristics are consistent with those of the family Paroviridae (Matthews, 1982) and thus we have concluded that IHHNV is a member of this family of viruses.

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References


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