Vol. 129: 183–191, 2018
https://doi.org/10.3354/dao03241

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published August 14

Presence of *Penaeus monodon* densovirus in the ovary of chronically infected *P. monodon* subadults

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ABSTRACT: Shrimp infected with *Penaeus monodon* densovirus (*Pmo*DNV) usually display no specific gross signs, but heavy infections can kill postlarvae and retard juvenile growth. In the present study, samples of hepatopancreas, feces, gonads and hemolymph were isolated from male and female *P. monodon* subadults chronically infected by *Pmo*DNV. Each sample of hepatopancreas and gonad was divided into 2 parts: one for *Pmo*DNV detection by polymerase chain reaction (PCR), and the other for routine histology and immunohistochemistry. The frequency of positive findings via PCR assays was 92% in the hepatopancreas, 57% in feces, 50% in ovary, 35% in hemolymph and 0% in the testis. Using the densitometric value (DV) of the specific band for *Pmo*DNV relative to that of the β-actin gene as an index of the viral load in the samples, no significant differences were observed among sample types and sexes. Hematoxylin-eosin staining of infected hepatopancreas revealed typical *Pmo*DNV inclusions in the nuclei of infected cells. The ovaries with high DV (>1) contained various types of inclusions along the row of the follicular cells or possibly in the connective tissue cells surrounding the oocytes. Using immunohistochemistry with specific probes to detect *Pmo*DNV proteins, a positive reaction was observed in viral inclusions found in infected hepatopancreas and in ovaries with high DV, specifically in the ovarian capsule, hemolymph, oocytes and nuclear inclusions. These results suggest that the localization of *Pmo*DNV in *P. monodon* is not confined to the hepatopancreas, but rather that the virus can also occur in the ovary; hence, trans-ovarian, vertical transmission of the virus is highly possible.

KEY WORDS: *Pmo*DNV · Shrimp virus · Hepatopancreas · Ovary · Hemolymph · Aquaculture

INTRODUCTION

Penaeus monodon densovirus (*Pmo*DNV), previously known as hepatopancreatic parvovirus (HPV), is an icosahedral, single-stranded linear DNA virus, with an approximate diameter of 22 nm (Bonami et al. 1995, Sukhumsirichart et al. 2006). The virus infects several types of penaeid shrimp, including *Fenneropenaeus chinensis* (Bonami et al. 1995), *Marsupenaeus japonicus* (Spann et al. 1997), *Penaeus monodon* (Sukhumsirichart et al. 1999), *P. semisulcatus* (Manjanaik et al. 2005, Tang et al. 2008), *F. merguiensis* (La Fauce et al. 2007) and several other shrimp species both in cultured and wild environ-

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ments (Safeena et al. 2012). Shrimp infected with *Pmo*DNV usually display no specific gross signs, but heavy infections can kill postlarvae (PL) (Spann et al. 1997) and retard the growth rate of juveniles (Flegel et al. 1999, Owens et al. 2011). The major target organ of *Pmo*DNV is the hepatopancreas, and the infection can be detected by simple smearing and staining of fresh hepatopancreatic tissue and observing under light microscopy, or by routine hematoxylin-eosin (H&E) staining. In either technique, the infected hepatopancreatic cells show single, large, basophilic intranuclear inclusions (Lightner 1996). A simple polymerase chain reaction (PCR) assay to detect *Pmo*DNV remains the routine method for its diagnosis (Sukhumsirichart et al. 1999, Pantoja & Lightner 2000, Phromjai et al. 2002, Umesha et al. 2003). Currently, samples used for diagnostic viral detection are the hepatopancreas, which is destructive, or feces, to avoid sacrificing the shrimp. The virus is released from infected hepatopancreatic cells into the lumen, and thereafter mixed with the gut content and expelled with the feces (Pantoja & Lightner 2000).

The horizontal mode of transmission of *Pmo*DNV in *P. monodon* is through oral feeding or co-habitation of the infected shrimp with non-infected ones (Catap & Travina 2005), or by feeding normal *P. monodon* PL with *Pmo*DNV-infected *Artemia* (Sivakumar et al. 2009). Empirical evidence suggests that vertical transmission, from parents to offspring, may also occur as *Pmo*DNV could be detected in PL from a hatchery (Manivannan et al. 2002, Umesha et al. 2003). However, it is possible that larvae and PL may ingest *Pmo*DNV-contaminated feces of the infected broodstock being kept in the same water environment. It has not been proven yet whether the virus can infect or interact with oocytes or sperm, thereby contributing to trans-ovarian transmission.

In this study, we investigated whether *Pmo*DNV is present in the hemolymph and gonads of *P. monodon* subadults, which would both indicate that the virus can infect tissues other than the hepatopancreas and that it can be transmitted vertically. In addition, the correlation between the presence of the virus in the feces and the hepatopancreas, as detected by PCR, was determined.

MATERIALS AND METHODS

Animals

Penaeus monodon subadults (10 mo old), consisting of 13 females (98.2 ± 6.6 g SD) and 12 males (84.1 ± 3.5 g), were randomly sampled from shrimp reared in 100 t, round canvas tanks. The average body weight (BW) of the female samples was significantly (*t*-test, $p < 0.05$) higher than that of the males. Those shrimp in which *Pmo*DNV infection was detected in the PL stage were reared to subadults in the tank for up to 10 mo and thus designated to be chronically infected with *Pmo*DNV. They were fed with commercial pellets and reared in 20 to 30 ppt seawater with adequate aeration. The rearing water was exchanged at 50–80% whenever water quality was not optimal, i.e. when total ammonia nitrogen or total nitrite exceeded 0.5 ppm. The water alkalinity was maintained at 120–150 ppm, dissolved oxygen above 4 ppm and pH at 8.0–9.0 (Aquacare 2000.4 PARA Test).

Fecal samples (10–20 mg) were collected in the morning from individual shrimp (total 23 shrimp) kept overnight in a 35 × 48 × 30 cm styrofoam box. Hemolymph (100–200 µl) was then withdrawn from the ventral hemolymph sinus into an anticoagulating shrimp salt solution (Vargas-Albores et al. 1993), and the hepatopancreas and gonads (ovary or testis) were dissected and isolated. Individual hepatopancreas and gonad samples were divided into 2 pieces, about 10–20 mg each, with one placed into Trizol solution (1 ml) for PCR determination, and the other in Davidson's fixative (1 ml) for histology and immunohistochemistry. The feces and hemolymph samples were individually placed in Trizol solution (1 ml) for PCR processing.

PCR assays

All samples were individually homogenized and incubated in Trizol reagent (1 ml) for 5–10 min at room temperature followed by DNA extraction as described by Chomczynski (1993). Briefly, 100 µl of chloroform were added into the samples. The mixture was incubated for 2–3 min at room temperature and centrifuged at 12 000 × *g* for 15 min. Following centrifugation, 3 phases appeared: a lower red, phenol–chloroform phase, a white interphase (debris or flocculent material containing DNA, lipids and carbohydrates) and a colorless, upper aqueous phase containing RNA. The intermediate phase was collected and washed with 100% ethanol and dissolved in 20 µl diethylpyrocarbonate (DEPC)-treated water. The concentration of the extracted DNA was measured using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific). The DNA was amplified by specific primers for *Pmo*DNV: vp-s (5′-AAT CTG CAG GGT ACG

GAA AAA AC-3′) and vp-a (5′-TGT GGA ACC ATC TCA AAT GCC-3′) (Chimwai et al. 2016). The primers for the shrimp genomic β-actin gene were Pm_actin-F (5′-GAC TCG TAC GTC GGG CGA CGA GG-3′) and Pm_actin-R (5′-AGC AGC GGT GGT CAT CAC CTG CTC-3′) (Chimwai et al. 2016). PCR reactions were performed in a 25 µl of total reaction mixture containing 1× PCR buffer, 10 mM dNTP mixture (Promega), 50 mM $MgCl_2$, 10 µM each primer, 1 µl of 200 ng Template DNA, and 1 U of *Taq* DNA polymerase (Thermo Scientific). The PCR program consisted of initial denaturation at 94°C for 2 min followed by 30 cycles of denaturation at 94°C for 10 s, annealing with primers at 55°C for 30 s and extension at 72°C for 30 s. The amplified products were visualized by agarose gel electrophoresis. The gel was stained with ethidium bromide for 5 min and de-stained with distilled water for 15 min before observation under UV light. The band intensity was expressed relative to staining of the band for the β-actin gene as an internal control, and densitometric analyses were performed using the Image J Software Package.

Histology and immunohistochemistry

The hepatopancreas and gonad tissue samples were fixed with Davidson's fixative and processed for paraffin embedding. Tissue blocks were sectioned at 5 µm thickness, processed for H&E staining, mounted with permount, dried, and then observed under a light microscope. Immunohistochemistry was performed in selected tissue sections of hepatopancreas and ovaries with strong PCR-positive signal for *Pmo*DNV (shrimp nos. 3 and 4). To enhance the immune reactivity, the slides were immersed in 0.01 M sodium citrate buffer, pH 6.0, and antigen retrieval was performed in a microwave oven (850 W) for 20 min. Blocking of endogenous peroxidase activity was performed by immersing the sections in 0.6% H_2O_2 in methanol for 15 min. The sections were incubated in blocking buffer (2% bovine serum albumin, 10% normal goat serum) for 1 h at room temperature, then incubated with primary antibodies, HPV 16-9C and HPV 17-iG (Rukpratanporn et al. 2005), for 1 h at 37°C. After washing, the slides were incubated with horseradish peroxidase-conjugated goat anti-mouse antibody. Immunoreactivity was visualized using Vector Novared. The sections were counter-stained with hematoxylin for 1 min, mounted with permount, and observed under a light microscope.

Statistical analysis

The data were expressed as arithmetic mean ± SEM and analysed by 1-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test, as well as by Student's *t*- and chi squared (χ^2) tests. Differences among or between samples were considered statistically significant at $p < 0.05$.

RESULTS

From the PCR results, the densitometric values (DVs) of the *Pmo*DNV amplicon (400 bp) relative to that of the β-actin gene (550 bp) were used as an indicator of the viral load (Table 1). Viral loads varied from 0 (negative) to 4.46. Considering any sample with DV >0 to be a positive case of viral infection, the frequency of the presence of the virus in the tissues studied was as follows: hepatopancreas, 23/25 (92%); feces, 13/23 (56.5%); ovaries, 6/12 (50%), hemolymph, 6/17 (35.3%); and testes, 0/11 (0%). Comparing between sexes, the frequencies of the positive cases of viral infection in the hepatopancreas, feces, ovary and hemolymph did not differ statistically (chi-squared). The average DV in the tissues, which was regarded as the viral load, was in the order of hepatopancreas > feces > ovary > hemolymph. However, due to the large variations of DV between the samples within each type of tissue, there was no statistical difference between the mean DVs among the tissues, except in the gonads where the virus was present only in the ovary, but not in the testis.

The relationship between DV in the hepatopancreas and the fecal samples varied between individual shrimp. However, 4 shrimp (nos. 64, 66, 76 and 82) had high and moderate DVs in the hepatopancreas but had low or 0 DVs in the feces, while 4 shrimp (nos. 3, 17, 51 and 73) had low or 0 DVs in the hepatopancreas but high DVs (>1) in the feces. There was no obvious pattern, as some shrimp had both positive DVs for the virus in the hepatopancreas and feces, whereas others did not show this pattern (DV was high in the feces, but absent from the hepatopancreas).

Investigating the relationship between DV in the hepatopancreas and the 6 ovarian samples that were PCR-positive (DV > 0), we found that 3 shrimp (nos. 3, 13 and 37) showed higher DVs and 3 shrimp (nos. 4, 10 and 29) lower DVs than those in the hepatopancreas of the same shrimp. The shrimp ovary has not been reported as being a target organ for *Pmo*DNV; thus the presence of this virus in the ovary was unexpected.

Table 1. Densitometric values from gel electrophoresis of the amplicons specific for *Penaeus monodon* densovirus (*Pmo*DNV) relative to the β-actin gene in hepatopancreas, feces, gonads and hemolymph of *P. monodon* subadults. (–) not sampled

Shrimp no.	Densitometric values in Hepatopancreas	Feces	Gonads	Hemolymph
Female				
1	0.00	0.00	0.00	0.00
2	1.00	1.07	–	3.18
3	0.06	1.52	2.16	–
4	4.46	2.49	1.76	0.05
5	0.48	0.26	0.00	0.00
10	1.47	1.40	0.43	0.00
13	0.04	0.00	0.38	0.00
17	0.00	0.85	0.00	0.00
29	0.36	0.00	0.05	–
32	0.09	–	0.00	–
35	0.01	0.00	0.00	0.00
37	0.52	0.60	2.06	–
81	0.22	0.00	0.00	–
Mean ± SEM	0.67 ± 0.34	0.68 ± 0.23	0.57 ± 0.25	0.40 ± 0.40
Male				
50	0.72	–	–	–
51	0.03	2.72	0.00	0.00
53	0.15	0.00	0.00	0.39
64	7.08	0.00	0.00	0.00
65	1.08	0.25	0.00	0.00
66	1.52	0.00	0.00	–
71	0.24	0.00	0.00	–
73	0.07	1.56	0.00	0.00
76	1.02	0.13	0.00	0.33
79	0.21	0.42	0.00	0.00
80	0.14	0.27	0.00	0.16
82	0.70	0.00	0.00	0.27
Mean ± SEM	1.08 ± 0.56	0.49 ± 0.26	0	0.13 ± 0.05

The relationship between DVs and shrimp BW was also determined. Since the mean BW (MBW) of the females was significantly higher than that of the males, the BWs of individual shrimp were expressed relative to the MBW of their sex. The result revealed that the shrimp that weighed less than MBW had significantly higher DV than those that weighed more than MBW (*t*-test, p = 0.0013; Fig. 1).

By H&E histology, the hepatopancreas of the shrimp with high DVs (nos. 4 and 10) for *Pmo*DNV revealed solitary inclusions in the nuclei of the E cells, the cell type known to be susceptible to the virus (Pantoja & Lightner 2001). Two observable types of inclusions (8–10 µm in size) showed either mixed eosinophilic/basophilic staining or basophilic staining surrounded by clear space (Fig. 2a). By immunohistochemistry using primary antibodies specific to *Pmo*DNV proteins, a positive reaction towards the virions was revealed by brownish stains observed in several, but not all, intranuclear inclusions (Fig. 2b). Positive reactions were also present in the intercellular spaces and within the lumen of the hepatopancreatic tubules (Fig. 2c). In the lumen, clumps

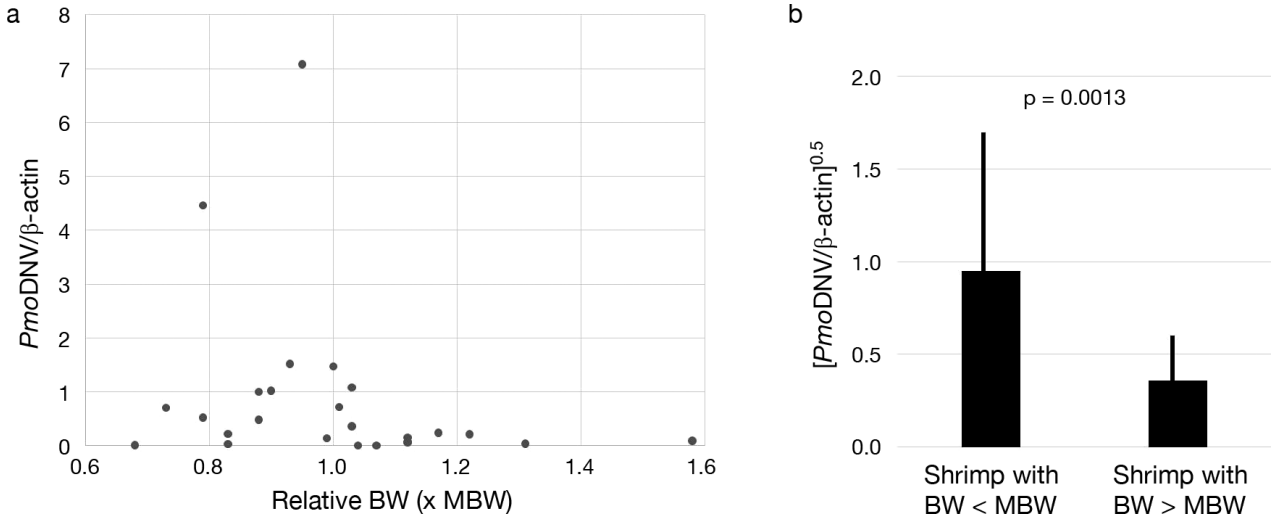

Fig. 1. (a) Densitometry of *Penaeus monodon* densovirus (*Pmo*DNV/β-actin) in the hepatopancreas in relation to body weights (BW) of individual *Penaeus monodon* (in terms of mean BW of all shrimp, MBW). (b) Densitometric values of *Pmo*DNV in shrimp with BW below MBW (<MBW) and above MBW (>MBW)

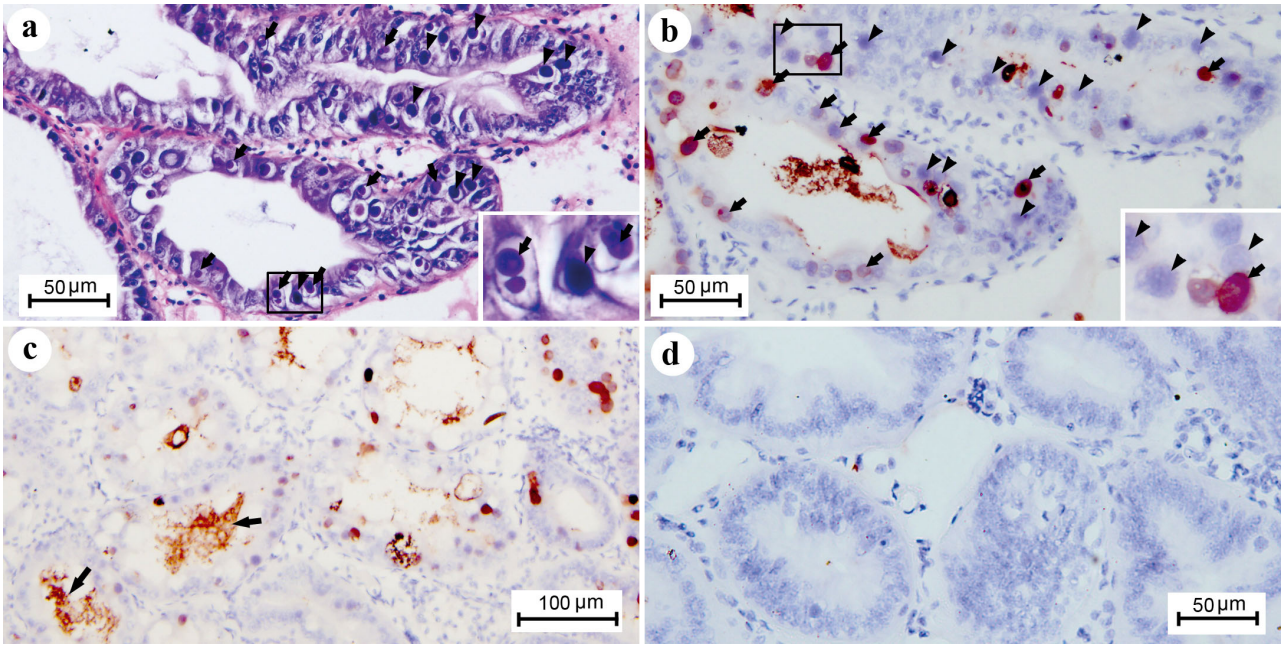

Fig. 2. (a) H&E stained section of the hepatopancreas (HP) of *Penaeus monodon* subadult with high densitometric values (>1) for *Penaeus monodon* densovirus (*Pmo*DNV). Intranuclear, eosinophilic inclusions were observed in the infected HP epithelial cells (arrows), which were turned into dense basophilic inclusions surrounded by clear space (arrowheads). Inset shows higher magnification. (b) Consecutive HP section immunostained with antibodies against *Pmo*DNV proteins. Brownish positive reactions with variable levels of intensity were observed in some intranuclear inclusions (arrows), whereas other inclusions had no positive reactions (arrowheads). Inset shows higher magnification. (c) *Pmo*DNV immunoreactive signal inside the HP lumen (arrows). (d) Negative control section showing no immunoreactive signal

of brown precipitates surrounded by dispersed brown particles were occasionally observed, which could be either the virus aggregate being released into the lumen and individual virions dispersing out from its core, or simply the primary antibodies being trapped by debris in the lumen. No positive reaction was observed in control slides with primary antibodies omitted (Fig. 2d).

Histological observations of the ovary revealed normal features of ovarian tissue, which is composed of a thin connective capsule, connective tissue cells, proliferating oogonia, immature oocytes, and follicular cells (Fig. 3a). However, at least 4 types of inclusions were observed in the ovaries with high DV (>1). These inclusions (approximately 10 µm) were observed mainly in the vicinity of the follicular cells (Fig. 3b,c) and were acidophilic (type i), basophilic (type ii), basophilic with condensed core (type iii) and dark basophilic surrounded by empty space (type iv). These inclusions were larger than the size of the follicular cells and many of them were localized in the follicular cells. It is possible that all of these 4 types were the same virally-induced inclusions, but in different chronological stages and were similar to those found in *Pmo*DNV-infected hepatopancreatic cells. No inclusions were observed in ovarian samples that did not have a positive viral load (Fig. 3d).

Immunohistochemical experiments revealed that positive reaction towards *Pmo*DNV in the ovary was not as intensive as in the hepatopancreas, yet was clearly observed in the connective tissue capsule, intercellular space (Fig. 4a), area surrounding the oocytes and within the inclusions (Fig. 4b). Positive reaction in the inclusions was light, but clearly presented (Fig. 4c). No positive reaction was observed in the control sections and in ovaries without the viral load (Fig. 4d,e, respectively).

DISCUSSION

A heavy infection by *Pmo*DNV retards the growth rate of *Penaeus monodon* juveniles (Flegel et al. 1999) and *Fenneropenaeus merguiensis* (Owens et al. 2011) in grow-out farms. However, it is not known if the virus would have a similar effect on subadult shrimp or broodstock. The finding that *P. monodon* subadults weighing less than MBW had significantly higher (*t*-test, $p = 0.0013$) DVs than those weighing more than MBW suggests that

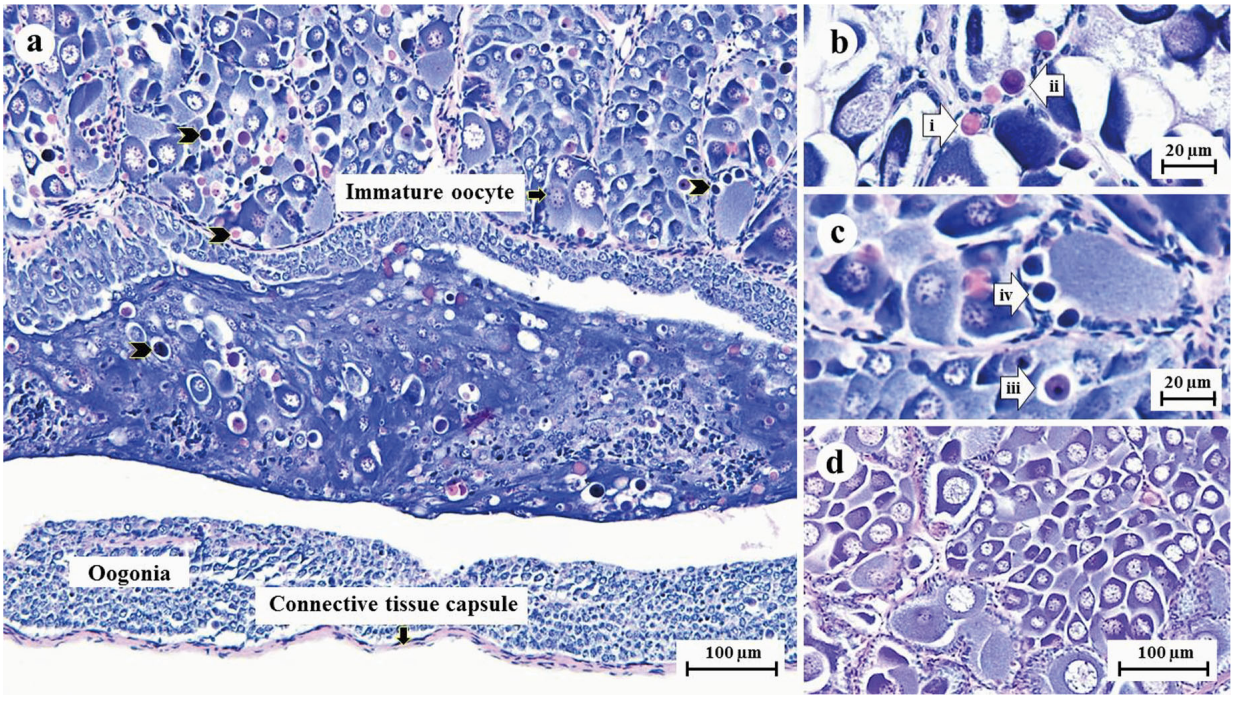

Fig. 3. (a) H&E stained ovarian section of *Penaeus monodon* with high densitometric values (>1) for *Penaeus monodon* densovirus (*Pmo*DNV). The ovary consisted of connective tissue capsule surrounding proliferating oogonia and immature oocytes. Several types of inclusions were observed (arrowheads). (b) Acidophilic (i) and basophilic (ii) inclusions. (c) Basophilic inclusion with condensed core (iii) and dark basophilic inclusion surrounded by empty space (iv). Several inclusions were lined in the same row of the follicular cells that surrounded the oocyte. (d) PCR-negative ovary showing no inclusion

*Pmo*DNV may retard growth rates in subadult shrimp. Alternately, the DV may not be an accurate index of the viral load of the hepatopancreas because the PCR sample is based on a small piece of tissue taken from a large organ, and may not be representative of the infection level.

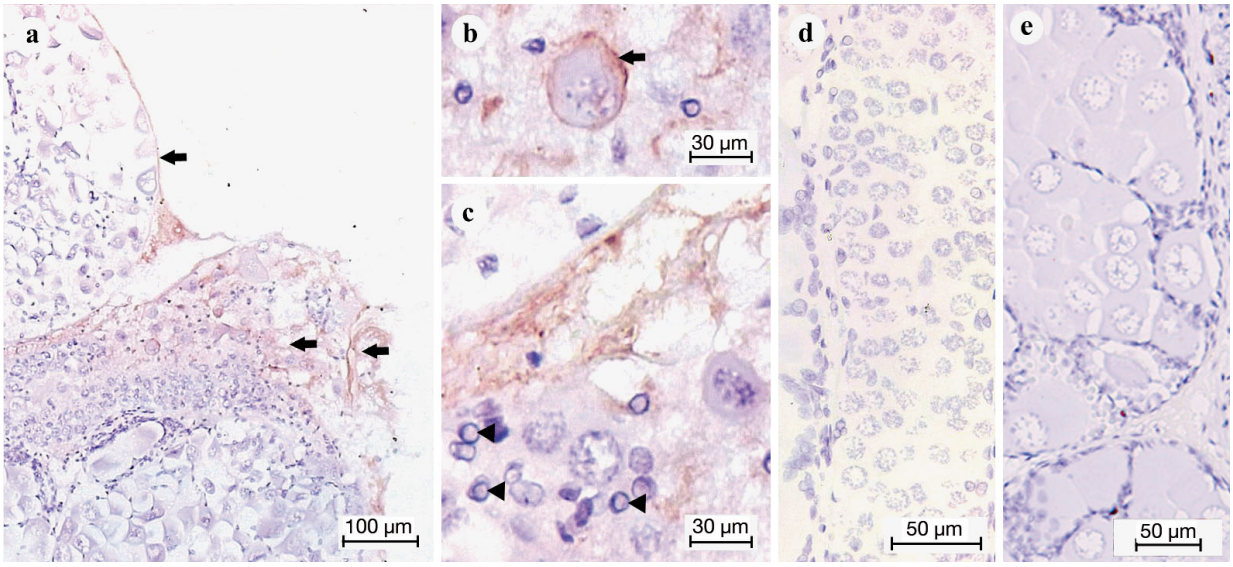

Fig. 4. (a) Immunohistochemistry specific for *Penaeus monodon* densovirus (*Pmo*DNV) of the ovary of *P. monodon* with high densitometric values (>1). The brownish positive signal was observed in the intercellular space and connective tissue capsule (arrows). (b) Immunoreactive signal in the periphery of oocytes (arrow). (c) Immunoreactive signal in the inclusions (arrowheads) along the row of the follicular cells. (d) Negative control section without antibody addition showing no immunoreaction signal. (e) Ovarian section of shrimp with negative PCR for *Pmo*DNV showing no immunoreactive signal

In commercial hatcheries, broodstock fecal samples are often used as representative samples for PCR detection of *Pmo*DNV infection in the hepatopancreas and to non-destructively sample valuable *Pmo*DNV-negative broodstock, especially those in special breeding programs. The present study reveals that if the test results of fecal samples are taken as an indicator of *Pmo*DNV infection of the hepatopancreas, it would result in 30% false negatives (negative predictive value = 0.3, sensitivity = 0.74, specificity = 1.0). Obviously, if hepatopancreatic tissue could be sampled for PCR without sacrificing the shrimp, the results would be more accurate. Hepatopancreatic biopsy of the shrimp has recently been achieved, and the diagnosis of *Pmo*DNV or other viral infections of this organ may be more accurate than employing feces as the representative sample (Kerdmusik et al. 2018).

We found that 4 out of 13 PCR-positive feces samples were from the shrimp that had low DV (<0.5) or were PCR-negative for *Pmo*DNV in the hepatopancreas. This observation prompts a question: Where did the virus appear, if not from the hepatopancreas? The answer might lie in one of the following non-exclusive possibilities: firstly, the small portion of hepatopancreatic samples (15–20 mg) taken from the whole organ (1–2 g) might have contained a low viral load simply by chance; secondly, the virus may have come from the infected cells of the gut wall of the shrimp; or thirdly, the virus in the hemolymph could have arrived at the gut lumen by some unknown pathway. Thus far, there has been no report of *Pmo*DNV infection in cells in the gut wall of shrimp. However, this could be easily examined in the future. More likely is that the PCR sample has significant variability. This could also be examined in the future by repeated sampling of affected tissues. Interestingly, Kanjanasopa et al. (2015) recently found that monodon baculovirus, another virus infecting hepatopancreatic cells, was also present in the oocytes and nauplii of *P. monodon*. The presence of *Pmo*DNV in the hemolymph of *P. monodon* in this study also supports that viremia occurred during the infection. To our knowledge, there has been no previous report that the shrimp ovary is the target organ for *Pmo*DNV. Therefore, our finding that approximately 50% of the ovaries were PCR-positive is a new and unexpected one. The PCR finding was supported by routine histology, which revealed several inclusions, and by immunohistochemistry, which revealed positive reactions in various components of the ovary, including the oocytes and the inclusions. The features of *Pmo*DNV infection in the ovarian tissue were similar to those in the hepatopancreas, which is a well-known target organ of the virus; the only difference was the intensity of the positive reaction in the inclusions. Because the inclusions were in the follicular cells surrounding the oocytes, it is likely that the inclusions represent the viral aggregates inside the nuclei of the follicular cells, and the cells were destroyed by viral infection.

The weak positive reaction of the immunohistochemistry in the inclusions could be due to the inaccessibility of the inclusions to some antibodies (Rukpratanporn et al. 2005), or the absence of the viral proteins in those inclusions. Further detailed observations of *Pmo*DNV in ovarian follicular cells require the resolution provided by transmission electron microscopy or *in situ* hybridization, which are the subject of on-going studies. The presence of a *Pmo*DNV-positive reaction on the periphery of several oocytes suggests that virions may attach to the oolemma but may not gain access into the cytoplasm or nuclei of the oocytes.

The observation of high viral load in the hepatopancreas but low load in the feces could be explained by the possibility that there was no shedding of the virus into the lumen at the time of feces sampling. The converse finding of high DVs in the feces but low levels in the hepatopancreas was surprising, but it is possible that the 2 groups of subadults were undergoing different periods of viral infection, where the first group was still in the pre-shedding stages while the second was already in the post-shedding period. The use of fecal samples for *Pmo*DNV detection as an indirect way to detect infection in the hepatopancreas is a common practice in the hatchery to avoid sacrificing the valuable broodstock (Pantoja & Lightner 2000). However, from the frequency of 56.5% positive cases found in the feces, compared to that of 92% in the hepatopancreas, the use of feces as representative samples may result in a large number of infected broodstock not being screened out.

In this study, not all *Pmo*DNV-positive ovaries were correlated to the presence of the virus in hepatopancreatic cells, as 3 ovarian samples had higher DVs than the hepatopancreas. This suggests that, besides being transported from the hepatopancreatic cells to the ovary, *Pmo*DNV might be specifically attracted to the ovary, which means that the ovary is another tropic site of viral infection. The presence of *Pmo*DNV in the ovary also indicates the possibility that the virus may be vertically transmitted through the trans-ovarian route. Thus far, direct evidence showing vertical transmission of this virus has not been shown. Horizontal transmission occurs via can-

nibalism (Catap & Travina 2005), immersion and feeding (Sun & Kusuda 1997). However, several studies detected the virus in PL by PCR, which was supportive of vertical transmission (Manivannan et al. 2002, Umesha et al. 2003). Nonetheless, it is too early to take the presence of *Pmo*DNV in the ovary as being direct evidence showing the vertical transmission of this virus.

At spawning, mature oocytes, or eggs, are released from the gonopore together with ovarian fluid. It could be inferred that several *Pmo*DNV virions attach to the outer oolemma at the time of spawning. In *P. monodon*, formation of the hatching envelope does not occur until the egg contacts with seawater (Pongtippatee-Taweepreda et al. 2004, Pongtippatee et al. 2013). During this process, the hatching envelope gradually surrounds the oolemma and completely covers it within 15 min after the beginning of its formation. Therefore, it is possible that *Pmo*DNV are trapped between the oolemma and the hatching envelope from the time of spawning to hatching, which is approximately 10 h. During that period, the development from embryo to nauplius occurs inside the envelope, and the virus may somehow enter the nauplius and infect the target cells at their earliest life stage. Obviously, further research is warranted to test this hypothesis that would unravel the exact mechanism and the specific route(s) for the vertical transmission of *Pmo*DNV in *P. monodon* and other penaeid shrimp.

CONCLUSIONS

*Pmo*DNV was detected by PCR in 4 tissues: hepatopancreas > feces > ovary > hemolymph; no PCR reaction was positive in samples of testes. Some shrimp with strongly positive signals by PCR in the feces and ovary had negative PCR results for the hepatopancreas, suggesting that the ovary and gut may be target organs of the virus as well. Histology of the ovarian tissues that were strongly PCR-positive for *Pmo*DNV revealed several types of inclusions associated with the follicular cells. By immunohistochemistry specific for *Pmo*DNV proteins, a reactive signal was found in the inclusions located in the nuclei of the infected hepatopancreatic cells, as well as in the intercellular space, connective tissue, oocytes and inclusions in the ovary. The presence of *Pmo*DNV in the ovary as detected by PCR and immunohistochemistry suggests the possibility of trans-ovarial transmission of *Pmo*DNV in *Penaeus monodon*.

Acknowledgements. The study was partially supported by the Office of the Higher Education Commission and Mahidol University under the National Research Universities Initiative and a Mahidol University Research grant. The AIT Fellowship for Master's study to S.F. by the Asian Institute of Technology is also gratefully acknowledged.

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Editorial responsibility: Jeffrey Shields, Gloucester Point, Virginia, USA

Submitted: November 15, 2017; Accepted: May 5, 2018
Proofs received from author(s): June 29, 2018