Diseases of Crustaceans – **Infection with Decapod Iridescent Virus 1 (DIV1)**

Signs of Disease

Infection with DIV1 is an emerging disease in farmed *Cherax quadricarinatus* and *Penaeus vannamei* suffering a high mortality in Zhejiang Province of China in 2014 (Xu et al., 2016; Qiu et al., 2017a). The following disease signs (Qiu et al., 2017a; Qiu et al., 2019) can be used for presumptive diagnosis of the disease.

Disease signs at pond level (Level I diagnosis)

- Diseased *P. vannamei* exhibit hepato-pancreatic atrophy with fading color.
- Upon dissection, the hepatopancreas of DIV1 infected shrimp appears pale.
- Shrimp shells are commonly soft.
- Empty stomach and guts.
- Some shrimp have slightly reddish bodies.
- Onset of clinical signs and mortality starting in few days after infection.
- Moribund shrimp sinks to bottom.
- A unique gross sign of infection with DIV1 can be observed with diseased *Macrobrachium rosenbergii*, which exhibit a typical white triangular area under the carapace at the base of rostrum.

Disease signs at animal level (Levels II and III diagnoses)

The following can be observed in infected shrimps:

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

Figure 1. *P. vannamei* from laboratory: left group (healthy); right group (infected with DIV1).
Source: Qiu et al., 2017

Figure 2. Faded hepatopancreas of *P. vannamei* infected with DIV1.
Source: Qiu et al., 2017

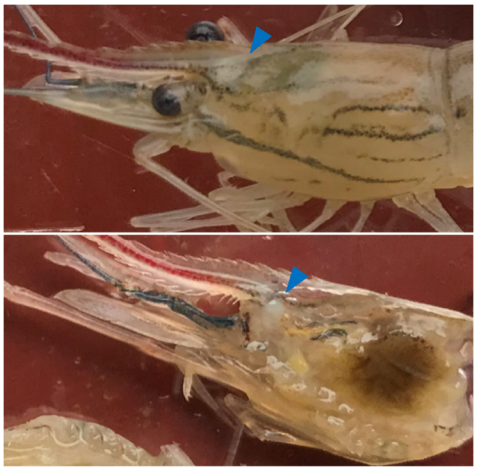

Figure 3. White area inside the carapace at the base of rostrum (blue arrows) of *M. rosenbergii* infected with DIV1.
Source: Qui et al., 2019

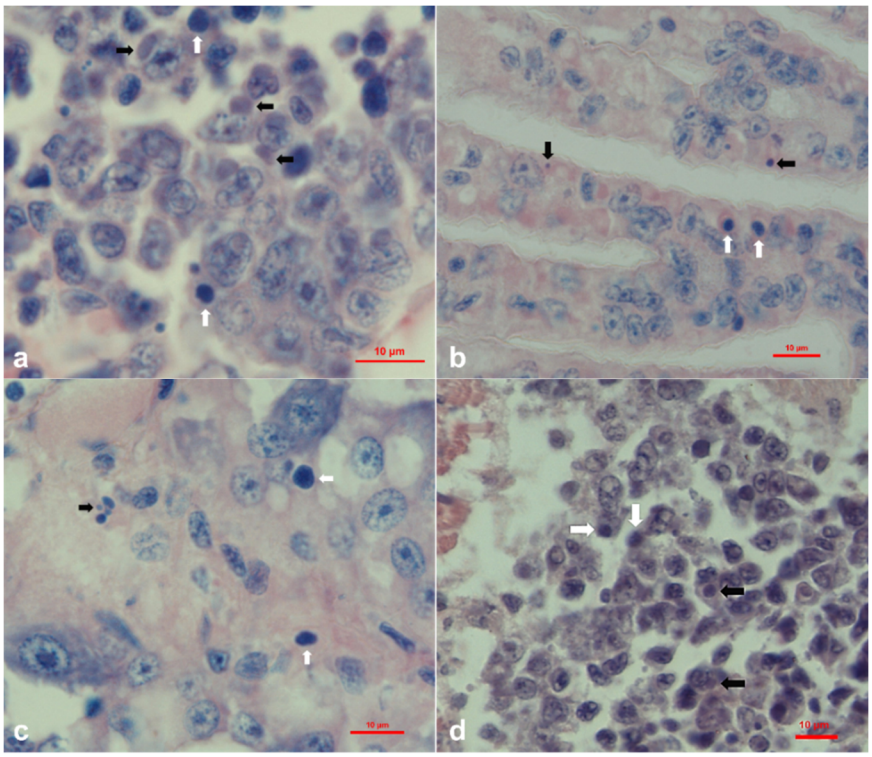

Figure 4. Histopathological features of *P. vannamei* infected with DIV1. Black arrows show the dark eosinophilic inclusions with tiny basophilic staining while white arrows show the karyopyknotic nuclei. a: hematopoietic tissue; b: gills; c: sinus in hepatopancreas, and d: pereiopods. H&E Stain, Bar=10 µm.

Source: Qui et al., 2017

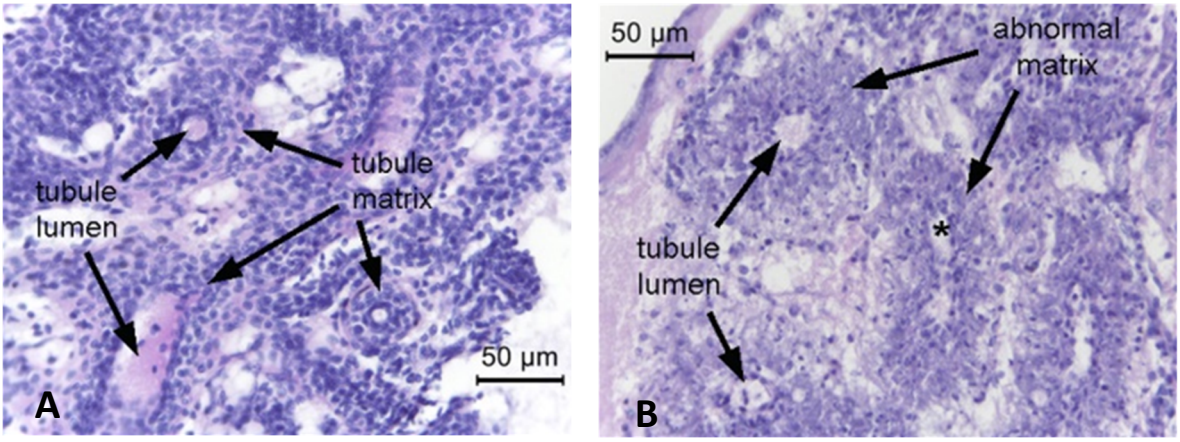

Figure 5. Photomicrographs of normal lymphoid organ (LO) tissue and abnormal LO also present in moribund shrimp from DIV1 challenge tests. (A) Normal LO tissue. (B) Low magnification of abnormal LO tissue from a farmed moribund shrimp showing a disorganized tubule matrix containing many pyknotic and karyorrhectic nuclei.

Source: Sanguanrut et al., 2020

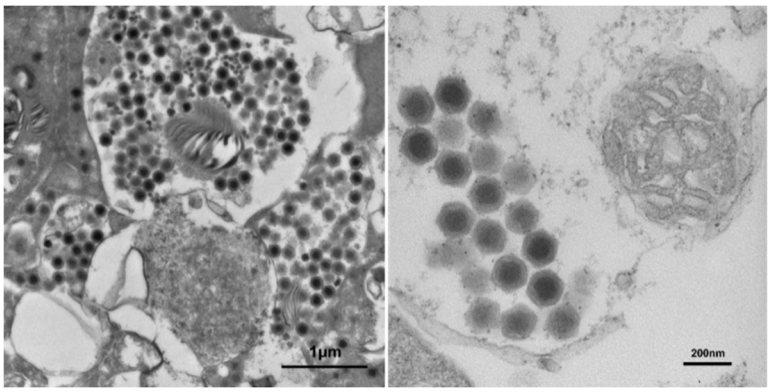

Figure 6. Typical icosahedral iridescent virions in hemocytes of *P. vannamei* infected with DIV1.
Source: Qui et al., 2017

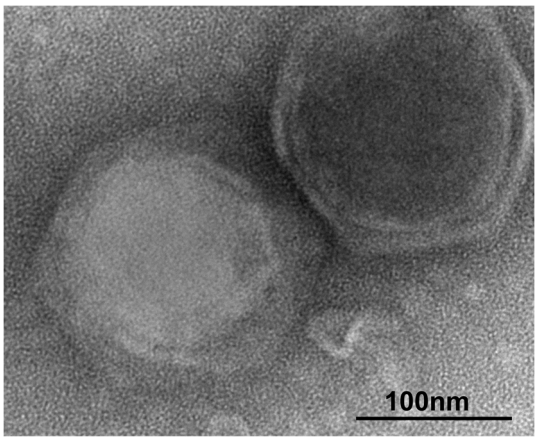

Figure 7. Purified DIV1 particles.
Source: Qui et al., 2017a

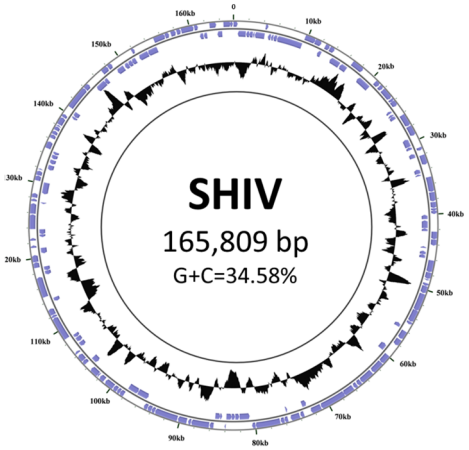

Figure 8. Circular map of the 165,809-bp DIV1 genome.
Source: Qui et al., 2018b

Disease Agent

DIV1 was assigned by the International Committee on Taxonomy of Viruses (ICTV) as the only member of the new genus *Decapodiridovirus* within the *Iridoviridae* family. Virions of DIV1 exhibit a typical icosahedral structure with a mean diameter around 150 nm. DIV1 has a double-stranded DNA genome of about 166 Kbp (Li et al. 2017; Qiu et al., 2018b). Shrimp hemocyte iridescent virus (SHIV 20141215) and *Cherax quadricarinatus* iridovirus (CQIV CN01) are two isolates. The complete genome of SHIV 20141215 is 99.97% identical to CQIV CN01.

Host Range

Currently known species susceptible to DIV1 include *P. vannamei*, *P. monodon*, *M. rosenbergii*, *Exopalaemon carinicauda*, *M. nipponense*, *Procambarus clarkii*, and *Cherax quadricarinatus* (Xu et al., 2016; Qiu et al., 2017; Qiu et al., 2019; Chen et al., 2019; Sanguanrut et al., 2020).

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Presence in Asia-Pacific

DIV1 was first reported in farmed *C. quadricarinatus* and *P. vannamei* in Fujian and Zhejiang Provinces of China, respectively, in 2014 (Qiu et al., 2017; Qiu et al. 2018c). China has extended the National Target Surveillance Program for aquatic animal diseases to include DIV1 since 2017. A total of 15 provincial administrative regions were involved in the program. The virus was detected in farmed *P. vannamei*, *P. japonicus*, *M. rosenbergii*, *M. nipponense*, *Procambarus clarkii* and *Exopaleomon carinicauda* sampled from nine provinces, including Tianjin, Shanghai, Jiangsu, Zhejiang, Anhui, Fujian, Hubei, Guangdong and Guangxi in 2018 (BoF et al., 2019). Wild caught *P. monodon* samples from the Indian Ocean were also detected positive for DIV1 (Srisala et al., 2020).

Molecular Diagnostic Methods (Level III diagnosis)

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
A new TaqMan assay (DIV1-MCP qPCR) 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. (Qiu et al., 2020).

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The earlier reported TaqMan assay (DIV1-ATPase qPCR) 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' (Qui et al., 2018a).

The DIV1-ATPase qPCR method is not recommended to be used as the second verification of the nested PCR method, as its 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 s 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).

List of Experts:

Dr. Liang Qiu
Maricultural Organism Diseases Control and Molecular Pathology Laboratory,
Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences,
#106 Nanjing Road, Qingdao, Shandong 266071, P. R. China
qiuliang@ysfri.ac.cn

Prof. Jie Huang
Network of Aquaculture Centres in Asia-Pacific (NACA)
NACA P. O. Box 1040, Kasetsart Post Office, Ladyao, Jatujak, Bangkok 10903, Thailand
jie.huang@enaca.org

Information presented is based on current knowledge and as reviewed by members of NACA's Asia Regional Advisory Group on Aquatic Animal Health (AG). As there are many unknowns regarding DIV1, the disease card will be updated as new information becomes available through peer-reviewed studies, expert opinions and experiences from primary producers.

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Department of Agriculture and Water Resources

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