NOTE

White tail disease of the giant freshwater prawn *Macrobrachium rosenbergii* in Thailand

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ABSTRACT: White tail disease (WTD) of the freshwater prawn *Macrobrachium rosenbergii* has recently been the cause of high mortalities in Thai prawn farms. The causative agents of this disease in other countries are *M. rosenbergii* nodavirus (*MrNV*) and extra small virus (*XSV*), which are usually detected using reverse transcriptase-polymerase chain reaction (RT-PCR) protocols. Using RT-PCR, most Thai post-larvae (PL) samples showing gross signs of WTD tested positive for *MrNV* but only a few were positive for XSV. In contrast, all tested brooder samples were positive for both *MrNV* and XSV. The possibility that brooders infected with *MrNV* and XSV could transmit the viruses to larvae and PL should be examined. Cloning, sequencing and comparison of deduced amino acid sequences of RT-PCR amplicons of WTD samples from Thailand with those of *MrNV* and XSV previously reported from the French West Indies and China revealed that the *MrNV* were closely related but not identical while those from XSV were identical. This is the first report of *MrNV* and XSV from Thailand.

KEY WORDS: White tail disease · *Macrobrachium rosenbergii* nodavirus · Extra small virus · RT-PCR detection · Brooder

INTRODUCTION

*Macrobrachium rosenbergii* is a native species of Thailand and other Southeast Asian countries (New 1990). It is considered to be a moderately disease-resistant aquaculture species when compared to penaeid shrimp (Nash et al. 1987) and has a high economic value. White tail disease (WTD) was first observed and reported in a hatchery in Guadeloupe; it was detected and reported later in Martinique, French West Indies (Arcier et al. 1999). It was then reported from Taiwan (Tung et al. 1999) and The People’s Republic of China in Zhejiang, Jiangsu, Guangdong and Shanghai provinces (Qian et al. 2003, Sri Widada et al. 2003) and finally from India (Sahul Hameed et al. 2004a). Typical gross signs of diseases in infected post larvae (PL) are white discoloration in the abdominal (tail) region. The causative agents of WTD are *M. rosenbergii* nodavirus (*MrNV*) and extra small virus (*XSV*) (Sri Widada et al. 2003).

*MrNV* is a small icosahedral non-enveloped virus, 26 to 27 nm in diameter that has been identified in the cytoplasm of connective tissue cells (Arcier et al. 1999). The capsid contains a single polypeptide of 43 kDa (Romestand & Bonami 2003). Based on these characteristics, the virus has been placed in the family *Nodaviridae* (Garzon & Charpentier 1992, Van Regenmortel et al. 2000, Romestand & Bonami 2003).
Recently, XSV and MrNV have been purified (Bonami et al. 2005).

Detection methods for MrNV include a double antibody sandwich enzyme-linked immunosorbent assay (DS-ELISA) (Romestand & Bonami 2003) and viral genome-based detection methods such as dot blot hybridization, in situ hybridization and reverse transcription-polymerase chain reaction (RT-PCR) amplification (Sri Widada et al. 2003). Similar genome-based detection methods are also available for XSV (Sri Widada et al. 2003, 2004). More recently a single-tube, duplex RT-PCR method has been developed for simultaneous detection of MrNV and XSV (Yoganandhan et al. 2005).

In the present study, farmed Macrobrachium rosenbergii showing gross signs of WTD and grossly normal brooders were tested for the presence of MrNV and XSV by RT-PCR (Sahul Hameed et al. 2004a), and selected amplicons were sequenced and compared to those previously reported for MrNV and XSV from other countries.

**MATERIALS AND METHODS**

**PL and brooders.** Infected PL with prominent signs of whitish muscle in the abdominal region were collected from different locations in Thailand (Table 1). In addition, 3 samples of grossly normal, pond-reared brooders were collected from culture ponds in Rachaburi, Thailand. These samples were transported to the laboratory on dry ice and stored at –20°C.

![Image](image_url)

<table>
<thead>
<tr>
<th>Sample Place of collection</th>
<th>Stage</th>
<th>Clinical signs</th>
<th>RT-PCR MrNV</th>
<th>XSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Petchaburi</td>
<td>9 d</td>
<td>White muscle</td>
<td>+++ –</td>
<td>–</td>
</tr>
<tr>
<td>(2) Petchaburi</td>
<td>15 d</td>
<td>Pale white</td>
<td>– –</td>
<td></td>
</tr>
<tr>
<td>(3) Petchaburi</td>
<td>28 d</td>
<td>White muscle</td>
<td>– –</td>
<td></td>
</tr>
<tr>
<td>(4) Petchaburi</td>
<td>19 d</td>
<td>White muscle</td>
<td>+++ –</td>
<td></td>
</tr>
<tr>
<td>(5) Petchaburi</td>
<td>12 d</td>
<td>White muscle</td>
<td>+++ –</td>
<td></td>
</tr>
<tr>
<td>(6) Ayuthaya</td>
<td>20 d</td>
<td>White muscle</td>
<td>+++ –</td>
<td></td>
</tr>
<tr>
<td>(7) Petchaburi</td>
<td>21 d</td>
<td>White muscle</td>
<td>+++ ++</td>
<td></td>
</tr>
<tr>
<td>(8) Ayuthaya</td>
<td>23 d</td>
<td>White muscle</td>
<td>– –</td>
<td></td>
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<tr>
<td>(9) Ayuthaya</td>
<td>10 d</td>
<td>White muscle</td>
<td>– –</td>
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</tr>
<tr>
<td>(10) Ayuthaya</td>
<td>23 d</td>
<td>Pale white</td>
<td>– –</td>
<td></td>
</tr>
<tr>
<td>(11) Ayuthaya</td>
<td>13 d</td>
<td>White muscle</td>
<td>+++ –</td>
<td></td>
</tr>
<tr>
<td>(12) Rachaburi</td>
<td>Brooder</td>
<td>–</td>
<td>+++ ++</td>
<td></td>
</tr>
<tr>
<td>(13) Rachaburi</td>
<td>Brooder</td>
<td>–</td>
<td>++ ++</td>
<td></td>
</tr>
<tr>
<td>(14) Rachaburi</td>
<td>Brooder</td>
<td>–</td>
<td>+++ ++</td>
<td></td>
</tr>
<tr>
<td>(15) Rachaburi</td>
<td>23 d</td>
<td>White muscle</td>
<td>++ +++</td>
<td></td>
</tr>
</tbody>
</table>

**RESULTS**

The typical gross signs of WTD in infected PL were lethargy and opaque abdominal muscles (white appearance). In all cases, mortality reached 100% within 2 to 3 d after the first appearance of prawns with whitish muscles. The brooder samples were grossly normal and showed no signs of WTD.

Both post-larval and brooder samples tested positive for MrNV and XSV by RT-PCR (Table 1). Both viruses were detected in various organs of brooders (Fig. 1). Comparisons revealed that the 3 sequences of Thai MrNV (GenBank Accession number DQ189990) were identical and very similar (98% cDNA identity) to those for MrNV reported from other geographical
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However, the minor changes in the Thai cDNA sequence led to 3 changes in deduced amino acids, one of which was a non-conservative change (Fig. 2). Thai XSV cDNA sequences (3) were also identical (GenBank Accession number DQ189991) and shared 98% identity to those reported from other regions. However, in contrast to Thai MrNV, deduced amino acid sequences showed 100% identity to those previously reported.

**DISCUSSION**

Sri Widada et al. (2003) suggested that both MrNV and XSV were associated with WTD in Chinese prawns. The simultaneous presence of more than 1 virus type in diseased crustaceans has previously been reported (Bonami 1980, Mari 1987). XSV has been described as a ‘satellite virus’ because it does not possess a gene coding for RNA polymerase and must therefore depend on that of MrNV or possibly another RNA virus for replication (Sri Widada & Bonami 2004).

In the brooder prawns, both MrNV and XSV could be detected, but the prawns showed no gross signs of disease. A similar type of tolerance has been reported for WSSV in *Macrobrachium rosenbergii* (Peng et al. 1998, Sahul Hameed et al. 2000) and for yellow head virus (YHV) in this and other palaemonid shrimp (Longyant et al. 2005). Tolerance in the latter is now known to be associated with low expression of the viral coat protein gp116 but not other viral proteins (P. Sithigorngul pers. comm.). As with these other viruses, the mechanism of tolerance to MrNV and XSV in adult prawns is not known.

The very similar amino acid sequences between Thai MrNV isolates and those reported from elsewhere suggests that all are very closely related. However, it is difficult to surmise at this time whether the close similarity indicates recent dispersal from a common origin. This might be suggested by the fact that the virus was first reported from the French West Indies and then sequentially from China, India and Thailand. On the other hand, it might also be that the disease was not recognized and reported from China, India and Thailand until after the initial report from the French West Indies. The fact that the Thai isolates differ by 3 amino acids from isolates previously reported suggests, at least, that if it was introduced from elsewhere, the introduction was probably not very recent.

![Fig. 1. *Macrobrachium rosenbergii*. Amplification of the RT-PCR products of MrNV and XSV in farm-cultured brooders collected from Rachaburi, Thailand. Lane M: marker; Lane 1: hemolymph; Lane 2: gill tissue; Lane 3: tail muscle; Lane 4: pleopod; Lane N: negative control](image)

![Fig. 2. *Macrobrachium rosenbergii*. Comparison of deduced amino acid (aa) sequences of MrNV capsid protein from various white tail disease (WTD) isolates. The non-conservative aa difference is indicated in bold](image)
A better knowledge of pathogen distribution in tissues and organs of affected animals helps us to understand pathology and transmission. It also assists in the isolation and detection of pathogens and in development of control measures. Tissue tropism of MrNV and XSV has been carried out by Sahul Hameed et al. (2004b), whose RT-PCR assays showed that both MrNV and XSV were present together in all positive tissues and organs. It is now known that XSV is a satellite virus dependent on the RNA-dependent RNA polymerase of MrNV for its replication (Qian et al. 2003). However, it is still not clearly understood whether both viruses are needed to cause WTD or whether MrNV alone is sufficient. Previous reports from India have shown that some WTD samples are positive for XSV only (Sahul Hameed et al. 2004a), and we found WTD samples positive for MrNV only. It is possible that failure to detect the dual infections was due to the fact that single-step RT-PCR protocols were used.

The presence of MrNV and XSV in brooders suggests that they are likely to transmit the viruses to the larvae and PL they produce, as is common for several penaeid shrimp viruses (Lightner 1996). In the interval while this is being determined, it would probably be prudent to screen brooders for MrNV and XSV before they are used for PL production.

LITERATURE CITED


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