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REVIEW ARTICLE

White Tail Disease of Freshwater Prawn, *Macrobrachium rosenbergii*

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Abstract *Macrobrachium rosenbergii* is the most important cultured freshwater prawn in the world and it is now farmed on a large scale in many countries. Generally, freshwater prawn is considered to be tolerant to diseases but a disease of viral origin is responsible for severe mortalities in larval, post-larval and juvenile stages of prawn. This viral infection namely white tail disease (WTD) was reported in the island of Guadeloupe in 1995 and later in Martinique (FrenchWest Indies) in Taiwan, the People's Republic of China, India, Thailand, Australia and Malaysia. Two viruses, *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus-like particle (XSV) have been identified as causative agents of WTD. MrNV is a small icosahedral non-enveloped particle, 26–27 nm in diameter, identified in the cytoplasm of connective cells. XSV is also an icosahedral virus and 15 nm in diameter. Clinical signs observed in the infected animals include lethargy, opaqueness of the abdominal muscle, degeneration of the telson and uropods, and up to 100 % within 4 days. The available diagnostic methods to detect WTD include RT-PCR, dot-blot hybridization, in situ hybridization and ELISA. In experimental infection, these viruses caused 100 % mortality in post-larvae but failed to cause mortality in adult prawns. The reported hosts for these viruses include marine shrimp, Artemia and aquatic insects. Experiments were carried out to determine the possibility of vertical transmission of MrNV and XSV in *M. rosenbergii*. The results indicate that WTD may be transferred from infected brooders to their offspring during spawning. Replication of MrNV and XSV was investigated in apparently healthy C6/36 *Aedes albopictus* and SSN-1 cell lines. The results revealed that C6/36 and SSN-1cells were susceptible to these viruses. No work has been carried out on control and prevention of WTD and dsRNA against protein B2 produced RNAi that was able to functionally prevent and reduce mortality in WTD-infected redclaw crayfish.

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Introduction

Macrobrachium rosenbergii, commonly known as 'scampi', is an economically important cultured freshwater prawn throughout the world. It is the most favoured species for farming and farmed on a large scale in many countries. The scampi is native to Southeast Asian countries and being cultured in Thailand, Vietnam, Kampuchea, Malaysia, Myanmar, Bangladesh, India, Sri Lanka, and the Philippines. It is also produced in Israel, Japan, Taiwan, and some African, Latin American, and Caribbean countries [10]. In the western hemisphere, examination of the possibility of intensive freshwater prawn culture began in the 1960s, but a considerable proportion of the global farmed freshwater prawn output still originates from the Indian sub-continent, Southeast Asia, and tropical areas of Latin America. Considering its high export potential, the giant freshwater prawn enjoys immense potential for culture in India. About 4 million hectares of impounded freshwater bodies in India offer great potential for freshwater prawn culture. In India its culture was introduced to compensate the heavy economic losses due to the epidemic white spot syndrome (WSS) in

penaeid shrimp farming, hypothesizing the resistance of the giant freshwater prawn to WSS [19]. Freshwater prawn farming in India has increased dramatically in the last decade and total production has reached 30,450 MT in 2003 in India. The prawns are exported to eastern European countries and the USA. Since the world market for scampi is expanding at attractive prices, there is great potential for scampi production and export.

Three species of *Macrobrachium* are very important for culture purpose and being cultured in different countries. *M. rosenbergii* is cultured in many countries, the oriental river prawn *M. nipponens* in China [9, 35], and the monsoon river prawn *M. malcolmsonii* in India [8, 9]. Disease has been considered as one of the important constraints to limit the production of freshwater prawn worldwide. Generally, *M. rosenbergii* is considered to be moderately disease-resistant in comparison to penaeid shrimp. Very few viral pathogens such as *Macrobrachium* hepatopancreatic parvo-like virus (MHPV), *Macrobrachium* muscle virus (MMV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), white spot syndrome virus (WSSV), *Macrobrachium rosenbergii* nodavirus (MrNV), and extra small virus like particle (XSV) have been reported in freshwater prawn. Vijayan et al. [33] have reported the incident of mortality in post-larvae of *M. rosenbergii* due to possible viral pathogen resembling to *Macrobrachium* muscle virus (MMV). Among these viruses, MrNV and XSV are important and responsible for huge economic losses, particularly in the hatchery and nursery phases. Hence this review will focus on white tail disease caused by MrNV and XSV.

Geographical Distribution

White tail disease (WTD) caused by MrNV and XSV is responsible for large-scale mortalities in hatchery and nursery phases of prawn culture system. The disease was first reported in the French West Indies [1], later in China [13], India [20], Chinese Taipei [34], Thailand [37], Australia [11] and Malaysia [17].

Clinical Signs and Histopathology

The clinical signs of WTD-infected post-larvae of prawn include lethargy and opaqueness of the abdominal muscle starting at second or third abdominal segment and gradually extend from the center to the anterior and the posterior sections of the muscle (Fig. 1). The whitish muscle appearance gave the name to the disease. Degeneration of the telson and uropods is observed in severe cases. Larvae, post-larvae and juveniles of *M. rosenbergii* are highly susceptible to WTD, which often causes high mortalities in these life stages. Mortality may reach a maximum in about 5 or 6 days after the appearance of the first gross signs. Very few post-larvae with WTD survive beyond 15 days in an outbreak, and post-larvae that survive may grow to market size like any other normal post-larvae. Adults are resistant to WTD, but act as carriers [13, 20].

The most affected tissue in infected PL is striated muscle of the cephalothorax, abdomen and tail. Histological features include the presence of acute Zenker's necrosis of striated muscles, characterised by severe hyaline degeneration, necrosis and muscular lysis. Moderate oedema and abnormal open spaces among the affected muscle cells are also observed. There are large oval or irregular basophilic cytoplasmic inclusion bodies with a diameter of 1–4 μm in infected muscles of the abdomen, cephalothorax, and intra tubular connective tissue of the hepatopancreas. No viral inclusions were observed in epithelial cells of the hepatopancreatic tubules or in midgut mucosal epithelial cells. Pathognomonic oval or irregular basophilic cytoplasmic inclusion bodies are demonstrated in the target tissues by histology [1, 6]. The presence of MrNV in infected cells can be demonstrated in histological sections using a DIG-labelled DNA in situ hybridisation probe specific for MrNV [24].

Causative Organisms

Two viruses namely *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus-like particle (XSV) are responsible for WTD [2]. MrNV is a small, icosahedral, non-enveloped particle, 26–27 nm in diameter (Fig. 2a). It contains two single-stranded RNAs: RNA1 (2.9 kb) and RNA2 (1.26 kb). Its capsid contains a single polypeptide of 43 kDa (Fig. 3). With these characteristics, it is closely related to the *Nodaviridae* family. XSV is also a non-enveloped icosahedral virus, 15 nm in diameter (Fig. 2b), with a linear ssRNA genome of 0.9 kb encoding two overlapping structural proteins of 16 and 17 kDa (Fig. 3). Because of its small size and absence of gene-encoding enzymes required for replication, it has been suggested that XSV may be a satellite virus and MrNV a helper virus.

Nucleotide sequencing of the MrNV genome indicated that RNA-1 was composed of 3,202 nucleotides (Acc. No. AY222839) and that RNA-2 contained 1,175 nucleotides (Acc. No. AY222840). Both RNAs appeared to be in sense orientation, allowing thus their direct translation. Partial amino-acid sequencing of the N-terminal end of CP-43 established that this structural protein was encoded by RNA-2 and its estimated size corresponds to that determined by SDS-PAGE analysis of CP-43. No other coding sequences were found in RNA-2. RNA-1 contained the coding sequences of two non-structural proteins, A protein,

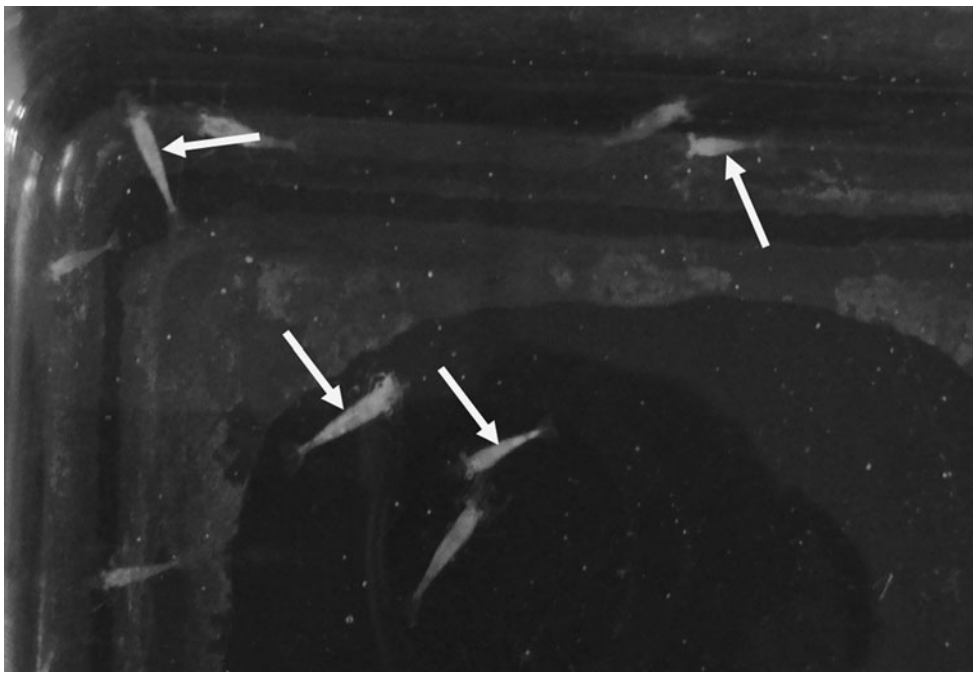

Fig. 1 Post-larvae of *Macrobrachium rosenbergii* with whitish muscle (*arrow*) and normal post-larvae with transparent body

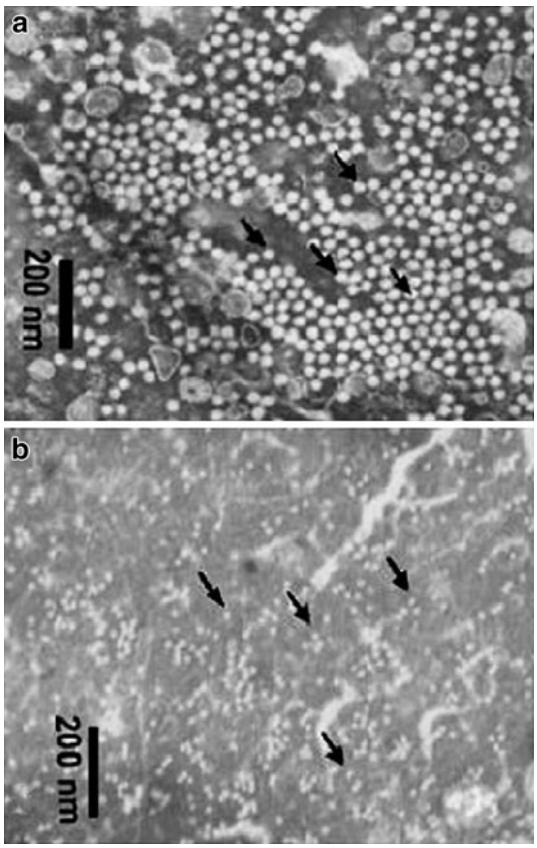

Fig. 2 Negatively stained purified MrNV (**a**) and XSV (**b**)

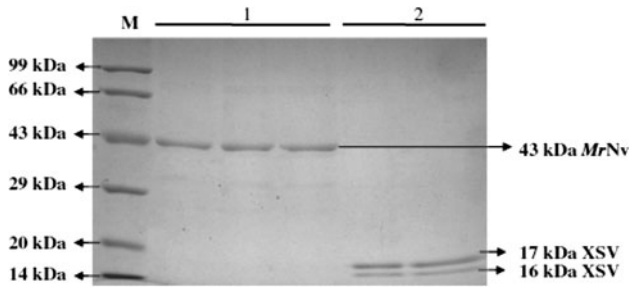

Fig. 3 SDS-PAGE analysis of MrNV and XSV particles purified by sucrose and CsCl density gradients. *Lane M* Marker; *Lanes 1* Purified MrNV (43 kDa); *Lanes 2* Purified XSV (17 and 16 kDa)

i.e. an RNA-dependent RNA polymerase (RdRp) consisting of ca 1,000 amino-acids (ca 100 kDa) and B protein with an estimated size of 13 kDa. B protein was encoded by the 3′ region of RNA-1 (2,725–3,126 nucleotides). It turned out therefore that the genome contains genes required for its replication and most probably also for the regulation of its development within host-cells as it codes for an RdRp which is absent in the host-cell, the capsid protein and most probably the regulator protein B. Except for the RdRp needed for the genome replication, all the necessary enzymes are provided by the host-cells. Nothing is yet known on the mechanism of the virus development. In other noda viruses so far studied, the regulation of RNA replication involves the sub genomic RNA-3 and the protein B [7]. The nucleotide sequence analysis of 850-bp segment of Indian isolate of MrNV showed 98 % nucleotide and 99 % amino acid sequence identity with the reported sequence of an MrNV isolate from the West Indies [22].

Nucleotide sequencing of the XSV genome showed that the viral RNA is composed of 796 nucleotides and in sense

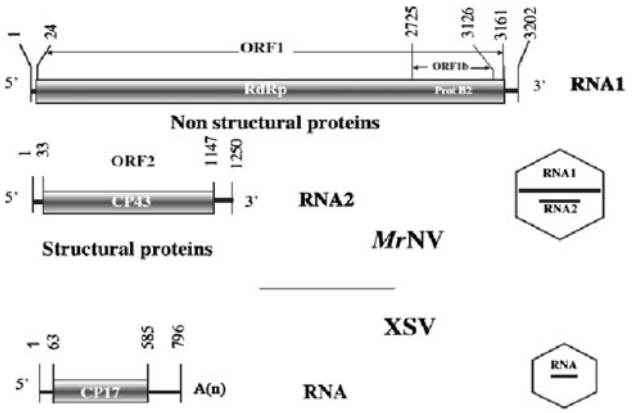

Fig. 4 Organization and encoded structural and non-structural proteins of MrNV and XSV genomes

orientation. A short poly (A) tail was found as well as a polyadenylation signal AAUAAA, localized 195 nucleotides downstream of the second stop codon and six nucleotides upstream of the poly(A) [25]. XSV genome contained the coding sequence of the capsid protein CP-17 or CP-16 and no other coding sequences were found. Amino acid sequencing of CP-17 and CP-16 clearly indicated that both polypeptides were encoded by the same reading frame and that CP-16 was a truncated form of CP-17, lacking the first 11N-terminal amino acids and it is worthwhile mentioning that the N-terminal end of both polypeptides is methionine. CP-16 was not a degraded form of CP-17 as both polypeptides were found in equimolar ratio and analysis was carried out by directly dissolving the virus particles in Laemmli buffer [2]. Besides, the ATG codon of CP-16 is localized in a favorable Kozak context as the ATG codon of CP-17 (7 out of 11 nucleotides matching with the Kozak consensus sequence). The organization of MrNV and XSV genomes and the encoded proteins are shown in Fig. 4.

Genes of MrNV such as RNA-dependent RNA polymerase (RdRp), B2 and capsid genes were amplified, cloned and sequenced. Expression of recombinant RdRP (44.5 kDa) B2 (32.2 kDa) and capsid (58.4 kDa) proteins was confirmed by Western blot analysis using anti-His mouse monoclonal antibodies [23].

Pathogenicity

These two viruses caused 100 % mortality in post-larvae 7–10 days post infection by immersion. In the virus-infected group, post-larvae started showing whitish muscles 5 days p.i. and reached the highest proportion at 7 days p. i. When infected by the intramuscular route, the viruses failed to cause mortality in adult prawns. RT-PCR analysis confirmed the infection in experimentally infected post-larvae [13, 21] and presence of viruses in gill tissue, head muscle, stomach, intestine, heart, hemolymph, pleopods, ovaries, and tail muscles of experimentally injected adult prawns [21]. MrNV and XSV were purified and post-larvae were challenged with different combinations of these two viruses by immersion [38]. Clinical signs of WTD were observed in post-larvae challenged with combinations containing a relatively high proportion of MrNV and low proportion of XSV. In contrast, there was little sign of WTD in post-larvae challenged with a higher proportion of XSV than MrNV, indicating that MrNV plays a key role in WTD of *M. rosenbergii*.

Transmission of Disease

Experiments were carried out to determine the possibility of vertical transmission of MrNV and XSV in *M. rosenbergii* [28]. Prawn brooders inoculated with MrNV and XSV by oral or immersion challenge survived without any clinical signs of WTD. Brooders spawned 5–7 days p. i. and eggs hatched. The survival rate of larvae gradually decreased, and 100 % mortality was observed at the post-larvae stage. Whitish muscle, the typical sign of WTD, was seen in advanced larvae stages. Ovarian tissue and fertilized eggs were positive for MrNV/XSV when tested by RT-PCR whereas larvae were positive by nested RT-PCR [28].

Host Susceptibility

The susceptibility of three species of marine shrimp (*Penaeus indicus*, *P. japonicus*, and *P. monodon*) to MrNV and XSV was tested by oral route and intramuscular injection [27]. The viruses failed to produce mortality in the shrimp but RT-PCR analysis revealed the presence of MrNV and XSV in the gills, abdominal muscle, stomach, intestine, and hemolymph of shrimp injected with the viruses. Re-inoculation using inoculum of MrNV and XSV prepared from the marine shrimp caused 100 % mortality in freshwater prawn post-larvae and moribund post-larvae showed positive for these viruses by RT-PCR. Our study indicates the possibility that marine shrimp act as a reservoir for MrNV and XSV and that these viruses maintain virulence in the shrimp tissue system. Nodavirus tentatively named PvNV (*Penaeus vannamei* nodavirus) was reported causing muscle necrosis in *P. vannamei* found in Belize in 2004 [31]. Numerous similarities were noted between PvNV and MrNV, particularly 69 % of similarity was found between the amino-acid sequence of the MrNV CP 43, the coat protein encoded by RNA-2 and the deduced amino-acid sequence of a PvNV cloned genome fragment. Based on alignment of known amino acid sequences of the coat protein

of alpha- and beta nodavirus, prawn Nodavirus (MrNV) and shrimp Nodavirus (PvNV), three distinct groups emerge from the phylogenetic tree obtained in the noda viridae family: alpha nodavirus (terrestrial environment of invertebrates—insects), betanodavirus (aquatic environment of vertebrates—fish) and prawn and shrimp nodavirus (aquatic environment of invertebrates—crustacean).

Some farmers have considered culturing shrimp (*P. monodon*) with *M. rosenbergii*, or crop rotation between these two species, as an alternative for sustenance and economic viability. However, such a possibility invites the transmitting of pathologically significant organisms from native to non-native hosts, as observed in our study. Recently, a natural WTD infection was observed in hatchery-reared *P. indicus* in a hatchery near Chennai where prawn and shrimp seed are produced simultaneously. Clinical signs included lethargy and opaqueness of the abdominal muscle. Opaqueness appeared at the centre of the abdominal muscle and gradually extended anteriorly and posteriorly with 100 % mortality within 2–3 days after appearance of the whitish muscle. RT-PCR assay confirmed the presence of MrNV and XSV [15].

In horizontal transmission experiments, five developmental stages of *Artemia* were exposed to MrNV and XSV by immersion and oral routes to determine whether *Artemia* acts as a reservoir or carrier of the viruses [26]. There was no significant difference in percent mortality between *Artemia* control groups and virus-challenged groups although *Artemia* of all stages tested positive for both viruses by nested RT-PCR, regardless of the challenge route. Mortality was 100 % in *M. rosenbergii* post larvae fed *Artemia* nauplii exposed to MrNV and XSV by either immersion or oral challenge routes while no mortality was observed in post-larvae fed virus-free *Artemia*. RT-PCR analysis of the *M. rosenbergii* post larvae confirmed the presence of MrNV and XSV in the challenge group and its absence in the control group. Thus, virus exposed *Artemia* are capable of transmitting the disease to *M. rosenbergii* post-larvae.

Among the 5 species of aquatic insects examined, the giant water bug *Belostoma* sp., dragonfly nymphs *Aesohna* sp., diving beetles *Cybister* sp. and back swimmers *Notonecta* sp. gave positive RT-PCR results for MrNV and XSV. By contrast, results for the water scorpion *Nepa* sp. were negative [30].

Susceptibility of Cell Lines to MrNV/XSV Infection

MrNV/XSV could be easily propagated in the C6/36 mosquito *Aedes albopictus* cell line [29] and this cell line can be cultured easily in Leibovitz L-15 medium containing 100 International Units ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 2.5 μg ml^{-1} fungizone supplemented with 10 % fetal bovine serum at 28 °C [29]. Other cell lines, namely the fish SSN-1 cell line, partially support the multiplication of these viruses [5].

Diagnostic Methods

The protocol for the RT-PCR for detection of MrNV/XSV developed by Sri Widada et al. [24] and Sahul Hameed et al. [20, 21] is recommended for all situations. MrNV and XSV can be detected by RT-PCR separately using a specific set of primers or these two viruses can be detected simultaneously using a single-tube one-step multiplex RT-PCR [32, 36]. Nested RT-PCR (nRT-PCR) is also available and recommended for screening brood stock and seed [26].

Quantitative RT-PCR (RT-qPCR) assay can be performed to quantify the MrNV/XSV in the infected samples using the SYBR green dye based on the method described by Zhang et al. [38].

Haridas et al. [3] and Pillai et al. [12] have applied loop-mediated isothermal amplification (LAMP) for rapid diagnosis of MrNV and XSV in the freshwater prawn. A set of four primers, two outer and two inner, have been designed separately for detection of MrNV and XSV. In addition, a pair of loop primers specific to MrNV and XSV has been used to accelerate LAMP reaction.

Antibody-based diagnostic methods for MrNV include the ELISA described by Romestand and Bonami [15] or the triple-antibody sandwich (TAS) ELISA based on a monoclonal antibody [14].

Control and Prevention

No work has been carried out on control and prevention of WTD. However, proper preventive measures, such as screening of brood stock and PL, and good management practices may help to prevent WTD in culture systems. As the life cycle of *M. rosenbergii* is completed under controlled conditions, specific pathogen free (SPF) brood stock and PL can be produced by screening using sensitive diagnostic methods such as reverse-transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) [16, 24, 36]. Hayakijkosol and Owens [4] reported a sequence specific dsRNA against protein B2 produced RNAi that was able to functionally prevent and reduce mortality in WTD-infected redclaw crayfish. RNAi is an effective tool to protect against MrNV infection. Significant up-regulation of immune-related genes was observed in prawn treated with recombinant RdRp protein and this indicates the possibility of inducing the resistance against WTD in prawn using viral proteins [18].

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