White Tail Disease (WTD) of *Macrobrachium rosenbergii*

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Introduction

*Macrobrachium rosenbergii* is the most favoured species for farming purposes and being cultured in different parts of the world.

The important freshwater prawn producing countries are China, Thailand, India, Taiwan, Bangladesh and Vietnam.

One of the major constraints limiting the prawn production all over the world is diseases.

Generally, *M. rosenbergii* is considered to be a moderately disease-resistant species when compared to penaeid shrimp.

No serious viral diseases have been reported so far in *M. rosenbergii* except WTD.
Viral Diseases of *Macrobrachium*

The following viruses have been reported in prawn:


2. *Macrobrachium* muscle virus (MMV) (Tung et al., 1999).

3. Infectious hypodermal and haematopoietic necrosis virus (IHHNV) (Hsieh et al., 2006).

4. White spot syndrome virus (WSSV) (Peng et al., 1998; Rajendran et al., 1999; Sahul Hameed et al., 2000; Waikhom et al., 2006; Yoganandhan and Sahul Hameed, 2007).

5. *Macrobrachium rosenbergii* nodavirus (*MrNV*) and extra small virus-like particle (XSV) (Arcier et al., 1999; Qian et al., 2003; Sahul Hameed et al., 2004a; Yoganandhan et al., 2006).
White Tail Disease (WTD) is responsible for high mortalities in hatchery and nursery-reared post-larvae of freshwater prawn and subsequent economic loses in prawn culture industry. This disease has been studied extensively by different workers.

**Distribution:** This disease was first reported in the French West Indies (Arcier et al., 1999), later in China (Qian et al., 2003), India (Sahul Hameed et al., 2004a) and very recently in Taiwan (Wang and Chang, 2006), Thailand (Yoganandhan et al., 2006) and Queensland, Australia (The Fishsite.com, 2008).
Clinical Signs

The clinical signs of WTD include lethargy and opaqueness of abdominal muscle. Opaqueness gradually extended anterior and posterior, and degeneration of telson and uropods was also observed in severe cases (Arcier et al., 1999; Sahul Hameed et al., 2004a).
Histological sign of WTD: Presence of basophilic cytoplasmic inclusions in striated muscles of abdomen and cephalothorax and intratubular connective tissue of the hepatopancreas and no viral inclusions were observed in epithelial cells of hepatopancreatic tubules or in midgut mucosal epithelial cells (Arcier et al., 1999).
Causative Organisms: Two viruses namely *Macrobrachium rosenbergii* nodavirus (*MrNV*) and extra small virus are responsible for WTD (Bonami et al., 2005; Sahul Hameed et al., 2004a).

*MrNV* is a small icosahedral non-enveloped particle, 26-27 nm in diameter. It contains two single-stranded RNAs, RNA1 (RdRP) and RNA2 (Capsid) of 2.9 and 1.26 kb, respectively. Its capsid contains a single polypeptide of 43 kDa. With these characteristics, it is closely related to the Nodaviridae family.
XSV is also non-enveloped icosahedral virus, 15 nm in diameter and possesses a linear ssRNA genome of 0.9 kb encoding 2 overlapping structural proteins of 16 and 17 kDa (Bonami et al., 2005). Because of small size and absence of gene-encoding enzymes required for replication, it has been suggested that XSV may be a satellite virus and MrNV is a helper virus.
Pathogenicity: These two viruses caused 100% mortality in post-larvae at 7 to 12 day post infection by immersion challenge. In virus-infected group, PL started showing whitish muscle at day 7 p.i. and reached highest proportion at 10 day p.i. (Qian et al., 2003; Sahul Hameed et al., 2004b). The RT-PCR analysis confirmed the infection in experimentally infected PL.

These viruses failed to cause mortality in adult prawn by intramuscular route (Sahul Hameed et al., 2004b).

![RT-PCR detection of MrNV and XSV in experimentally infected post-larvae. M: marker; Lane 1: virus suspension prepared from infected post-larvae; Lane 2: healthy post-larvae; Lanes 3, 4 and 5: different groups of post-larvae exposed to MrNV and XSV](image-url)
Tissue tropism of *MrNV/XSV*: The RT-PCR assay revealed the presence of *MrNV/XSV* in gill tissue, head muscle, stomach, intestine, heart, hemolymph, pleopods, ovaries and tail muscle of experimentally injected adult prawn (Sahul Hameed et al., 2004b). The virus multiplies in cytoplasm of connective cells of the most organs and tissues.

Our results on tissue distribution indicate that pleopods would be a convenient source of RNA for non-destructive screening of *MrNV* and *XSV* without stress to the prawns.

Fig. 3. *Macrobrachium rosenbergii*. RT-PCR detection of *MrNV* and *XSV* in different organs of experimentally infected adult prawns. M: marker; Lane 1: hemolymph; Lane 2: gill tissue; Lane 3: hepatopancreas; Lane 4: heart; Lane 5: stomach; Lane 6: eyestalk; Lane 7: head muscle; Lane 8: abdominal muscle; Lane 9: tail muscle; Lane 10: ovarian tissue; Lane 11: intestine; Lane 12: pleopod
Transmission of *MrNV* and *XSV*

Our pathogenicity experiments conducted on post-larvae by immersing challenge, oral route and brooders by i.m. route indicates the possibility of horizontal and vertical transmissions (Sahul Hameed et al., 2004b; Sudhakaran, Sahul Hameed et al., 2007)
Host range for *MrNV* and *XSV*  
(Sudhakaran, Sahul Hameed et al., 2006b, c)

**Penaeid Shrimp:** *Penaeus indicus, P. japonicus* and *P. monodon*  
(Sudhakaran, Sahul Hameed et al., 2006a)

**Artemia** (Sudhakaran, Sahul Hameed et al., 2006b)

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**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of individuals used</th>
<th>Viral inoculum from different hosts</th>
<th>Cumulative mortality (%)</th>
<th>WTD detection by RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 day</td>
<td>3 days</td>
</tr>
<tr>
<td>I</td>
<td>90</td>
<td><em>Penaeus monodon</em></td>
<td>0</td>
<td>23.33</td>
</tr>
<tr>
<td>II</td>
<td>90</td>
<td><em>Penaeus indicus</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>90</td>
<td><em>Penaeus japonicus</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>90</td>
<td>Naturally infected post-larvae of <em>M. rosenbergii</em></td>
<td>0</td>
<td>76.67</td>
</tr>
<tr>
<td>V</td>
<td>90</td>
<td>Tissue filtrate from normal post-larvae</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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*WTD* = Waterborne Toxoplasma Detection; *RT-PCR* = Reverse Transcription Polymerase Chain Reaction.

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a = negative RT-PCR reaction; “+” MrNV or XSV positive by RT-PCR.

a Experiments were carried out in triplicate.
The results of our study on marine shrimp with these viruses indicate the possibility of marine shrimp acting as reservoir for *MrNV* and XSV or getting natural infection and maintaining their virulence in tissue system of marine shrimp.

Some percentage of our farmers have considered either mixed culture of shrimp (*P. monodon*) with *M. rosenbergii* or by crop rotation between these two species as a viable alternative for their sustenance and economic viability. This situation invites the possibility of transmitting pathologically significant organisms from native to non-native hosts as observed in the present study.
We observed natural infection of WTD in hatchery reared *P. indicus* and *P. monodon* in one hatchery, near Chennai (seeds of prawn and shrimp are produced simultaneously in the same hatchery) (Ravi, Sahul Hameed et al., 2009).

The clinical signs observed were lethargy, opaqueness of abdominal muscle. Opaqueness appear at the centre of abdominal muscle and gradually extended anterior and posterior, and 100% mortality within 2-3 day after appearance of whitish muscle. The RT-PCR assay confirmed the presence of MrNV and XSV.
Confirmation of WTD infection by RT-PCR

Fig. 2. Amplification of the RT-PCR products of MrNV and XSV in naturally infected post-larvae of marine shrimp collected from a hatchery. Lane M: marker; Lane N: negative control; Lane 1: positive control; Lane 2: *P. monodon*; Lane 3: *P. indicus*.
Pathogenecity of *MrNV/XSV* inoculum prepared from naturally infected marine shrimp PL

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**Fig. 3.** Cumulative percent mortality of post-larvae of *Macrobrachium rosenbergii* infected with inoculum prepared from *MrNV* and XSV infected *P. monodon*, *P. indicus* and *M. rosenbergii* by immersion challenge. Normal tissue extract prepared from healthy post-larvae was used as negative control.

**Fig. 4.** Amplification of the RT-PCR products of *MrNV* and XSV in experimentally-infected post-larvae of *M. rosenbergii* infected with inoculum prepared from infected post-larvae of marine shrimp. Lane M: marker; Lane N: negative; Lane 1: positive control; Lane 2: Post-larvae of *M. rosenbergii* infected with inoculum of *P. monodon*; Lane 3: Post-larvae of *M. rosenbergii* infected with inoculum of *P. indicus.*
A nodavirus (tentatively named *PvNV*, *Penaeus vannamei* nodavirus) that causes muscle necrosis in *P. vannamei* was observed by Tang et al. (2007) with clinical signs of white, opaque lesions in the tails. One clone with a 928 bp insert was sequenced and found to be similar (69% similarity) to the capsid protein gene of *MrNV*.
Diagnostic Methods for *MrNV* and *XSV*

The available diagnostic methods for *MrNV* and *XSV* include genome based and protein based diagnostic methods.

Genome based diagnostic methods developed for the detection of *MrNV* and *XSV* are dot-blot hybridization, *in situ* hybridization, Reverse Transcriptase-polymerase chain reaction (RT-PCR) (Sri Widada et al., 2003; Sahul Hameed et al., 2004a) and LAMP (Pillai et al., 2006).

![Image of gel electrophoresis](image)
To avoid the necessity of carrying out two separate RT-PCR reactions, we have developed a modified method for simultaneous detection of *MrNV* and XSV in a single tube, one-step multiplex RT-PCR assay. Using mRT-PCR, naturally infected and experimentally infected post-larvae and adult prawns showed two prominent bands of 681 and 500 bp for *MrNV* and XSV, respectively, and these concurred with bands obtained in separate RT-PCR assays for the two viruses.

mRT-PCR for *MrNV* and XSV
(Yoganandhan, Sahul Hameed et al., 2005)
Dot-blot hybridization has been applied to detect *MrNV* in field samples by Sri Widada et al. (2003). This method could detect the *MrNV* at the level of 0.8 pg of viral RNA (Sri Widada et al., 2003).

*In situ* hybridization using *MrNV* probe was developed by Sri Widada et al. (2003) is also available.
Protein based diagnostic methods for *MrNV*

A sandwich enzyme-linked immunosorbent assay (S-ELISA) has been developed to detect *MrNV* using polyclonal antiserum raised against purified *MrNV* (Romestand and Bonami, 2003).

Qian et al. (2007) have developed monoclonal antibodies against *MrNV* and triple antibody S-ELISA has been developed using these Mabs.
Separation and purification of \( MrNV \) and XSV, and their Pathogenicity

\( MrNV \) caused 100% mortality in PL at 7 to 10 d p.i.
XSV did not cause significant mortality in PL
\( MrNV \) and XSV also caused 100% mortality in PL in the same period of 7 to 10 d p.i.
Protein pattern of purified MrNV and XSV, and Western blot for detection of MrNV

MrNV

XSV

Western Blot
Thank You