Diseases of Crustaceans – **Viral Covert Mortality Disease (VCMD)**

Signs of Disease

Important: affected animals may show one or more of the signs below, but the infection may be present in the absence of any signs, especially during the early phase of infection.

Disease signs at pond level (Level I diagnosis)

- Most of moribund shrimp stay at the bottom and die; moribund and dead shrimp can be observed daily;
- High mortality follows a rapid change in water temperature, especially at above 28°C.

Disease signs at animal level (Level I diagnosis)

The following can be observed in infected shrimps:

- Hepatopancreatic atrophy and necrosis (Figures 1 and 2);
- Empty stomach and gut;
- Soft shell;
- Slow growth;
- In many cases, abdominal muscle whitening and necrosis (Figures 1 and 2).

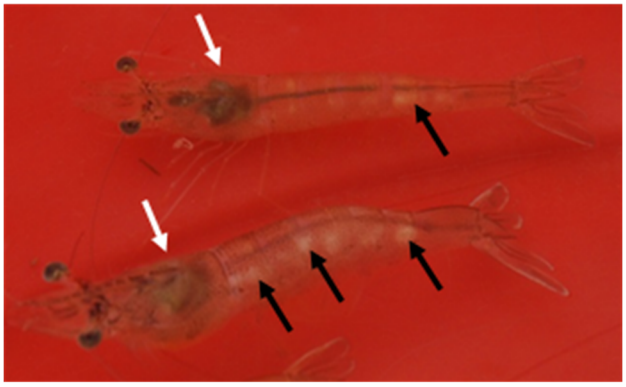

Figure 1. VCMD in cultured white shrimp (*Penaeus vannamei*). White arrows indicate atrophy and a faded colour to the hepatopancreas. Black arrows show whitening of abdominal muscle segments.
Source: QL Zhang

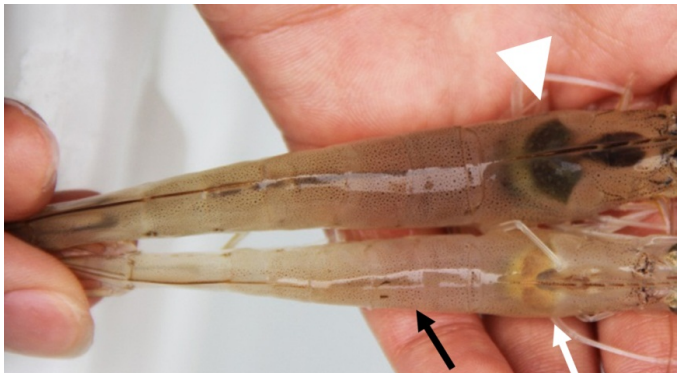

Figure 2. VCMD in experimentally infected whiteleg shrimp (*P. vannamei*). White arrow indicates atrophy and color fading of the hepatopancreas compared to the normal shrimp with dark heaptopancreas (white arrow head).
Source: QL Zhang

Disease Agent

VCMD is caused by covert mortality nodavirus (CMNV), a positive single strand RNA virus that has been classified in the family Nodaviridae.

Similar Diseases

- Infection with Infectious myonecrosis virus (IMNV)
- White tail disease (Infection with *Penaeus vannamei* nodavirus)

Viral Covert Mortality Disease

Host Range

Crustaceans known to be susceptible to infection with covert mortality nodavirus (RT-PCR and ISH positive) include *Penaeus vannamei, P. chinensis, P. japonicus, P. monodon, Macrobrachium rosenbergii, Procambarus clarkii, Exopalaemon carinicauda, Ocypode cordimanus, Diogenes edwardsii, Corophium sinense, Parathemisto gaudichaud,* and *Tubuca arcuate* (Zhang et al., 2014; 2017a; Li, et al., 2018a; Liu et al., 2018). Fish species, including *Mugilogobius abei*, *Carassius auratus*, and *Paralichthys olivaceus* may also be susceptible to the virus, according to the results from ISH (Zhang et al., 2018; Wang et al., 2018, 2019).

Presence in Asia-Pacific

Shrimp samples collected from China were found positive for CMNV by RT-PCR, ISH (Zhang et al., 2014; Zhang et al., 2017; Zhang 2019). Shrimp samples collected from Thailand were found positive for CMNV by RT-PCR and ISH (Pooljun et al., 2016; Thitamadee et al., 2016). Material collected from Thailand, however, consisted of only non-diseased, grossly normal shrimp. They showed no ISH reactions in the muscle tissue and the positive ISH reactions were relatively weak and occurred only in nuclei of the tubule epithelial cells of the hepatopancreas. This differed from the Chinese samples where the positive signals arose from the cytoplasm of such cells (Fig. 5).

Epidemiology

- Infection with CMNV usually occurs within 30-80 days post stocking, with cumulative mortality up to 80%. In serious cases, it occurs within 10-20 days of stocking post larvae into grow-out ponds. Cases of asymptomatic infection detected with CMNV kits were also found in farms.
- Horizontal transmission through cannibalistic behavior of shrimp.
- Vertical transmission of CMNV via sperm or oocytes in *Exopalaemon carinicauda* (Liu et al., 2017).
- Some wild crustaceans in the ponds are vectors of the disease (Liu et al., 2018).
- Migratory birds, aquatic insects and humans are likely mechanical vectors of the disease.

Horizontal Transmission

Eleven species of invertebrates inhabiting shrimp ponds were found to be CMNV positive using RT-nPCR or RT-LAMP. These include brine shrimp *Artemia sinica*, barnacle *Balanus sp.*, rotifer *Brachionus urceus*, amphipod *Corophium sinense*, Pacific oyster *Crassostrea gigas*, hermit crab *Diogenes edwardsii*, common clam *Meretrix lusoria*, ghost crab *Ocypode cordimundus*, hyperiid amphipod *Parathemisto gaudichaudi,* fiddler crab *Tubuca arcuata*, and an unidentified gammarid amphipod (Liu et al., 2018). Five wild crustacean species, including *Cor. sinense*, *Dio. edwardsii*, *Ocy. cordimanus*, *Par. gaudichalldi*, and *Tub. arcuata*, were also tested to be ISH positive of CMNV. These five species might also act as reservoir hosts of CMNV in horizontal transmission of the disease (Liu et al., 2018).

Histological Images (Level II diagnosis)

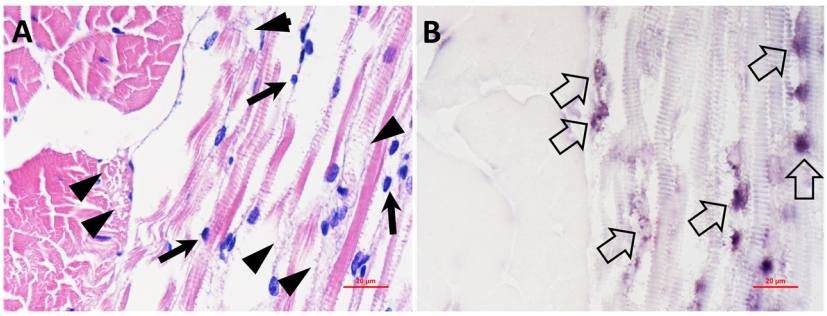

Figure 3. H&E staining and *in situ* hybridization (ISH) for necrotic muscle of *P. vannamei* naturally infected with CMNV. The muscle showed fragmentation tending towards coagulative and dissolving necrosis (black triangles). The black arrows and the open arrows indicated the karyopyknotic nuclei and purple hybridization signal of the CMNV RNA probe, respectively. Scale bars = 20 µm (A), 20 µm (B). Source: QL Zhang

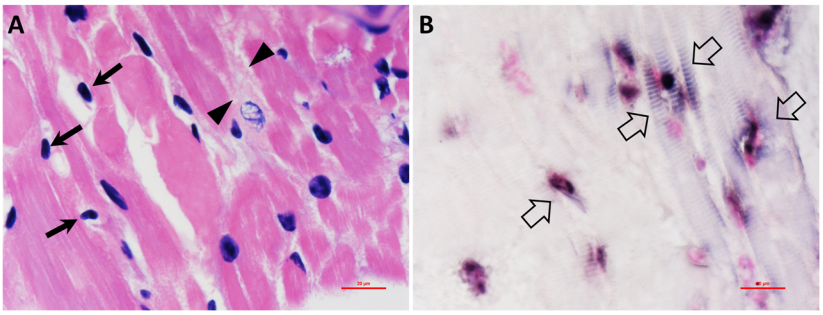

Figure 4. A and B: H&E staining and ISH for necrotic muscle of *E. carinicauda* naturally infected with CMNV. The muscle showed fragmentation tending towards coagulative and dissolving necrosis (black triangles). The black arrows and open arrows indicated the karyopyknotic nuclei and the purple hybridization signal of CMNV probe, respectively. Scale bars =20 µm (A and B). Source: QL Zhang

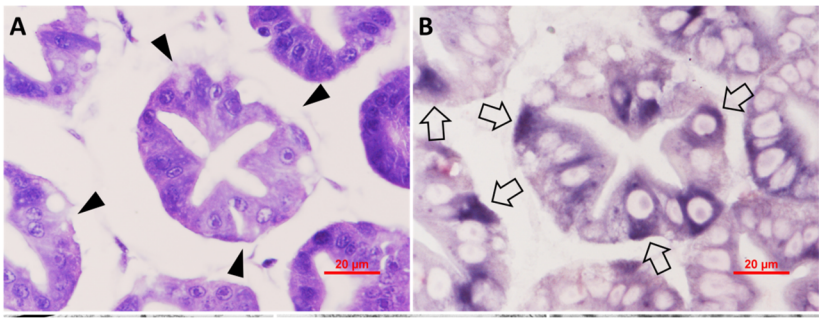

Figure 5. A and B: H&E staining and ISH for atrophied and necrotic hepatopancreas epithelium of *P. monodon* naturally infected with CMNV. The black triangles and open arrows indicated the necrotic epithelium and the purple hybridization signal of CMNV probe, respectively. Scale bars =20 µm (A and B). Source: QL Zhang

Molecular Diagnostics (Level III diagnosis)

Reverse transcription amplification based methods

Different molecular diagnostic methods were established based on the sequence of RNA-dependent RNA polymerase (RdRp). The reported methods include:

- A reverse transcriptase, nested PCR (RT-nPCR) (Zhang, et al., 2014).
- A RT-LAMP method (Zhang et al., 2017b).
- A sensitive TaqMan RT-PCR (Pooljun et al., 2016).
- A high compatibility TaqMan real time RT-PCR (Li et al., 2018b)

Nucleic acid probe based in situ hybridization (ISH) methods

- An ISH method for CMNV was initially described in 2014 (Zhang, et al., 2014).
- An alternative ISH method was published (Zhang, et al., 2017).

There is no sequence overlap between the RT-nPCR and any of the TaqMan RT-PCR method or the RT-LAMP. Therefore, RT-nPCR results can be verified with any of the TaqMan RT-PCR methods or the RT-LAMP tests. It is not valid to verify the results between RT-nPCR and ISH, or between the RT-LAMP and any of TaqMan RT-PCR methods, as there are overlaps over the targeting sequences of the relevant methods. ISH and RT-nPCR may bring a high risk of contamination to each other. It is discouraged to run these two tests in the same room or without a diagnostic laboratory Quality Management System.

Due to the lack of proofreading activity of RdRp, CMNV genomes have characteristically high mutation rates, which bring the risk of false negatives. Touch down PCR combining with sequencing may be applied to find out false negatives caused by possible mutations.

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Table 1. Primers and probes for the above-mentioned molecular diagnostic methods.

Methods	Primers/Probe	Sequences (5'-3')	Ta	Target region
RT-nPCR [1]	CMNV-7F1 CMNV-7R1	AAATACGGCGATGACG ACGAAGTGCCCACAGAC	45°C	619 bp (232-850)
	CMNV-7F2 CMNV-7R2	CACAACCGAGTCAAACC GCGTAAACAGCGAAGG	50°C	165 bp (256-420)
RT-LAMP [2]	F3 B3 FIP BIP LF LB	TGCCAAGCAAATACGAGCT CATCAGCGATGTCACGGC GTCGTCGACGGTTAGGTTGCGTTTTCCAAGCAC TTCCCGACAA CGTCCAAAAGGACCTCCGCATTTTTGGAGACCT TGGTCACGC GCTCACGGCTTTGGATACC GATTGCATGCGTCAACCTCA	65°C	179 bp (873-1051)
TaqMan RT-PCR [3]	CMNV-F CMNV-R CMNV-TaqMan	ACCTCCGCAATCTGATTG GGGTCTACTTTCGTTGGA FAM-CGCTACCACTGTCGGCTTGT-TAMRA	60°C	130 bp (981-1110)
TaqMan RT-PCR [4]	CMNV-Taq-F CMNV-Taq-R CMNV-Taq-P	CGAGCTAATCCAAGCACTTC ACCTGTTAGGTACGCTACCA FAM-CGCTCACGGCTTTGGATACCTT-TAMRA	52.7°C	198 bp (886-1084)
PCR for ISH probe [5]	CMNV-7Probe-F CMNV-7Probe-R	GGCGATGACGGCTTGA GGCGGTGAGATGGATTTT	By Labeling Kit	201 bp (238-438)
PCR for ISH probe [6]	CMNV-ISH-F CMNV-ISH-R	CTACTGCAGAATACGGCGATGACGG CAGAAGCTTAAAGGGACGGAAGGGTT	By Labeling Kit	227 bp (233-459)

[1] Zhang, et al., 2014; [2] Zhang et al., 2017b; [3] Pooljun et al., 2016; [4] Li et al., 2018; [5] Zhang, et al., 2014; [6] Zhang et al., 2017a.

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 VCMD, 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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