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Experimental transmission of *Macrobrachium rosenbergii* nodavirus (*Mr*NV) and extra small virus (XSV) in three species of marine shrimp (*Penaeus indicus*, *Penaeus japonicus* and *Penaeus monodon*)

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Abstract

The susceptibility of three species of marine shrimp (*Penaeus indicus*, *Penaeus japonicus* and *Penaeus monodon*) to *Macrobrachium rosenbergii* nodavirus (*Mr*NV) and extra small virus (XSV) was tested by oral route and intramuscular injection. The results revealed that these marine shrimp were not susceptible to these viruses which failed to produce mortality in shrimp. RT-PCR analysis revealed the presence of *Mr*NV and XSV in different organs such as gill, abdominal muscle, stomach, intestine and hemolymph of three species of shrimp injected with viruses. These viruses were also found in different tissues of shrimp fed with WTD-infected prawn meat, but not in control groups fed with uninfected meat. The reinoculation studies using the inoculum of *Mr*NV and XSV prepared from marine shrimp caused 100% mortality in the post-larvae of freshwater prawn and the moribund post-larvae showed positive for these viruses by RT-PCR. The results of present study indicate the possibility of marine shrimp acting as reservoir for *Mr*NV and XSV and maintaining their virulence in tissue system of marine shrimp.

Keywords: *Mr*NV and XSV; Susceptibility; Shrimp; RT-PCR; Reinfection

1. Introduction

Macrobrachium rosenbergii is the most important and economically cultured palaemonid in the world and it is now farmed in large scale in different parts of the world including India. In 2002, the freshwater prawn production showed a significant increase, reaching an all time high of 20,000 tonnes in India. Infectious diseases caused by viruses and bacteria constitute the main barrier to the development and continuation of crustacean aquaculture, each cultivated species being sensitive to several types of pathogens. A new viral disease similar to white tail disease (WTD), reported by Arcier et al. (1999) has been observed in freshwater prawn hatcheries and nursery ponds in different parts in India, causing high mortalities and huge economic losses (Sahul Hameed et al., 2004a). Before its occurrence in India, this

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disease was reported from the French West Indies (Arcier et al., 1999), Taiwan (Tung et al., 1999) and China (Qian et al., 2003). The causative agent of WTD was originally reported to be a virus, subsequently identified as *M. rosenbergii* nodavirus (*Mr*NV) (Arcier et al., 1999). *Mr*NV is a small, icosahedral, non-enveloped virus 26–27 nm in diameter. The genome is formed by two pieces of ssRNA (RNA1 and RNA2) of 2.9 and 1.26 kb, respectively, and there is a single polypeptide of 43 kDa in the capsid. Qian et al. (2003) subsequently reported the occurrence of an additional extra small virus (XSV) in prawns with WTD collected from China. Sahul Hameed et al. (2004a) have reported the presence of XSV in addition to *Mr*NV in WTD-infected post-larvae of freshwater prawns in India.

Various diagnostic methods have been developed to detect these viruses including histopathology, immunological methods, reverse transcriptase-polymerase chain reaction technique (RT-PCR) and in-situ dot blot hybridization method using nucleic acid probes. Romestand and Bonami (2003) have developed a sandwich enzyme-linked immunosorbent assay to detect *Mr*NV in freshwater prawns. Recently, genome-based methods, dot-blot hybridization and RT-PCR have been developed to detect *Mr*NV (Sri Widada et al., 2003) and XSV (Sri Widada et al., 2004; Sahul Hameed et al., 2004a,b). The pathogenicity of these two viral particles in post-larvae and adult freshwater prawns, and distribution of these two viruses in different tissues and organs of experimentally infected prawns have been studied out using RT-PCR assay (Sahul Hameed et al., 2004b). Our previous studies revealed that these viruses failed to infect the adult freshwater prawn and may not be suitable for propagating these viruses in adult prawn (Sahul Hameed et al., 2004b). Lack of established prawn cell lines is also a hurdle in propagating these viruses in large quantity of viruses for various purposes including preparation of diagnostic reagents. We are screening different arthropod species to find out the host range and suitable proliferating system in crustacean species for these viruses. While screening, three species of marine shrimp (*Penaeus indicus, Penaeus japonicus* and *Penaeus monodon*) were found to be very useful to proliferate these viruses. Hence, the present study was carried out to examine the infectivity and pathogenicity of the *Mr*NV and XSV to three species of marine shrimp and examine the target organs of these viruses in these shrimp to determine whether these target organs are same as in freshwater prawn. We also studied the possibility of using these marine shrimp to proliferate *Mr*NV and XSV instead of adult freshwater prawn.

2. Materials and methods

2.1. Preparation of viral inoculum

Naturally WTD-infected post-larvae (PL) with prominent sign of whitish muscle in the abdominal region were collected from hatcheries located near Nellore, Andhra Pradesh and used as the source of viral inoculum for infectivity experiments. Frozen infected PL were thawed and homogenized in a sterile homogenizer. A 10% (w/v) suspension was made with TN buffer (20 mM Tris–HCl and 0.4 M NaCl, pH 7.4). The homogenate was centrifuged at 4000×*g* for 20 min at 4 °C and its supernatant was recentrifuged at 10 000×*g* for 20 min at 4 °C before the final supernatant was filtered through a 0.22-μm pore membrane. Then, the presence of *Mr*NV and XSV in tissue suspension was checked by RT-PCR. The filtrate was then stored at −20 °C for infectivity studies.

2.2. Collection and maintenance of experimental animals

P. indicus, *P. japonicus* and *P. monodon* (10–15 g body weight) were collected from grow-out ponds or the sea, and maintained in a 1000-l fiberglass tank with an airlift biological filter at room temperature (27–30 °C), with salinity between 20 and 25 ppt. Natural seawater was used in the experiments. It was pumped from the adjacent sea and allowed to sediment to remove the sand and other suspended particles. The animals were fed with commercial pellet feed (CP shrimp feed, Thailand). Dissolved oxygen, salinity, pH and temperature were measured in alternate days during the experimental period. Salinity was measured with a salinometer and dissolved oxygen was estimated by the Winkler method. From these animals, five per species were randomly selected and screened [gill tissue, stomach, intestine, abdominal tissue (25 mg) or hemolymph (50 μl)] for *Mr*NV and XSV by RT-PCR (Sahul Hameed et al., 2004a).

For experimental transmission, healthy post-larvae (PL 10) of *M. rosenbergii* were collected from a hatchery in a locality with no record of WTD. They were randomly sampled and screened for WTD by RT-PCR assay prior to challenge experiments. After collection, the post-larvae were washed with sterile freshwater to remove food and other materials adhering to the body. The washed post-larvae were maintained in glass aquaria (25 l) containing aerated freshwater at a temperature of 27–30 °C and fed twice a day with *Artemia* nauplii.