Asia Regional Aquatic Animal Health Programme

DISEASE ADVISORY

Decapod Iridescent Virus 1 (DIV1): an emerging threat to the shrimp industry

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The shrimp industry has been beset by many devastating diseases in the last three decades, which has caused severe production and economic losses and even caused the collapse of the industry in some countries. These include viral (WSSV, TSV, YHV), bacterial (luminous vibriosis, AHPND), and parasitic (EHP) diseases. Recently, another emerging shrimp viral disease is threatening the shrimp industry in China, one of the top shrimp producers in the world. The virus, formally named as Decapod iridescent virus 1 or DIV1 by ICTV, was first detected as early as 2014 from *Cherax quadricarinatus* samples in Fujian Province, and temporarily named the new virus as *Cherax quadricarinatus* iridovirus (CQIV) (Xu et al., 2016). In December 2014, Qiu et al. (2017) identified a new iridescent virus in farmed white leg shrimp *Penaeus vannamei* from Zhejiang Province and named it Shrimp hemocyte iridescent virus (SHIV) based on the infected tissues and susceptible species. The disease has occurred in farmed *P. vannamei* and giant freshwater prawn *Macrobrachium rosenbergii* in some provinces (Qiu et al, 2018c, 2019) and again in February this year, affecting about a quarter of the area under shrimp production in the south of Guangdong Province (He, 2020). The virus infects all stages of shrimps (PLs, juveniles, adults) and has been observed to affect the Pacific white shrimp, crayfish, and giant freshwater prawn.

Currently known susceptible species of DIV1 include *P. vannamei*, *M. rosenbergii*, *Exopalaemon carinicauda*, *M. nipponense*, *Procambarus clarkii*, and *C. quadricarinatus* (Xu et al., 2016; Qiu et al., 2017; Qiu et al., 2019a; Chen et al., 2019). Two species of crab, *Eriocheir sinensis* and *Pachygrapsus crassipes* could be infected with DIV1 in experimental challenge through intramuscular injection (Pan et al., 2017), but cannot yet be identified as susceptible species. DIV1 could also be detected in *P. chinensis*, *P. japonicus*, *M. superbum*, *Nereis succinea* or some cladocera by PCR method (Qiu et al., 2017; Qiu et al., 2018a; Qiu et al., 2019a; Qiu et al., 2019b). Infection with DIV1 has been reported in some provinces of P.R. China since 2014. China has extended the National Targeted Surveillance Program to cover DIV1 since 2017 and revealed that DIV1 has been detected in 9 of 15 provincial administrative regions (Qiu et al., 2018a; Qiu et al., 2019b; BoF et al., 2019). Positive cases have been reported in the wild populations of *P. monodon* caught in Indian Ocean (Srisala et al., 2020). The geographic distribution of DIV1 may be wider than currently known, since mortality may not have been investigated in other countries or regions (NACA, 2019).

Signs of the Disease

Clinical signs of infected *P. vannamei* are not typical, including slightly reddish body, hepatopancreatic atrophy with colour fading, and empty stomach and guts. A unique gross sign of DIV1 can be observed in diseased *M. rosenbergii*, which exhibit a typical and whitish area under the carapace at the base of rostrum. Moribund individuals sink to the bottom in deep water and dead individuals can be found every day, with a cumulative mortality up to 80% (Chen et al. 2019; Qiu et al. 2017; Qiu et al. 2019a). The half lethal time (LT50) of DIV1 per os challenge was 8.11 ± 0.81 d (Qiu et al., 2017) which is about double of that of WSSV.

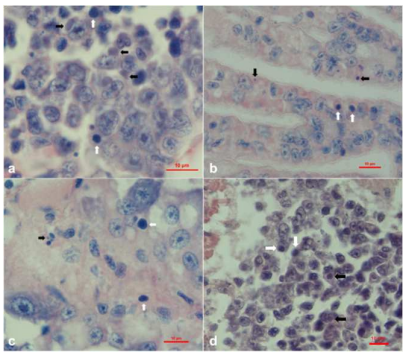
***P. vannamei* from laboratory: left group (healthy); right group (infected with DIV1).**
Source: Qiu et al., 2017

Faded hepatopancreas of *P. vannamei*, infected with DIV1.
Source: Qiu et al., 2017

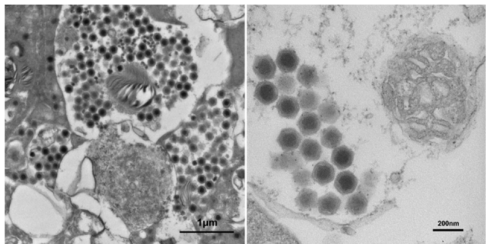
White area inside the carapace at the base of rostrum of *M. rosenbergii* infected with DIV1.
Source: Qui et al., 2019a

Internally, the following can be observed in infected shrimps:

- Dark eosinophilic inclusions mixed with basophilic tiny staining and karyopyknosis in hematopoietic tissues, lymphoid organs, and hemocytes in gills, hepatopancreatic sinus and pereiopods in histopathological sections stained by H&E (Qiu et al., 2019a; Chen et al., 2019; Sanguanrut et al., 2020).
- Typical icosahedral iridescent virions occur in the cytoplasm of the above-mentioned tissues observed with ultrathin sections under transmission electron microscope (Qiu et al., 2017).



Histopathological features of *P. vannamei* infected with DIV1. Black arrows show the dark eosinophilic inclusions with tiny basophilic staining, while white arrows show the karyopyknotic nuclei. a: hematopoietic tissue; b: gills; c: sinus in hepatopancreas, and d: pereiopods. H&E Stain, Bar=10 µm.
Source: Qui et al., 2017



Typical icosahedral iridescent virions in hemocytes of *P. vannamei* with DIV1.
Source: Qui et al., 2017

PCR Detection Methods

Nested PCR (Qiu et al., 2017)

Nested PCR is carried out in two separate steps using two pairs of primers targeting the ATPase gene of DIV1. In the first step, the PCR amplifies a 457 bp amplicon. In the second step, a 129 bp amplicon is amplified. To visualize the amplicons, the PCR product (5 µL of each) was analyzed in a 1% agarose gel containing GeneFinder (Bio-V, China).

The first-step PCR:

i) The 25 µL of PCR reaction mixture contained 2.5 µL of 10× Ex Taq Buffer (Mg^{2+} free), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM primers (SHIV-F1: 5′ GGG CGG GAG ATG GTG TTA GAT-3′ and SHIV-R1: 5′-TCG TTT CGG TAC GAA GAT GTA-3′), 0.625 U TaKaRa Ex Taq DNA polymerase (TaKaRa, Dalian, Liaoning, China), and 1 µL of template DNA.

ii) The PCR was performed at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 59 °C for 30 s and 72 °C for 30 s, ending at 72 °C for 2 min.

The second step of the (nested) PCR:

i) A PCR mixture the same as above but with 1 µL of the 1st step PCR product as templates and different primers (SHIV-F2: 5′-CGG GAA ACG ATT CGT ATT GGG-3′ and SHIV-R2: 5′-TTG CTT GAT CGG CAT CCT TGA-3′).

ii) The amplification was performed with the following cycling parameters: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 59 °C for 30 s and 72 °C for 20 s, with a final extension at 72 °C for 2 min.

Real-time PCR

Primers and probe for DIV1-MCP qPCR (Qiu et al., 2020)

A new TaqMan assay was established using a pair of primers (142F/142R) targeting a amplicon of 142 bp in the DIV1 MCP gene. The sequences of primers are 142F: 5′- AAT CCA TGC AAG GTT CCT CAG G -3′ and 142R: 5′- CAA TCA ACA TGT CGC GGT GAA C -3′. The sequence of TaqMan probe is 5′- CCA TAC GTG CTC GCT CGG CTT CGG -3′ labeled with 6-FAM at the 5′ end and TAMRA at the 3′ end.

Primers and probe for DIV1-ATPase qPCR (Qiu et al., 2018b)

The earlier reported TaqMan assay used a pair of primers (SHIV-F/SHIV-R) targeting a amplicon of 188 bp in the DIV1 ATPase gene. The sequences of primers are SHIV-F: 5′- AGG AGA GGG AAA TAA CGG GAA AAC-3′, SHIV-R: 5′- CGT CAG CAT TTG GTT CAT CCA TG-3′. The TaqMan Probe has the sequence: 5′- CTG CCC ATC TAA CAC CAT CTC CCG CCC-3′.

The DIV1-ATPase qPCR method is not recommended to be used as the second verification of the nested PCR method, as it's amplicon has partial overlap with the target sequence of the nested PCR.

The amplification can be carried out following the protocol:

i) Real-time PCR amplification was performed in a 20-µL reaction system consisting of 10 µL 2× Master Mix (FastStart Essential DNA Probes Master mix, Roche), 500 nM of each primer, 200 nM TaqMan probe and 1µL DNA template.

ii) The PCR profile is one cycle of 95 °C for 10 min for initial denaturation, followed by 40 cycles of 95 °C for 10 seconds and 60 °C for 30s to 1 min.

The methods can detect 1.2 copies of target DNA. These two Real-time PCR methods can verified each other. The diagnostic sensitivity and diagnostic specificity are 97.2% and 98.7%, respectively, when the DIV1-MCP qPCR was compared with the DIV1-ATPase qPCR (Qiu et al., 2020).

Suggested Preventive Strategies

The establishment of biosecurity system for the industry is the principle of prevention and control of DIV1 (Huang et al., 2017). Surveillance program, broodstock/postlarvae quarantine, and health certification for the disease are urgently needed in major shrimp producing countries. For these purposes, regional or national capacity building for DIV1 testing and proficiency testing should be carried out as soon as possible. In addition, notification and reporting of any outbreak or viral detection should be promoted. There is no typical clinical sign for infected penaeid shrimps. Alternatively, giant freshwater prawn *M. rosenbergii* can be used as an indicator species for suspected cases, as a typical white hematopoietic tissue can be observed in the diseased prawns. Confirmatory diagnosis should rely on molecular detection methods. Unlike WSSV, DIV1 can easily cause lethal infection to the species of genus *Macrobrachium*. Thus, polyculture with different crustaceans (e.g. *P. vannamei* and *M. rosenbergii*) will bring high risk of DIV1 transmission and should not be recommended (Qiu et al., 2019). However, as diseased shrimp can be removed by predatory fish (Jang et al., 2007), polyculture of shrimp with a small number of fish is recommended for prevention of the disease. A high-density nursery of postlarva with a second testing before stoking to the grow-out pond can be considered as a quarantine approach to increase biosecurity (Clausen et al., 2016). Studies have shown that live polychaete used as feed for broodstock has been found positive for DIV1, and may pose risk of introducing the pathogen. Therefore, it is suggested that shrimp breeding and hatchery facilities should use an alternative feed or adopt some treatment approaches to decontaminate live feeds prior to use (Qiu et al., 2018c).

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