

Multiple infections of a new-type decapod hepanhamaparvovirus (DHPV) and *Enterocytozoon hepatopenaei* in Korea and DHPV infectivity in *Penaeus vannamei*

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ABSTRACT

Decapod hepanhamaparvovirus (DHPV) is a viral pathogen known to cause slow growth and reduced productivity in penaeid shrimp. In this study, a new type of suspected DHPV was detected in cultured Pacific white shrimp (*Penaeus vannamei*) collected from 14 farms in Korea, where growth retardation was observed. DHPV was detected in 98 of 113 collected shrimp (86.7%), and 26 of 30 collected pond water (86.7%). Overall, DHPV was detected in 14 farms (100%), and coinfection of DHPV and *Enterocytozoon hepatopenaei* (EHP) was observed in 10 out of 14 farms (71.4%). We sequenced the presumptive viral DNA fragment at the junction of the genes encoding the NS2 and NS1 proteins of DHPV, and the nucleotide and deduced amino acid sequences which showed less than 89% and 86% identity, respectively to those of other DHPVs available in GenBank. Similarly, comparisons of the sequenced VP region of the DHPV from *P. vannamei* with those of other DHPVs indicated that the nucleotide and deduced amino acid sequences showed less than 79% and 83% identity, respectively. Phylogenetic analyses based on comparisons of the two DHPV fragments indicated that the newly detected DHPV in *P. vannamei* can be clearly differentiated from the DHPVs currently available in GenBank. Using a laboratory bioassay, the potential risk of DHPV transmission was examined by feeding virus-infected shrimp tissue to healthy *P. vannamei*, and potential DHPV infection was confirmed within 7 days based on PCR analysis and histopathological examination. The emergence of a new type of DHPV and its potential coinfection with EHP would have a significant impact on the global shrimp industry, and consequently, the presence of this virus in cultured shrimp as well as in the rearing pond water should be closely monitored to control its potential spread.

1. Introduction

Although shrimp is among the most valuable aquaculture species cultivated worldwide, they are also susceptible to disease outbreaks that can result in substantial economic losses (Asche et al., 2021). The findings of studies reported to date have indicated that diseases of viral origin are a major factor contributing to the disruption of the shrimp aquaculture industry in many parts of the world (Flegel, 2019). At present, more than 22 viruses are known to infect shrimp (Walker and Winton, 2010), among which, 7 types have been confirmed to affect penaeid shrimp (Singaravel et al., 2021).

Parvoviruses that infect the hepatopancreas of penaeid shrimp have previously been described as hepatopancreatic parvoviruses (HPV) (Lightner, 1996), and are now classified in the family *Parvoviridae*, subfamily *Hamaparvovirinae* as members of a single species named decapod hepanhamaparvovirus 1 (DHPV) (Pénzes et al., 2020; Srisala et al., 2021). The range of known DHPV hosts includes at least 10 species of penaeid shrimp worldwide (Yan et al., 2010), among which, 8 full sequences derived from three penaeid shrimp species (*Penaeus monodon*, *P. chinensis*, and *P. merquensis*) are listed in GenBank as *Hepandensovirus* (Srisala et al., 2021).

DHPVs are small icosahedral, non-enveloped, linear negative single-

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stranded DNA viruses of between 4 and 6 kbp in size (Sukhumsirichart et al., 2006). These viruses infect the epithelial cells of the hepatopancreas and midgut of shrimp, with infected individuals showing non-specific gross signs, including an atrophied hepatopancreas, anorexia, retarded growth, and reduced preening activities (Lightner and Redman, 1985; Lightner et al., 1992; Madan et al., 2013). Although DHPV is not a cause of significant mortalities, infections are associated with reduced growth rates, with corresponding increases in feed conversion efficiency, potentially contributing to significant economic losses on farms (OIE, 2007).

Cultured shrimp can be infected with multiple viruses or different strains of the same virus, either simultaneously or sequentially, and multiple infections with several pathogens can cause substantially greater losses in shrimp farming than single infections (Walker and Winton, 2010). Several studies have reported significant increases in the cumulative mortality rate in co-infected groups compared with those singularly infected (Manivannan et al., 2002; Phuoc et al., 2008; Jang et al., 2014; Han et al., 2019), and there have been reports of coinfection of DHPV and *Enterocytozoon hepatopenaei* (EHP) in *P. vannamei*, and DHPV and monodon baculovirus (MBV) in *P. monodon* (Flegel et al., 1999; Chayaburakul et al., 2004; Singaravel et al., 2021).

In this study, we detected the emergence of a new-type DHPV in cultured Pacific white shrimp (*P. vannamei*) whose infection is characterized by retarded growth, and also observed the same virus to be widespread in Korean shrimp culture farms, with occasional detection of coinfections with EHP. Moreover, we confirmed the potential transmissibility and infectivity of this new-type DHPV in *P. vannamei* based on a laboratory bioassay. This discovery is of particular interest in at least three respects. First, to the best of our knowledge, there have been no previous reports on the simultaneous occurrence of major shrimp pathogens (DHPV and EHP) on Korean farms; second, we observed that the newly identified DHPV isolated from *P. vannamei* differs genetically from other DHPVs available in GenBank; and third, the new-type DHPV is highly infectious and contagious in shrimp.

2. Materials and methods

2.1. Sample collection

In 2021, a total of 113 specimens of cultured shrimp (*P. vannamei*) characterized by retarded growth were collected from 14 anonymous farms located in Chungcheongnam-do (Taejeon-gun, Seosan-si), Incheon (Ganghwa-gun), Jeollabuk-do (Gochang-gun, Gunsan-si), and Jeollanam-do (Sinan-gun, Hampyeong-gun), Korea. These specimens were transported on ice to the Laboratory of Aquatic Biomedicine, College of Veterinary Medicine, Kyungpook National University, Daegu, Korea, at which they were stored at -80°C until use for analysis.

In addition, in the aforementioned farms, pond water ($N = 30$), shrimp feces ($N = 4$), crab ($N = 9$, *Macrophthalmus japonicus*), barnacle ($N = 3$, *Chthamalus challengeri*), and commercial feed ($N = 8$) samples were collected. Detailed information on the collected samples is presented in Table 1.

2.2. DNA extraction

For DNA extraction, the different samples (shrimp, pond water, feces, crab, barnacle, and feed) were prepared as follows. The hepatopancreases (average 30 mg) of shrimp were pooled from 3 representative shrimp collected from each of the sampled rearing ponds, and DNA was extracted using a DNeasy Blood & Tissue kit (Qiagen Korea Ltd., Seoul, Korea) according to the manufacturer's instructions. Similarly, samples of pond water (300 μL) and feces (30 mg), commercial feed (30 mg), pooled crab hepatopancreases (average 30 mg, $N = 3$), pooled barnacle soft tissues (average 30 mg, $N = 3$) were used for DNA extraction.

2.3. Detection of DHPV and sequence comparison

For DHPV detection, PCR analysis was performed using the primers 1414F/1750R (Jeeva et al., 2014) (Table 2). Each PCR mixture was prepared in a final volume of 25 μL , which included 1 μL of extracted DNA, 1 μL (0.05 μM) of each of the two primers, 22 μL of DNase-free water, and AccuPower[®] PCR Premix (Bioneer, Daejeon, Korea). The PCR amplification consisted of an initial denaturation for 3 min at 95°C , followed by 35 cycles of 30 s at 95°C , 30 s at 57°C , and 60 s at 72°C , and a final extension for 10 min at 72°C . In addition, using the primer pair F1/R2 described by Tang et al. (2008), DNA extracted from a representative DHPV-infected shrimp (Sample No. 21-044B1) was used to amplify sequences targeting the 5' end of the VP region of DHPV (Table 2).

The positive amplicons of DHPV thus obtained were sequenced commercially by Bioneer Inc. (Daejeon, Korea), and representative nucleotide and deduced amino acid sequences were compared directly with those of other DHPVs hosted on GenBank. For phylogenetic analyses, the DHPV datasets of nucleotide and amino acid sequences from 9 other complete or almost complete DHPV genomes of isolates from different geographical regions and host species and newly obtained sequences in this study were subjected to maximum-likelihood estimations with 1000 bootstrap replications using MEGAX (ver. 10.0) (Kumar et al., 2018).

2.4. Detection of EHP

For EHP detection, PCR analysis was performed using the spore wall protein (SWP) primers (Jaroenlak et al., 2016) (Table 2). The 25 μL reaction mixture contained 1 μL of the extracted DNA, 1 μL (0.05 μM) of each of the two primers, and AccuPower[®] PCR Premix (Bioneer, Daejeon, Korea). For the 1st-step PCR amplification was performed using primers SWP-1F and SWP-1R. For the 2nd-step PCR, as a template, we used 1 μL of the final reaction mixture obtained from the 1st-step, which was amplified using primers SWP-2F and SWP-2R. Some of the samples used had previously been confirmed as EHP based on PCR analysis (Kim et al., 2022).

2.5. DHPV infectivity test in *P. vannamei*

For the infectivity test, healthy Pacific white shrimp (approximately 0.8 g) purchased from a local shrimp farm (Jeju, Korea) was transferred to the Laboratory of Aquatic Biomedicine, College of Veterinary Medicine, Kyungpook National University (Daegu, Korea). The shrimp ($N = 60$) were divided into two groups (control and challenge), each comprising 30 shrimp. The shrimp in each group were further subdivided to obtain triplicates batches, each comprising 10 shrimp, which were placed in separate 27 L glass tanks maintained at a salinity of 25 ppt and a temperature of approximately $26\text{--}28^{\circ}\text{C}$. prior to conducting the infectivity test, representative shrimp ($N = 5$) were randomly selected for the confirmation of negative DHPV status based on PCR analysis (Jeeva et al., 2014).

For infection, whole body of the DHPV-infected shrimp ($N = 8$, sample no. 21-084B3) (4.52×10^7 DHPV copies/ μL) was prepared by chopping without the cuticle, and frozen at -80°C until use. DHPV tissue (0.4 g) was mixed with commercial feed (0.4 g), which was fed to shrimp in challenge tanks once daily for 3 days (10% of total body weight). Experimental shrimp in control tanks were fed with comparable amounts of healthy shrimp muscle tissue mixed in a commercial feed. During the infectivity test, except for the period of supply of DHPV uninfected or infected tissue, shrimp were fed a commercial feed twice a day (9:00 and 18:00 h). Uneaten feed and waste were removed daily by siphoning, and survival was recorded daily (termination day, Day 16).

Live shrimp from control tanks ($N = 3$, Day 7), and dead shrimp ($N = 6$, Day 7 to Day 15), live shrimp from challenge tanks ($N = 3$, Day 7) were collected to confirm DHPV infection based on PCR analysis (Jeeva

Table 1

Sample information and PCR results for the decapod hepanhamaparvovirus (DHPV) and *Enterocytozoon hepatopenaei* (EHP) were detected in samples collected from 14 shrimp farms in Korea.

Aquafarm	Sample ID	Year /Month	Province/area	Sample types (number / length, weight)	PCR detection	
					DHPV	EHP
Farm 1	21-022A1	2021/04	Chungcheongnam-do/Taeon-gun	Pond water (N = 1)	+	–
	21-022A2	2021/04		Pond water (N = 1)	+	–
	21-022B1*	2021/04		Shrimp (N = 3)	+	+
	21-022B2*	2021/04		Shrimp (N = 3)	+	–
	21-023F1	2021/04		Feces (N = 2)	+	–
	21-055A	2021/07		Pond water (N = 1)	+	+
	21-055B1	2021/07		Shrimp (N = 3 / 10.8 cm, 10.5 g)	+	+
	21-055B2	2021/07		Shrimp (N = 3 / 12.1 cm, 13.7 g)	+	+
Farm 2	21-042A	2021/06	Chungcheongnam-do/Taeon-gun	Pond water (N = 1)	+	–
	21-042B	2021/06		Shrimp (N = 3 / 8.2 cm, 5.5 g)	+	–
Farm 3	21-015A	2021/04	Incheon/Ganghwa-gun	Pond water (N = 1)	–	–
	21-015B	2021/04		Shrimp (N = 3 / 1.0 cm, 0.01 g)	–	–
	21-015C	2021/04		Feed (N = 1)	–	–
	21-044A1	2021/06		Pond water (N = 1)	+	+
	21-044A2	2021/06		Pond water (N = 1)	+	+
	21-044B1*	2021/06		Shrimp (N = 3 / 9.5 cm, 6.9 g)	+	+
	21-044B2*	2021/06		Shrimp (N = 3 / 8.5 cm, 5.2 g)	+	+
	21-044C	2021/06		Feed (N = 1)	+	–
Farm 4	21-046A	2021/07	Incheon/Ganghwa-gun	Pond water (N = 1)	+	+
	21-046B	2021/07		Shrimp (N = 3 / 7.3 cm, 3.4 g)	+	–
	21-046C	2021/07		Feed (N = 1)	+	–
Farm 5	21-051A1	2021/07	Jeollabuk-do/Gochang-gun	Pond water (N = 1)	+	+
	21-051A2	2021/07		Pond water (N = 1)	+	–
Farm 6	21-051B	2021/07	Jeollabuk-do/Gunsan-si	Shrimp (N = 3 / 11.3 cm, 11.7 g)	+	–
	21-053A	2021/07		Pond water (N = 1)	+	–
	21-053B	2021/07		Shrimp (N = 3 / 10.0 cm, 8.1 g)	+	–
	21-053C	2021/07		Feed (N = 1)	+	–
Farm 7	21-058A	2021/08	Jeollanam-do/Sinan-gun	Pond water (N = 1)	+	–
	21-058B1	2021/08		Shrimp (N = 3 / 9.0 cm, 6.0 g)	+	–
	21-058B2	2021/08		Shrimp (N = 3 / 9.4 cm, 7.3 g)	+	–
Farm 8	21-002A	2021/03	Incheon/Ganghwa-gun	Pond water (N = 1)	–	–
	21-002B1	2021/03		Shrimp (N = 3 / 3.4 cm, 0.5 g)	–	–
	21-002B2	2021/03		Shrimp (N = 3 / 4.0 cm, 0.7 g)	–	–
	21-005A4	2021/04		Pond water (N = 1)	–	–
	21-005A5	2021/04		Pond water (N = 1)	–	–
	21-005B1	2021/04		Shrimp (N = 3 / 4.2 cm, 0.7 g)	–	–
	21-005B2	2021/04		Shrimp (N = 3 / 5.0 cm, 1.3 g)	–	–
	21-060A	2021/08		Pond water (N = 1)	+	+
	21-060B1	2021/08		Shrimp (N = 3 / 6.0 cm, 2.0 g)	+	+
	21-060B2	2021/08		Shrimp (N = 3 / 11.0 cm, 10.9 g)	+	–
	21-098I1	2021/11		Barnacle (N = 3)	+	–
Farm 9	21-007C	2021/04	Jeollanam-do/Sinan-gun	Feed (N = 1)	+	–
	21-038B	2021/05		Shrimp (N = 3 / 7.0 cm, 3.1 g)	+	–
	21-063A1	2021/08		Pond water (N = 1)	+	–
	21-063A2	2021/08		Pond water (N = 1)	+	+
	21-063A3	2021/08		Pond water (N = 1)	+	–
	21-063B1	2021/08		Shrimp (N = 3 / 8.1 cm, 4.5 g)	+	+
	21-063B2	2021/08		Shrimp (N = 3 / 14.3 cm, 24.2 g)	+	–
	21-063C	2021/08		Feed (N = 1)	+	–
Farm 10	21-028A3*	2021/04	Chungcheongnam-do/Seosan-si	Pond water (N = 1)	+	–
	21-028B1*	2021/04		Shrimp (N = 3 / 1.8 cm, 0.04 g)	+	–
	21-028C1	2021/04		Feed (N = 1)	+	–
	21-064A1	2021/08		Pond water (N = 1)	+	+
	21-064A2	2021/08		Pond water (N = 1)	+	–
	21-064A3	2021/08		Pond water (N = 1)	+	–
	21-064A4	2021/08		Pond water (N = 1)	+	–
	21-064A5	2021/08		Pond water (N = 1)	+	–
	21-064A6	2021/08		Pond water (N = 1)	+	–
	21-064B1	2021/08		Shrimp (N = 3 / 9.5 cm, 7.1 g)	+	–
	21-064B2	2021/08		Shrimp (N = 3 / 9.0 cm, 6.0 g)	+	+
	21-064B3	2021/08		Shrimp (N = 3 / 8.4 cm, 5.0 g)	+	+
	21-064B4	2021/08		Shrimp (N = 3 / 8.3 cm, 4.8 g)	+	+
	21-084B3	2021/09		Shrimp (N = 11 / 10.4 cm, 9.6 g)	+	+
	21-073B	2021/08		Shrimp (N = 3 / 9.12 cm, 6.26 g)	+	+
Farm 11	21-077A1	2021/08	Chungcheongnam-do/Taeon-gun	Pond water (N = 1)	+	–
Farm 12	21-077A2	2021/08		Pond water (N = 1)	+	–
	21-077B1	2021/08		Shrimp (N = 3 / 9.1 cm, 6.8 g)	+	–
	21-077B2	2021/08		Shrimp (N = 3 / 6.3 cm, 2.3 g)	+	–
	21-077C	2021/08		Feed (N = 1)	+	–
	21-077F1	2021/08		Feces (N = 1)	+	–
	21-077F2	2021/08	Jeollanam-do/hampyeong-gun	Feces (N = 1)	+	–
Farm 13	21-078A1	2021/08		Pond water (N = 1)	+	–

(continued on next page)

Table 1 (continued)

Aquafarm	Sample ID	Year /Month	Province/area	Sample types (number / length, weight)	PCR detection	
					DHPV	EHP
Farm 14	21-078A2	2021/08	Chungcheongnam-do/Taejeon-gun	Pond water (N = 1)	+	–
	21-078B1	2021/08		Shrimp (N = 3 / 7.2 cm, 3.1 g)	+	–
	21-078B2	2021/08		Shrimp (N = 3 / 4.5 cm, 1.0 g)	+	+
	21-078B3	2021/08		Shrimp (N = 3 / 8.7 cm, 5.8 g)	+	–
	21-080	2021/09		Crab (N = 3)	+	–
	21-086	2021/09		Crab (N = 3)	+	–
	21-087G	2021/09		Crab (N = 3)	+	–
	21-090B	2021/09		Shrimp (N = 3 / 10.0 cm, 8.1 g)	+	+

* These samples were used in previous studies (Kim et al., 2022).

Table 2

The sequences of the primers used for PCR amplification of the decapod hepanhamaparovirus (DHPV) and *Enterocytozoon hepatopenaei* (EHP) in this study.

Primer	Sequence 5' to 3'	Amplicon size (bp)	Description	Reference
1414F	GTTAGGAATGGAAGATGTGT	350 (approx.)	DHPV NS2 and NS1 protein genes detection	Jeeva et al. (2014)
1750R F1	CTCCAATAAAATGTGTAATGAGG TGAGACAGGGCCATATGCTA	750 (approx.)	DHPV VP region detection	Tang et al. (2008)
R2 SWP-1F	TTGTCATGTCTGAATCCTTGG TTGCAGAGTGTGTTAAGGGTTT	514	EHP spore wall protein gene detection	Jaroenlak et al. (2016)
SWP-1R SWP-2F SWP-2R	CACGATGTGTCTTTCGAATTTTC TTGGCGGCACAATTCTCAAACA GCTGTTTGTCTCCAACGTATTGA	148		

et al., 2014). Furthermore, live shrimp were also collected from control (N = 3, Day 7 and Day 14) and challenge (N = 3, Day 7 and Day 14) tanks, which were fixed in Davison's fixative (Moore and Barr, 1954). The hepatopancreas tissues of these specimens were subsequently deparaffinized, rehydrated in an alcohol series, and then stained with hematoxylin and eosin.

3. Results

3.1. Clinical signs of shrimp growth

Diseased shrimp collected from all farms were observed to have whitish atrophied hepatopancreases, and shrimp from Farms 6 and 7 were visually smaller (total length: 9–10 cm; average weight: 6–8 g) than healthy shrimp obtained from non-affected farms (total length: 10.5–12.5 cm; weight: 10–15 g) 3 months after stocking. Differences were even more pronounced among shrimp coinfecting with DHPV and EHP from Farms 8 and 10; these shrimp were characterized by severe growth retardation (total length: 6–8 cm; average weight: 2–5 g).

3.2. DHPV and EHP detection by PCR analysis

In addition to shrimp, PCR analyses were also performed to evaluate the presence of DHPV and EHP in a range of other samples collected from aquaculture farms (pond water, feces, crabs, barnacles, and feed).

Among the collected shrimp (N = 113), DHPV was detected in 98 samples (86.7%), and both DHPV and EHP were detected in 50 samples (44.2%). Among the collected pond water (N = 30), DHPV was detected in 26 samples (86.7%), and both DHPV and EHP were detected in 8 samples (26.7%). Additionally, DHPV was detected from feces (N = 4), crab (N = 9), barnacle (N = 3) and feed (N = 7) around shrimp farms affected by DHPV, but EHP was not detected from those samples. Overall, DHPV was detected in 14 farms (100%), and coinfection of DHPV and EHP was observed in 10 out of 14 farms (71%) (Table 1).

3.3. DHPV sequence analysis

We subsequently analyzed sequences of the DHPV detected in

P. vannamei. Among the assessed samples, the nucleotide sequences obtained from DHPV-positive *P. vannamei* using 1414F/1750R PCR primers, targeting the presumptive viral sequence at the junction of the genes encoding NS2 and NS1 protein, were observed to be almost identical. Therefore, representative nucleotide and deduced amino acid sequences obtained from DHPV-positive *P. vannamei* (Sample Nos. 21-044B1, 21-077B1, 21-078B2, and 21-084B3) were analyzed by direct sequence comparison and phylogenetic analysis. The results revealed that the presumptive viral sequence from *P. vannamei* was similar to that of other DHPVs available in GenBank at the nucleotide (86.4–89.3%) and deduced amino acid levels (82.7–86.3%). Moreover, phylogenetic analysis based on this sequence indicated that the viral fragment could be clearly differentiated from other currently available DHPV genomes in GenBank (Fig. 1 and Supplementary data 1).

We also successfully amplified a partial sequence of the VP region of the DHPV infecting *P. vannamei* using the primer pair F1/R2 previously described by Tang et al. (2008) and preliminarily analyzed the derived nucleotide and deduced amino acid sequences obtained from the representative DHPV-positive *P. vannamei* (Sample Nos. 21-044B1, 21-077B1, 21-078B2, and 21-084B3) by direct sequence comparisons with those of other DHPVs available in GenBank. This revealed that the partial VP region of the DHPV infecting *P. vannamei* is similar to that of other DHPVs at the nucleotide (70.8–78.6%) and deduced amino acid levels (64.1–82.6%); phylogenetic analysis indicated that the VP region of the DHPV infecting *P. vannamei* can be clearly differentiated from that of other currently available DHPV sequences (or genomes) hosted in GenBank (Fig. 2 and Supplementary data 1).

3.4. Confirmation of the infectivity of DHPV in *P. vannamei*

A laboratory shrimp assay was performed to determine the infectivity of DHPV in *P. vannamei*. During the initial stages of the infectivity test, both control and challenge tanks shrimp showed normal appearance and feeding behavior.

From day 7 after feeding with the DHPV-infected shrimp tissue, mortality was observed among shrimp of challenge tanks, whereas all control tanks individuals appeared healthy. At the time of assay termination (Day 16), the survival rates of shrimp from control and challenge

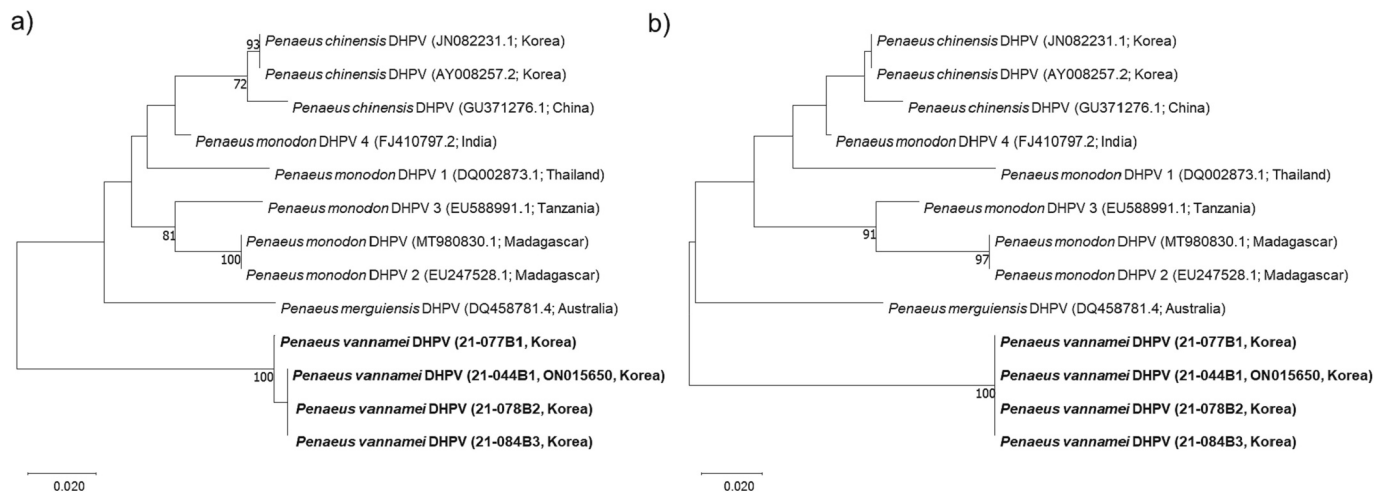


Fig. 1. The maximum-likelihood phylogeny of the decapod hepanhamaparvovirus (DHPV) based on the sequences of 9 complete or almost complete genomes of DHPV isolated from different geographical regions and host species in the GenBank database. These include the sequences of the presumptive viral DNA fragment (a) and the deduced amino acid sequences (b) at the junction of the genes encoding NS2 and NS1 proteins of DHPV detected in *Penaeus vannamei* (sample Nos. 21-044B1, 21-077B1, 21-078B2, and 21-084B3) cultured in Korea. Bootstrap values were calculated from 1000 replicates. The scale bars (0.020 each) represent 1 substitution per 100 nucleotides and the number of amino acid substitutions per site, respectively.

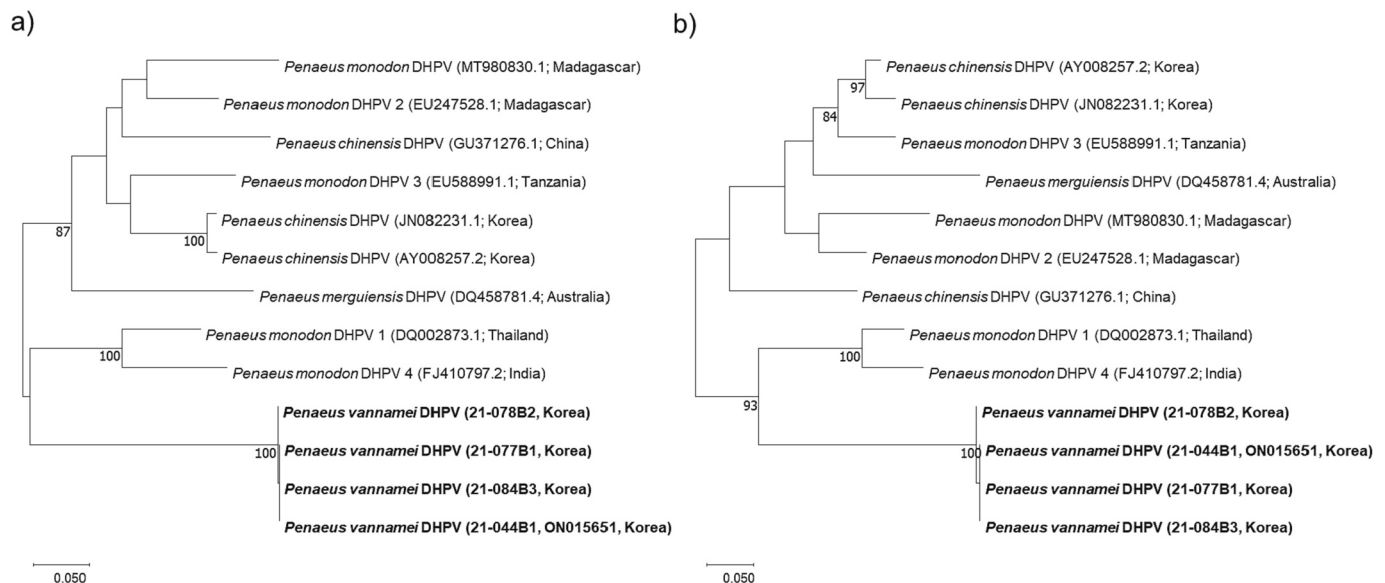


Fig. 2. The maximum-likelihood phylogeny of the decapod hepanhamaparvovirus (DHPV) based on the sequences of 9 complete or almost complete genomes of DHPV isolated from different geographical regions and host species in the GenBank database. These include the viral DNA fragment (a) and the deduced amino acid sequences (b) of the VP gene of DHPV detected in *Penaeus vannamei* (sample Nos. 21-044B1, 21-077B1, 21-078B2, and 21-084B3) cultured in Korea. Bootstrap values were calculated from 1000 replicates. The scale bars (0.050 each) represent 1 substitution per 100 nucleotides and the number of amino acid substitutions per site, respectively.

tanks were 100% and 80%, respectively (Fig. 3).

PCR revealed the presence of DHPV in all deceased shrimp ($N = 6$), as well as in 3 representative surviving shrimp from challenge tanks. However, DHPV was not detected in the representative survival shrimp ($N = 3$) from control tanks by PCR analysis (data not shown).

Histopathological examination confirmed the presence of basophilic intranuclear inclusion bodies (typical evidence of DHPV infection) in challenged shrimp from the challenge tanks (OIE, 2007; Safeena et al., 2012) (Fig. 4). In contrast, in the hepatopancreas of control tanks shrimp, we observed normal tubule structures and epithelial cells, including large amounts of lipid droplets (R-cells) and secretory vacuoles (B-cells) (Fig. 4).

3.5. Nucleotide sequence deposition

Representative DHPV sequences obtained from *P. vannamei* (sample no. 21-044B1) cultured in Korea have been deposited in GenBank under accession Nos. ON015650 (amplified with primer pair 1414F/1750R) and ON015651 (amplified with primer pair F1/R2), respectively.

4. Discussion

Although *P. vannamei* aquaculture is increasing on a global scale, in recent years, there has been a concomitant increase in severe growth retardation in these shrimp (Kooloth Valappil et al., 2021). EHP, a microsporidian responsible for causing hepatopancreatic dysfunction that mainly infects penaeid shrimp including *P. vannamei* is a causal

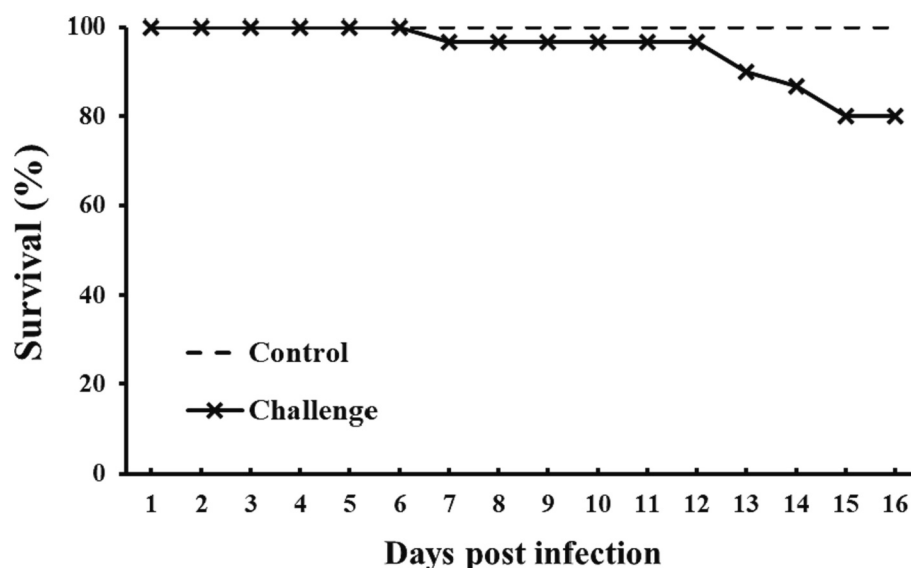


Fig. 3. Survival of the Pacific white shrimp (*Penaeus vannamei*) infected with the decapod hepanhamaparvovirus (DHPV). Shrimp were initially fed DHPV-infected shrimp for 3 days and were thereafter fed twice daily with a commercial feed during the challenge period. Control tanks fed with healthy shrimp tissue; Challenge tanks fed with DHPV-infected shrimp.

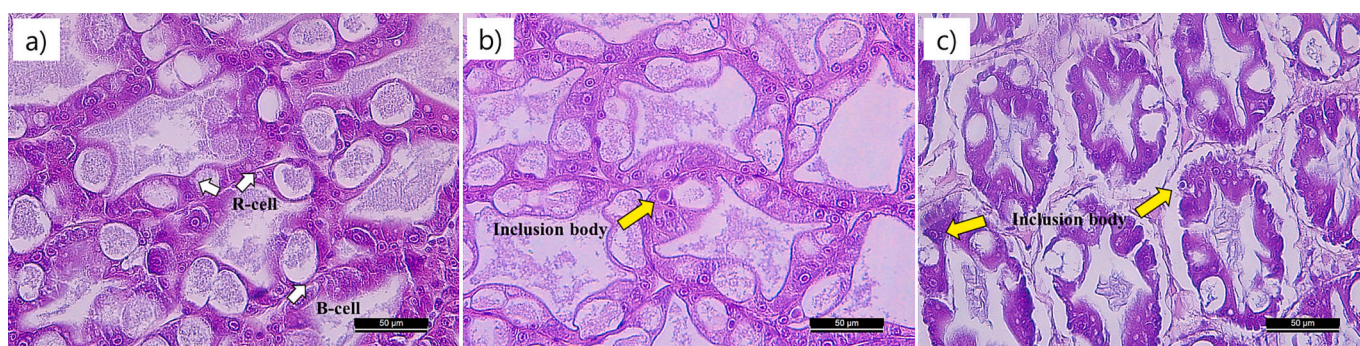


Fig. 4. Histopathological features (hematoxylin and eosin staining) of the hepatopancreas of representative shrimp (*Penaeus vannamei*) collected on 7 and 14 days after feeding with healthy shrimp tissue (Control tank) or decapod hepanhamaparvovirus (DHPV)-infected shrimp tissue (Challenge tank). (a) non-infected tissue $\times 400$ (control tank, Day 7), (b) DHPV-infected tissue $\times 400$ (challenge tank, Day 7), (c) DHPV-infected tissue $\times 400$ (challenge tank, Day 14).

organism associated with disease outbreaks in farmed shrimp; the affected individuals are characterized by a soft shell, lethargy, reduced feed intake, and an empty midgut (Tang et al., 2016; Caro et al., 2020). EHP infection is often associated with the co-occurrence of other infectious diseases, including acute hepatopancreatic necrosis disease and septic hepatopancreatic necrosis (Aranguren et al., 2017). DHPV, a new emerging viral pathogen detected in farmed *P. vannamei*, has to date primarily been reported in *P. monodon* (Sukhumsirichart et al., 2006) and *P. chinensis* (Jeeva et al., 2012), although severe growth retardation has recently (between 2018 and 2019) been detected in *P. vannamei* shrimp farms in India by Singaravel et al. (2021), who confirmed multiple infections with DHPV and EHP based on histopathological examination and transmission electron microscopy. These authors also reported that DHPV-infected *P. vannamei* is characterized by non-specific signs, including atrophied hepatopancreases, anorexia, retarded growth, and reduced preening activities, similar to those associated with EHP infection (Singaravel et al., 2021).

Our findings in the present study indicate that DHPV has already been extensively transmitted among Korean shrimp farms, as evidenced the detection of this virus in pond water, shrimp feces, feed, crab, and barnacle collected from all 14 shrimp farms surveyed in this study. This suggests that untreated water as well as other crustacean species should be monitored for DHPV management. Dhar et al. (2014) demonstrated

that DHPV can be transmitted via untreated pond water and cannibalization of moribund or dead shrimp.

Interestingly, the newly detected DHPV from *P. vannamei* in the present study shows relatively low sequence identity with other DHPVs deposited in GenBank. Direct comparisons of the viral nucleotide and deduced amino acid sequences revealed that the presumptive viral DNA fragments obtained in this study were notably dissimilar to those of previously sequenced DHPVs and that the VP region is more variable than the sequence coinciding with the junction of the genes encoding NS2 and NS1 proteins of this virus. Moreover, phylogenetic analyses based on these two DHPV fragments indicated that the DHPV newly detected in *P. vannamei* is clearly different from other currently available DHPV genomes. Based on these observations, we propose that the virus detected in *P. vannamei* cultured on Korean shrimp farms and characterized by retardant growth could be classified as a new type of DHPV. Further studies aimed at determining and analyzing the complete genome of this new-type DHPV are currently in progress.

In addition to the molecular characterization of this DHPV, we also examined the infectivity of the virus in *P. vannamei* using a shrimp laboratory bioassay. After feeding DHPV-infected shrimp tissue, infected shrimp showed mortality of only 20% during the bioassay period. However, DHPV infection was confirmed in shrimp within 7 days, by PCR and histopathology examination. In many countries, slow growth is

considered among the most problematic production-limiting conditions affecting shrimp farming, although, in this respect, the detrimental effects of DHPV on *P. vannamei* have been largely overshadowed by the effects of EHP infection. Previously, Tang et al. (2016) reported that EHP was detected from day 15 after feeding the shrimp with EHP-infected tissue (although EHP was not detected in samples collected on days 5 and 10), and notably did not cause any mortality during the laboratory bioassay period. The results obtained in the present study indicate that DHPV is a highly contagious pathogen in *P. vannamei*, and accordingly serve to emphasize that rapid and accurate diagnosis is necessary to manage the adverse effects associated with DHPV infection in shrimp farms. Thus, regular shrimp disease surveillance is recommended for the early detection of infection, along with the monitoring of the disease status and initiation of immediate action for controlling this and other pathogens (Babu et al., 2021).

DHPV has recently been removed from the World Organization for Animal Health (OIE) list of reportable pathogens, as this virus is no longer considered to be associated with any significant negative economic repercussions in the aquaculture industry (Thitamadee et al., 2016). However, given that emergence of a new type of DHPV and its possible coinfection with EHP may have a significant impact on the global shrimp industry, thus there is a need to develop advanced diagnostic methods and management strategies for this viral pathogen. Adding the testing protocol of a new virus to the routine basis causes a significant increase in the production cost since it requires time and labor. However, to prevent further economic damage due to reduced production, measures for controlling and preventing DHPV infection should include the screening of post-larval stages for detecting aggressive vertical transmission, the establishment of biosecurity measures, such as emergency harvest, and appropriate quarantine procedures to curtail and prevent the horizontal spread of this virus.

CRedit authorship contribution statement

Chorong Lee: Writing – original draft. **Hye Jin Jeon:** Formal analysis. **Bumkeun Kim:** Formal analysis. **Seong-Kyoon Choi:** Formal analysis. **Ji Hyung Kim:** Supervision. **Jee Eun Han:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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